



Effects of arginine on inflammation and renal injury in rats with streptozotocin-induced Type 2 Diabetes



Kuan-Hsun Huang,¹ Man-Hui Pai,² and Sung-Ling Yeh¹

¹School of Nutrition and Health Sciences and ²Department of Anatomy, Taipei Medical University

Introduction

Advanced glycation end product (AGE) is the ligand of its cellular surface receptor (receptor of AGE, RAGE). AGEs activate the expression of RAGE and may consequently result in inflammatory reaction and organ injury in diabetes mellitus (DM). Arginine (Arg) is a amino acid which possess immunomodulatory effect. Previous *in vivo* and *in vitro* studies showed that Arg inhibits AGEs formation. However, the effects of Arg on RAGE expression and inflammation in diabetic condition are not clear. This study investigated the effects of dietary Arg supplementation on renal RAGE expressions and inflammatory mediator production in diabetic rats.

Study design

There were 1 normal control (NC) group and 2 diabetic groups in this study. Rats in NC group were fed with chow diet. One diabetic group (DM) was fed a common semipurified diet whereas the other diabetic group received a diet in which part of the casein was replaced by Arg (DM-Arg) (Table 1) for 8 wk. Diabetes was induced by intraperitoneal injection of nicotinamide (150 mg/kg) followed by streptozotocin (65 mg/kg). Rats with blood glucose levels exceeding 180 mg/dl were considered diabetic. Blood samples were collected and plasma fructosamine (AGEs), C-reactive protein (CRP), interleukin (IL)-6, prostaglandin (PG)E₂, monocyte chemotactic protein (MCP)-1, intercellular adhesion molecule (ICAM)-1 levels were analyzed by ELISA. The kidneys of the animals were harvested at the end of the study for further analysis.



Measurements of the kidneys

Nitrotyrosine ELISA
RAGE Immunohistochemistry stain
Nuclear factor (NF)- κ B p65 Western blot

Results

Plasma CRP, MCP-1, PGE₂ and fructosamine levels were significantly higher in the diabetic groups than those in the NC group. plasma CRP and fructosamine concentrations were significantly lower in the DM-Arg group than in DM group. No differences in plasma IL-6, MCP-1 and PGE₂ levels were found between the DM and DM-Arg groups at the end of the study (Fig 1 & Table 2). Immunohistochemistry stain showed that the expressions of RAGE in kidney were significantly lower in DM-Arg group than in DM group (Fig. 2). Kidney nitrotyrosine concentrations (Fig. 3) and NF- κ B p65 protein expressions (Fig. 4) were significantly lower in DM-Arg group than in DM group.

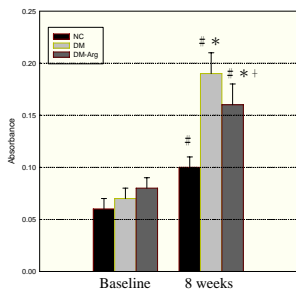


Figure 1. Production of fructosamine as measured by nitroblue tetrazolium (NBT) assay. (n = 13)
*: Significantly different from the NC group at the same time point. (p < 0.05) #: Significantly different from baseline at the same group. (p < 0.05) + : Significantly different from DM group at the same time point. (p < 0.05)

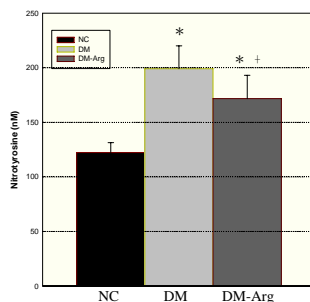


Figure 3. Concentrations of nitrotyrosine in kidney homogenates. (n = 10) *: Significantly different from the NC group. (p < 0.05) #: Significantly different from DM group. (p < 0.05)

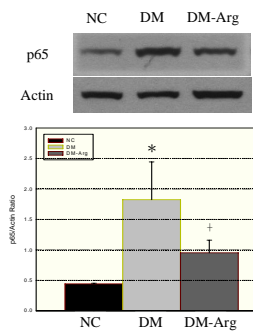


Figure 4. Kidney p65 (NF- κ B subunit) expressions of the 3 groups. (n = 8)
*: significantly different from the NC group. (p < 0.05) + : Significantly different from DM group. (p < 0.05)

Table 1. Composition of the semipurified diet (g / kg)

gram/kilogram	control	Arginine
soybean oil	100	100
casein	200	158
arginine	0	20.9
salt mixture	35	35
vitamin mixture	10	10
methyl cellulose	31	31
choline chloride	1	1
methionine	3	3
corn starch	620	641.1

Table 2. Concentrations of plasma C-reactive protein (CRP), interleukin(IL)-6, prostaglandin(PG) E₂, monocyte chemotactic protein (MCP)-1, intercellular adhesion molecule (ICAM)-1 in the groups at the end of the experimental period.

	NC	DM	DM-Arg
CRP (mg/dl)	22.29 ± 2.12	37.56 ± 4.18 *	33.17 ± 4.67 *#
IL-6 (pg/ml)	128.92 ± 14.61	117.4 ± 15.05	115.35 ± 8.38 *
PGE ₂ (ng/ml)	2.38 ± 0.87	4.83 ± 1.84 *	4.98 ± 1.66 *
MCP-1 (ng/ml)	143.81 ± 11.66	320.30 ± 129.62 *	286.31 ± 123.67 *
ICAM-1 (ng/ml)	25.91 ± 3.36	30.9 ± 7.11	31.9 ± 8.73

*: Significantly different from the NC group. (p < 0.05) #: Significantly different from the DM group. (p < 0.05)

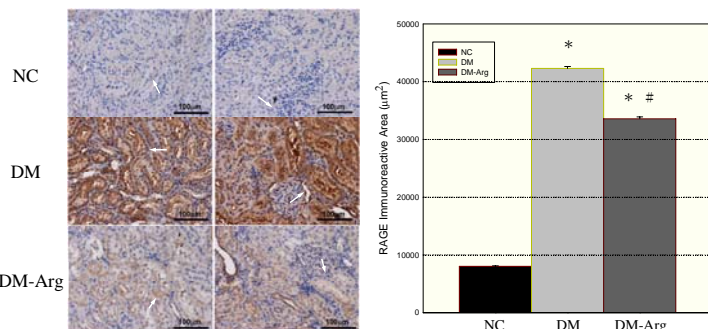


Figure 2. The RAGE expressions in kidney. (n = 3) *: p < 0.05 compared with NC. #: p < 0.05 compared with DM.

Conclusions

These results suggest that dietary Arg supplementation reduced inflammatory reaction and may ameliorate renal damage in rats with type 2 diabetes.