European Journal of Pharmacology 689 (2012) 233–240

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and inflammation

Inflammatory milieu as an early marker of kidney injury in offspring rats from diabetic mothers ☆

Matheus Correa-Costa [a,1], Maristella A. Landgraf [a,1], Maria F. Cavanal [b], Patricia Semedo [c], Daniel A.G. Vieira [b], Davi T.K. De Marco [b], Aparecida E. Hirata [b], Niels O.S. Câmara [a,c,*,1], Frida Z. Gil [b,1]

[a] Department of Immunology, Institute of Biomedical Sciences IV, University of São Paulo (USP), 05508-000 São Paulo, Brazil
[b] Department of Physiology, Federal University of São Paulo, São Paulo 04039-000, Brazil
[c] Nephrology Division, Federal University of São Paulo (UNIFESP), São Paulo 04039-001, Brazil

ARTICLE INFO

Article history:
Received 23 November 2011
Received in revised form
29 April 2012
Accepted 15 May 2012
Available online 28 May 2012

Keywords:
Fetal programming
Maternal diabetes
Renal inflammation
L-arginine

ABSTRACT

The present study investigated the early presence of inflammatory response in renal tissue of young offspring from diabetic mothers. The effect of L-arginine (L-arg) supplementation was also investigated. The offspring was divided into four groups: group CO (controls); group DO (diabetic offspring); group CA (CO receiving 2% L-arg solution) and group DA (DO receiving the 2% L-arg solution). Glycemia, arterial pressure and renal function were evaluated; gene and protein expression of pro-inflammatory cytokines were also measured. Blood pressure levels were significantly increased in 2 and 6 month-old DO rats, whereas L-arg administration caused a significant decrease in the DA group, at both ages. DO rats showed a significantly blunted glycemic response to exogenous insulin. In 2 month-old DO animals, renal protein expression of pro-inflammatory molecules was significantly increased. At six months of age, we also observed an increase in gene expression of pro-inflammatory molecules, whereas L-arg supplementation prevented this increase at both ages. Our data suggest that activation of inflammatory pathways is present early in the kidney of DO rats, and that L-arg can attenuate the expression of these markers of tissue inflammation. Our results also reinforce the concept that intrauterine environmental factors are a fundamental determinant in the development of metabolic and vascular diseases later in life.

1. Introduction

Gestational diabetes mellitus can impose several threats both to the mother and to the conceptus. Diabetic pregnancy increases the risk of intrauterine death, prematurity, perinatal mortality and congenital malformations (Lynch and Wright, 1997; Martinez-Frias, 1994; Nold and Georgieff, 2004). It has also been demonstrated by both epidemiological and experimental studies that the offspring of diabetic mothers has an increased risk for development of cardiovascular disease and insulin resistance in adulthood (Boloker et al., 2002; Carlsson et al., 1999; Holemans et al., 1999; Manderson et al., 2002; Pettitt et al., 1983). In previous studies, we have demonstrated that maternal diabetes promotes remarkable changes in kidney morphology and function and in vascular reactivity in mature offspring (Rocha et al., 2005). In young animals, we found an altered response to a glucose tolerance test and an inability to excrete a salt overload (Magaton et al., 2007; Rocco et al., 2008). Our previous study showed that in the diabetic offspring model, the early onset of hypertension was, at least partially, related to an insufficient basal production of nitric oxide (NO), since after the administration of L-arginine (L-arg) blood pressure levels and vascular reactivity returned to normal levels (Cavanal Mde et al., 2007).

Activation of inflammatory pathways has been demonstrated in several pathologies, among them metabolic and cardiovascular diseases. Insulin resistance, impaired endothelium-dependent relaxation and up-regulation of inflammatory markers were shown to be present in spontaneously hypertensive rats (SHR) and in a salt-sensitive model of hypertension, the Dahl rats (Delano et al., 2010; Potenza et al., 2005; Zhou et al., 2010). In these hypertensive states, free radical production is enhanced (DeLano et al., 2005; Swei et al., 1999). On the other hand, alterations in NO synthase (NOS) isoforms in brain and kidney

☆This study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Grant numbers: 2007/07139-3, 2010/17782-3, 2012/02270-2 and 2010/52180-4), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Instituto Nacional de Ciência e Tecnologia de Fluídos Complexos (INCT Complex Fluids).

* Correspondence to: Laboratory of Transplantation Immunobiology, Department of Immunology, Institute of Biomedical Sciences IV, University of São Paulo, 05508-000 Av Prof Lineu Prestes, 1730 SP, Brazil.
Tel./fax: +55 11 3091 7388.
E-mail address: niels@icb.usp.br (N.O.S. Câmara).

[1] The authors contributed equally to the work.

0014-2999
http://dx.doi.org/10.1016/j.ejphar.2012.05.024

of rats with genetic and salt-induced hypertension were shown by Hojna et al. (2010). Therefore, in the present study, we hypothesized that inflammatory response can be detected early in renal tissue of young DO rats, and in mature DO animals, this inflammatory response could be even enhanced. Since L-arg has been shown to attenuate both renal injury and hypertension in DO group (Cavanal Mde et al., 2007), the role of L-arg was also investigated in our study.

2. Materials and methods

All procedures used in this study were approved and performed in accordance with the guidelines of the Ethics Committee of Biomedical Institute, Federal University of São Paulo (document number 0518/06) and were conducted following the *Guide for Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996). Wistar rats from our colony (Federal University of São Paulo) were maintained in a room at 22 ± 1 °C with a 12 h light cycle and 60% humidity.

2.1. Animal model

Diabetes mellitus was induced by streptozotocin, (50 mg/kg), given by a single intraperitoneal injection to female Wistar rats (250–300 g). Control animals were given an equivalent amount of citrate buffer. Diabetic state was confirmed 48 h after, by measuring blood glucose. Only those animals with glycemic levels above 250 mg/dl were considered for mating. After 3–7 days of diabetic state confirmation, the rats were caged overnight with a male. Vaginal smears were taken the following morning and a positive smear was considered as day 0 of gestation. All dams were housed and fed individually with the same diet. After birth, each litter, consisting of 6 male rats, was left with the mother for 21 days; if the male number was not enough to complete 6, females were used but discarded at weaning. Male pups from different mothers were randomly divided into four subgroups: group CO (controls); group DO (diabetic offspring); group CA (controls receiving 2% L-arg solution dissolved in 2% sucrose in drinking water) and group DA (diabetic offspring receiving L-arg solution dissolved in 2% sucrose in drinking water). A number of 5 rats were used for each group. Rats in the CO and DO groups received a 2% sucrose-only solution. The L-arg dose was similar to that used in a previous study (Reckelhoff et al., 1997), and the sucrose solution was used as a vehicle in order to improve L-arg ingestion. Supplementation with the L-arg solution or the sucrose-only solution began immediately after weaning and was continued until the end of the experimental protocol. Study rats received ~1.2 g L-arg/kg of body weight/day. Glycemia was measured in newborn rats, 12 h after delivery, and in 12-h fasted adult rats, every month. Arterial pressure was evaluated monthly, from 2 months of age, by tail plethysmography.

For protein excretion determination, rats were placed in metabolic cages and a 24-h urine collection was performed. Protein concentration was measured by precipitation with 3% sulfosalicylic acid.

To assess the response to insulin, an insulin overload test was performed in all the experimental groups. After 8 h of food deprivation, 2 month-old rats were anesthetized (50 ml/kg ketamine and 20 ml/kg xylazine; 0.2 ml/100 g), and then insulin (Humulin, Eli Lilly Indianapolis, 0.75 Unity/kg) was administered into the penile vein. This test comprised 6 blood glucose measures using test strips (Accu-check Advantage, Roche, Mannheim, Germany) at baseline (before insulin administration), 4, 8, 12 and 16 min post-insulin application. Glucose measures were then converted into natural logarithm (Ln); the slope was calculated using linear regression (time × Ln (glucose)) and multiplied by 100 to obtain the glucose decay constant rate during the insulin tolerance test (kITT) per minute (%/min). Tryglicerides were determined by the GPO-PAP (Glycerol Phosphate Oxidase) enzymatic test.

2.2. mRNA-expression levels

Kidney samples were quickly frozen in liquid nitrogen. Total RNA was isolated from kidney tissue using TRizol Reagent (Invitrogen, USA) methodology, and RNA concentration was determined by spectrophotometer readings at an absorbance of 260 nm. First-strand cDNA was synthesized using MML-V reverse transcriptase (Promega, Madison, WI, USA). All experimental protocols of real-time PCR were based on the manufacturer's recommendations using the TaqMan gold RT-PCR Core Reagents Kit (Applied Biosystems, Foster City, CA, USA). Primers and probes to hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Rn01527838_g1), TNF-α (Rn99999017_m1), IL-1β (Rn00580432_m1), IL-6 (Rn00561420_m1) inducible Nitric Oxide Synthase (iNOS) (Rn00561646_m1) and endothelial Nitric Oxide Synthase (eNOS) (Rn02132634_s1) were purchased from Applied Biosystems. Cycling conditions were as follows: 10 min at 95 °C, followed by 45 cycles of 15 s at 95 °C and 1 min at 60 °C. The amount of the target gene was normalized first to an endogenous reference (HPRT) and then relative to a calibrator (CO group), using the 2-DDCt method. Hence, mRNA levels were expressed as an *n*-fold difference relative to the calibrator. Analyses were performed with the Sequence Detection Software 1.9 (SDS).

2.3. Cytokine assay

A Bio-Plex assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to measure tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, IL-17, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-1 and leptin in kidney samples. The kit was used according to the manufacturer's instructions. The assay was read on the Bio-Plex suspension array system, and the data were analyzed using Bio-Plex Manager software version 4.0. Standard curves ranged from 32,000 to 1.95 pg/ml. Total protein of kidney tissue was measured and the results are indicated as pg of specific molecule/ug of total protein.

2.4. Statistical analysis

All data were described as mean ± S.E.M. Statistical evaluation of the data was carried out using the *t*-test or the One Way Analysis of Variance (ANOVA) followed by Tukey's post-test. A *P*-value lower than 0.05 was considered to be significant. All statistical analyses were performed with the aid of GraphPad software (San Diego, CA, USA).

3. Results

3.1. Effect of maternal diabetes and L-arginine supplementation on body and kidney weights, glycemia, triglycerides levels, proteinuria and blood pressure levels in offspring

No differences concerning kidney weight were observed among all groups (data not shown). The values for body weight, blood pressure, proteinuria, glycemia and triglycerides are depicted in Tables 1 and 2. Regarding this last parameter, although there is no difference between 2 month-old DO and CO groups, at the age of 6 months, the DO group showed higher levels of triglycerides, and the administration of L-arg led to a significant decrease of it both in

Table 1
Body weight, mean arterial blood pressure, triglycerides, 24 h proteinuria and glycemia in 2 month-old rats.

Groups	Body weight (g)	Systolic blood pressure (mmHg)	Triglycerides (mg/dl)	Proteinuria (mg/24 h)	Glycemia (mg/dl)
CO	242.0 ± 4.41	121.2 ± 2.72	91.5 ± 2.24	3.15 ± 0.72	100.1 ± 4.22
CA	281.6 ± 9.62	110.07 ± 1.19	44.5 ± 0.71[a]	4.42 ± 0.9	92.42 ± 1.49
DO	228.6 ± 5.67	150.74 ± 3.59[a]	90.5 ± 5.5	9.88 ± 0.81[a]	112.4 ± 3.59
DA	265.3 ± 13.4	112.43 ± 3.52[b]	54.0 ± 1.2[b]	5.7 ± 0.9	90.33 ± 3.07

Values are means ± S.E.M.; n=6–15.
[a] Significance level $P<0.05$ vs. CO group.
[b] Significance level $P<0.05$ vs. DO group.

Table 2
Body weight, mean arterial blood pressure, triglycerides, 24 h proteinuria and glycemia in 6 month-old rats.

Groups	Body weight (g)	Systolic blood pressure (mmHg)	Triglycerides (mg/dl)	Proteinuria (mg/24 h)	Glycemia (mg/dl)
CO	449.7 ± 8.89	110.33 ± 3.51	98.0 ± 3.0	5.96 ± 0.58	102.0 ± 2.69
CA	437.7 ± 4.19	111.35 ± 1.19	47.0 ± 1.5[a]	6.53 ± 0.54	109.4 ± 3.10
DO	449.5 ± 12.28	159.89 ± 1.96[a]	154.7 ± 30.33[a]	4.9 ± 0.3	115.3 ± 5.43
DA	443.12 ± 14.73	114.86 ± 4.47[b]	58.67 ± 6.5[b]	9.75 ± 1.1[b]	110.0 ± 3.21

Values are means ± S.E.M.; n=6–12.
[a] Significance level $P<0.05$ vs. CO group.
[b] Significance level $P<0.05$ vs. DO group.

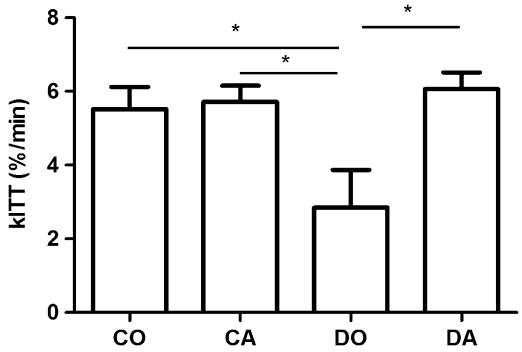

Fig. 1. Glycemic response to exogenous insulin in 2 month-old diabetic offspring, with or without L-arg supplementation. Two month-old diabetic offspring shows a blunted response to exogenous insulin. L-arginine reversed the insulin resistance in DO animals, but had no effect in control animals. kITT=glucose decay constant rate. n=5; *$P<0.05$.

CO and DO groups. Blood pressure levels were significantly increased in 2 month-old DO rats, when compared to the CO group. This profile was maintained and even enhanced at six months. L-arg administration did not change the blood pressure levels in CA animals but caused a significant decrease in DA group, at both ages. As shown in Fig. 1, DO rats showed a significantly blunted glycemic response to exogenous insulin. In DA and CA groups, a normal response to insulin was observed.

3.2. Expression of mRNA of cytokines in kidney.

We assessed the expression of inflammatory cytokines TNF-α, IL-1β and IL-6, as well as iNOS and eNOS, by real-time PCR in the kidneys. No significant difference was observed in the gene expression of TNF-α, IL-1β and IL-6, when comparing 2 month-old DO rats to CO animals at the same age.

L-arg treatment promoted NOS expression, since iNOS and eNOS expressions were significantly higher in CA and DA groups, when compared to CO and DO rats, respectively (Fig. 2). At six months of age, the mRNA-expression levels of TNF-α, IL-1β and IL-6 were significantly higher in DO group than CO group, and as in the younger group, the L-arg administration reduced the expression of these inflammatory markers. For iNOS, both treated groups showed significantly higher gene expression than untreated ones. eNOS mRNA levels were significantly higher in the DA group when compared to the DO group (Fig. 3). It is important to highlight that, when observing iNOS and eNOS expression, DO group showed significantly less gene expression than CO group, which were restored by L-arg treatment (Figs. 2 and 3).

3.3. Expression of cytokines, chemokines and hormone in renal tissue.

Levels of TNF-α, IL-1β, IL-6, IL-17, IFN-γ, MIP-1 and leptin were quantified in the kidney of the offspring. For IL-1β and leptin, the protein expression in 2 month-old DO group was not significantly different from those from CO animals. However, at this age, levels of TNF-α, IL-6, IFN-γ, MIP-1 and IL-17 were significantly increased in DO rats, and the L-arg treatment abolished this effect (Fig. 4). In 6-month-old rats, all the inflammatory markers evaluated were enhanced in the DO group, with the exception of IL-6; as observed in two-month-old rats, the L-arg supplementation prevented this increase (Fig. 5).

4. Discussion

The present study indicates that activation of inflammatory pathways is present early in the kidney from DO animals, and that L-arg can attenuate these markers of tissue inflammation. Moreover, we reinforce the importance of intrauterine environmental factors in determining metabolic disturbances in offspring.

In our model, the offspring was exposed to high glycemic levels since conception, but the direct harmful effect of streptozotocin to the fetus was avoided. Among the known changes caused by sustained hyperglycemia, we can list some of the main metabolic disturbances: increased production of reactive oxygen species, impaired insulin action in vascular tone, enhanced production of advanced glycosylation end-products, lipid and protein abnormalities. All these metabolic changes can occur during pregnancy, inducing fetal abnormalities (Muniyappa et al., 2007).

The decline in triglyceride levels after L-arg deserves comment. It is known that insulin resistance causes visceral fat accumulation,

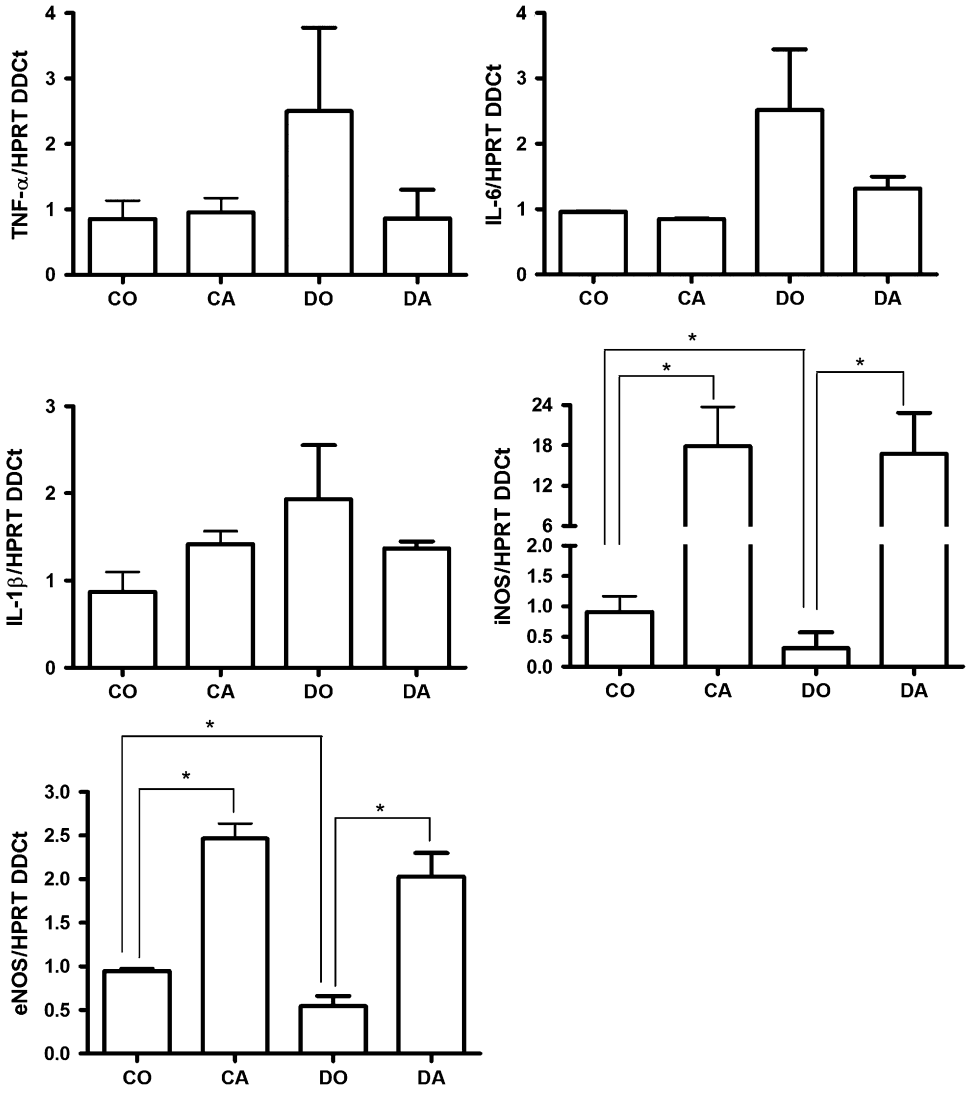

Fig. 2. Expression of pro-inflammatory cytokines and NOS isoforms in the kidney from rats at 2 month-old. Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, iNOS and eNOS were measured by real time PCR in the kidney from 2 month-old rats. Although not significant, an increase in gene inflammatory cytokines expression was observed. The NOS isoforms showed significantly higher gene expression than untreated ones. $^{*}P < 0.05$; $n = 5$.

which may play a key role in the development of a pro-inflammatory state. Drugs such as Fenofibrate, which are used in hypertriglyceridemic patients, increase NO bioavailability (Koh et al., 2006). It is possible that L-arg, acting as an anti-inflammatory agent, could decrease triglyceride levels by improving the metabolic response to insulin, as shown in Fig. 1.

Although we did not measure insulinemia, it is likely that normal glycemic levels are the result of a stimulated secretion of insulin. Hyperinsulinemia is usually present in other pathologic situations, as in metabolic syndrome. The most widely accepted hypothesis for beta-cell disfunction during diabetic pregnancy is that increased free radical reactive oxygen species induce damage to the developing fetus, resulting in both early and late metabolic disturbances. Insulin resistance is characterized by pathway-specific impairment in phosphatidylinositol 3-kinase-dependent signaling that in vascular endothelium contributes to a reciprocal relationship between insulin resistance and endothelial dysfunction (Muniyappa et al., 2007). The clinical importance of this connection is the finding that specific therapeutic interventions targeting insulin resistance often improve endothelial function (and vice versa). L-arg supplementation has shown protective effects in a model of sucrose-induced insulin resistance in rats, decreasing the hyperlipidemia and the insulin resistance (Monti et al., 2008). In our present study, insulin resistance and blood pressure levels returned to normal levels in DO rats treated with L-arg, reinforcing the role of NO in our model.

In the present study, we have confirmed that, in the DO model, there is a clear resistance to insulin in the young offspring, associated with increased pro-inflammatory cytokines levels. In fact, insulin resistance is associated with chronic activation of the innate immune system (Ehses et al., 2009). Moreover TNF-alpha and IL-6 are positively correlated with insulin resistance, and these cytokines can impair biological effects of insulin in skeletal muscle and adipose tissue (Emanuelli et al., 2001; Monroy et al., 2009; Nieto-Vazquez et al., 2008).

In this study, we could demonstrate that the protein expression of some inflammatory cytokines was already increased in 2 month-old DO animals, suggesting that the inflammatory process can be triggered early in offspring life, at a time when kidney injury has not been detected. This can be suggested by the normal levels of proteinuria, since in rats, only values above 15 mg/24 h are considered significantly elevated (Suzuki et al., 2006). However, at this age, DO group has already shown an

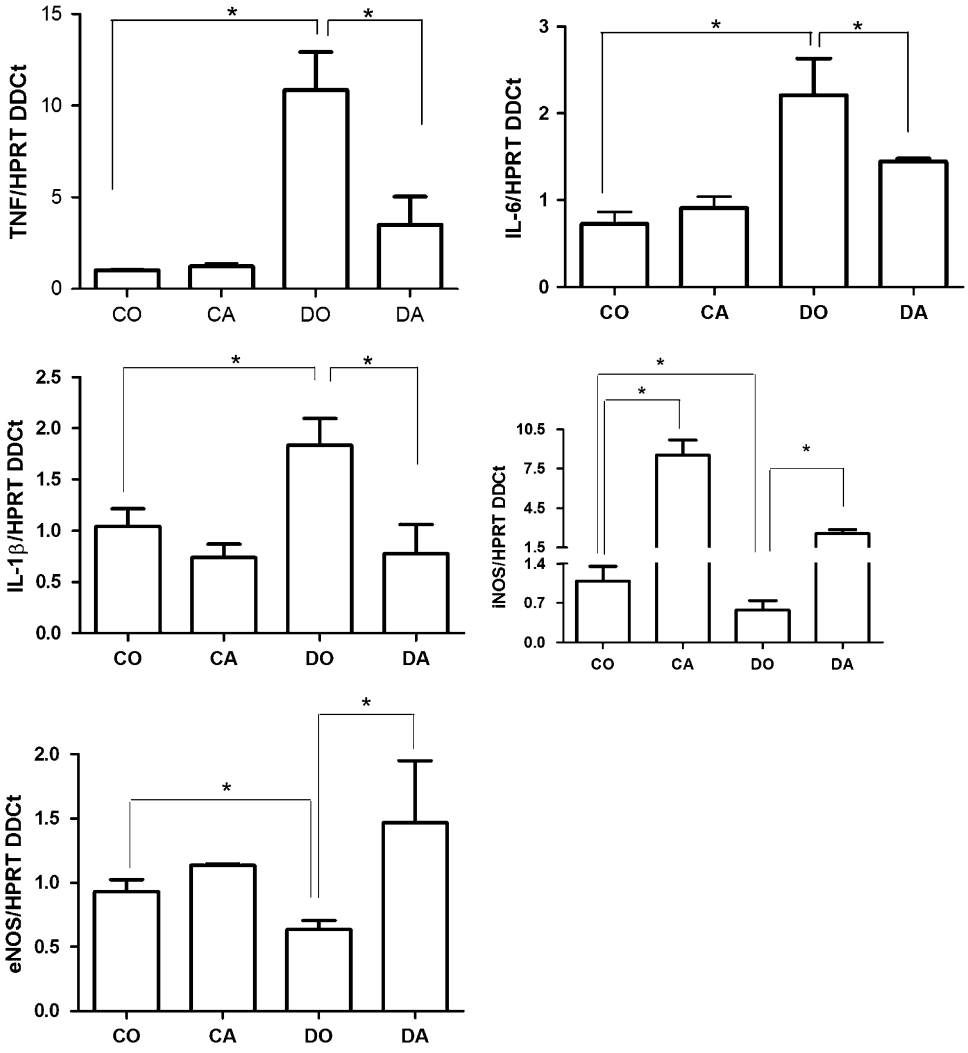

Fig. 3. Expression of pro-inflammatory cytokines and NOS isoforms in the kidney from rats at six months of age. Tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, IL-1β, iNOS and eNOS were measured by real time PCR in the kidney from six months of age rats. At six months of age, the expression of TNF-α, IL-1β and IL-6 was significantly higher in DO than CO rats, and the L-arg administration reduced the expression of these inflammatory markers. For iNOS, both treated groups showed significantly higher gene expression than untreated ones. For eNOS, the DA group showed significantly higher mRNA expression than DO group. $^{*}P<0.05$; $n=5$.

inability to excrete a salt overload, altered glucose tolerance tests and hypertension linked to decreased vascular NO (Cavanal Mde et al., 2007; Magaton et al., 2007; Potenza et al., 2005) which altogether, could characterize hypertension linked to insulin resistance and to oxidative stress. In the older group, the inflammatory mechanism persists and other inflammatory cytokines are detected, suggesting that aging could amplify this process. Rocha et al. (2005) has found that a significant decrease in nephron number is only seen in 12 month-old DO animals, while at six months of age the glomeruli count is within normal range (Rocha et al., 2005). This change in nephron number in older rats could be, at least, explained by an exacerbated inflammatory response, since pro-inflammatory cytokines are important mediators of tissue damage and long-term exposure to molecules, such as TNF-α, induces glomerular endothelial cells apoptotic death (Messmer et al., 1999, 2000).

The increased production of inflammatory cytokines was demonstrated to play an important role in the development and progression of diabetic nephropathy (Matavelli et al., 2010; Navarro-Gonzalez and Mora-Fernandez, 2008). But as far as we know, this is the first study demonstrating the involvement of inflammatory markers in the kidney of offspring from diabetic mothers and the beneficial effects of L-arg.

Several studies have shown that NO promotes inflammation (Kroncke et al., 2001; Langrehr et al., 1993), whereas an equal number of studies demonstrate an anti-inflammatory role, decreasing production of inflammatory molecules (Angele et al., 1999; Meldrum et al., 1997). These conflicting results may be due to concentration-dependent effects of NO, the variety of in vivo or in vitro experimental models, and/or differences in cell sensitivity to NO. In our study, L-arg showed an anti-inflammatory role, and decreased the protein expression of TNF-α, IFN-γ, MIP-1, IL-6 and IL-17 in 2 month-old DO group, and TNF-α, IFN-γ, MIP-1, IL-1β, IL-2, IL-17 and leptin in 6 month-old age DO rats. Gene over-expression of TNF-α, IL-1β and IL-6 observed in DO animals was also attenuated by L-arg, suggesting that diabetic offspring may represent an experimental model where an overall inflammatory state is present.

Some of the up-regulated molecules present in the DO group have been linked to metabolic disease. IL-17 has been associated to renal inflammation and other metabolic disorders, and its presence leads to a worse prognosis of the disease (Ahmed and Gaffen, 2010; Kitching and Holdsworth, 2011; Pini and Fantuzzi, 2010; Shin et al., 2009; Turner et al., 2010; Winer et al., 2009). The Th1 pattern molecule IFN-γ has been described as an important mediator of "fat inflammation", and its presence leads to an

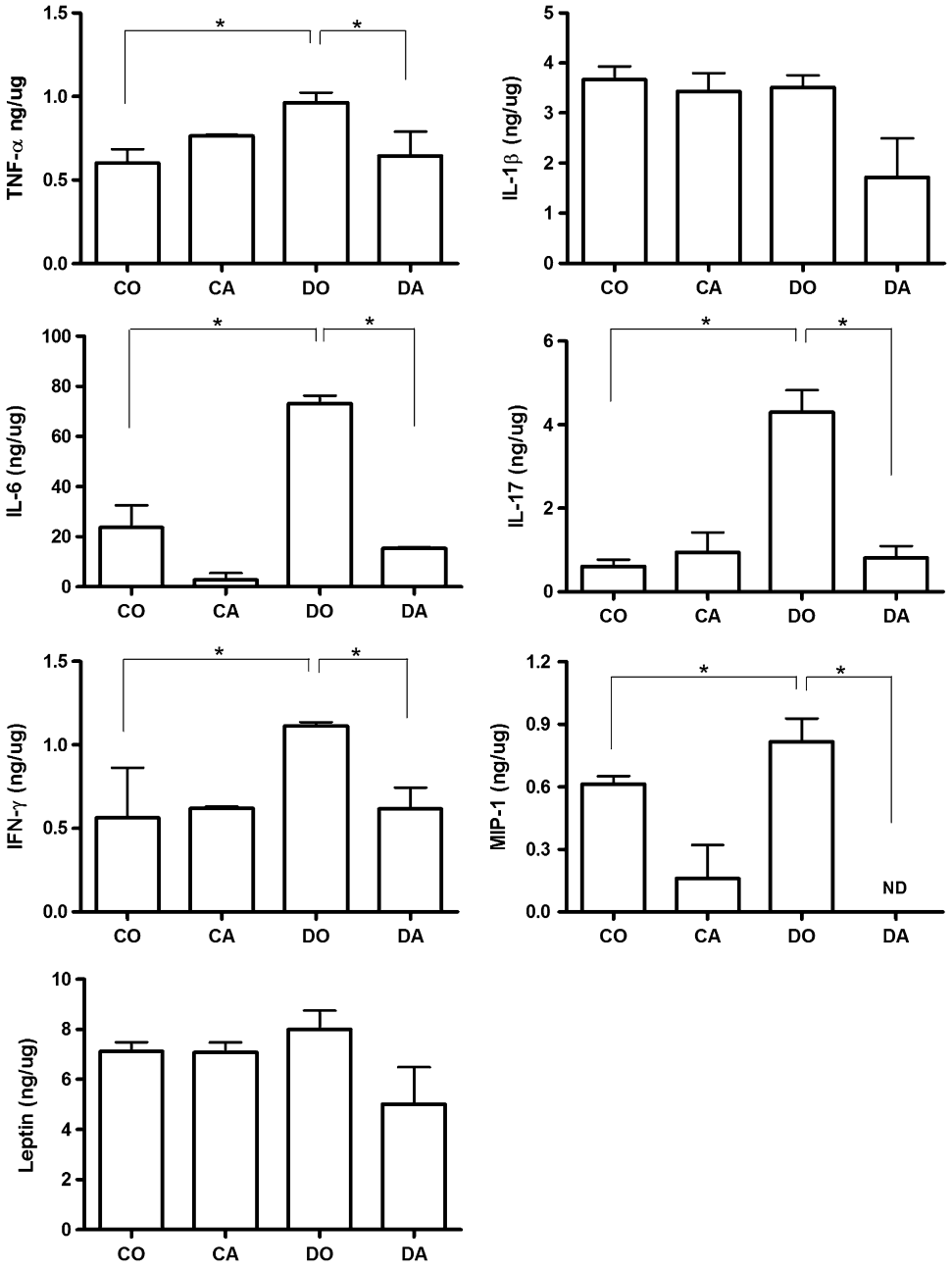

Fig. 4. Pro-inflammatory cytokines in the kidney from rats at 2 month-old. All molecules were quantified by Bioplex. At 2 month-old, DO rats did not differ from CO rats in interleukin (IL)-1β and leptin protein expression. Renal levels of tumor necrosis factor-alpha (TNF-α), IL-6, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-1 and IL-17 were significantly increased in DO rats, and the L-arg treatment prevented this effect. $^{*}P < 0.05$; $n = 5$. ND = not detectable.

increased recruitment of macrophages and production of chemokines and TNF-α. Moreover, leptin is produced mainly in adipose tissue, and acts as a pro-inflammatory adipokine. Studies with leptin showed its capacity of inducing pro-inflammatory cytokines (Loffreda et al., 1998), and stimulating chemotaxis in polymorphonuclear cells (Brennan and Mantzoros, 2006; Caldefie-Chezet et al., 2003). Furthermore, leptin promotes Th1 cell differentiation and cytokine production (Matarese et al., 2005). All these data corroborate our results and confirm our idea that, in our model, L-arg has an anti-inflammatory property.

The renal cells, as a response to the environmental changes, might initiate the production of the several cytokines, promoting inflammation. Later, there must occur the recruitment of immune cells, which will increase and exacerbate the inflammatory process. The recruitment of immune cells is evident when we observe the presence of the chemokine MIP-1 in renal tissue. Among such cells, we could imagine that the M1 macrophages, or classically activated macrophages, may contribute to the disease, as they are known as inflammatory macrophages. In this sense, probably there is also a decrease in M2 macrophages. As these macrophages have a well known anti-inflammatory profile, the lack of them probably influences the pro-inflammatory environment (Anders and Ryu, 2011).

Furthermore, talking about the reduced expression of inflammatory markers after L-arg supplementation, Tousoulis and colleagues have shown that supplementation of L-arg in high risk patients increased NO production and reduced leukocyte adhesion (Tousoulis et al., 2007). Moreover, Michell and colleagues suggest that high blood pressure induces increase in reactive oxygen species production in endothelial cells that will promote

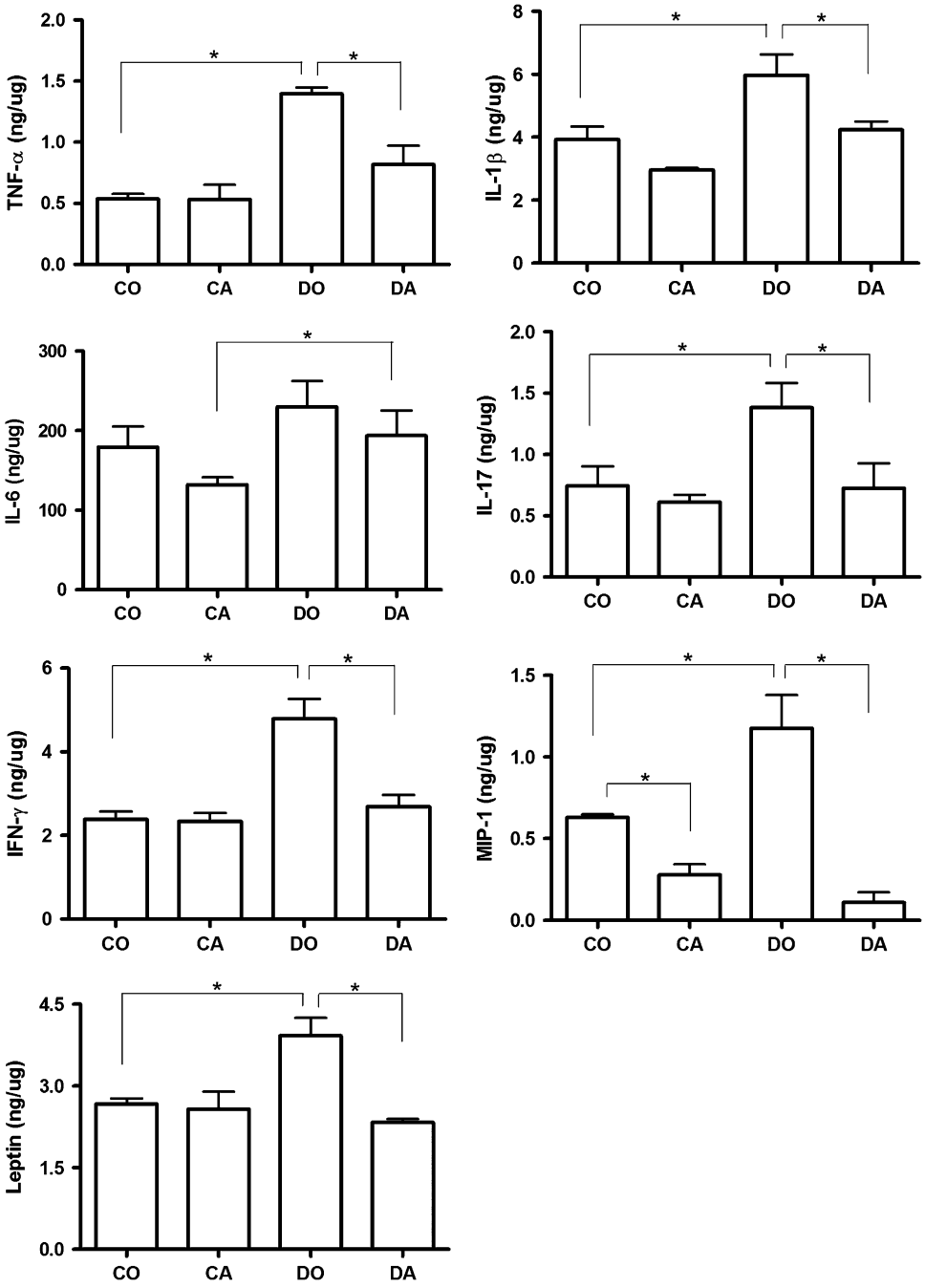

Fig. 5. Pro-inflammatory cytokines in the kidney from rats at six months of age. All molecules were quantified by Bioplex. At six months of age, tumor necrosis factor-alpha (TNF)-α, interleukin (IL)-1β, IL-17, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-1 and leptin enhanced in DO group and the L-arg supplementation prevented this increase. $^{*}P < 0.05$; $n = 5$.

increased endothelial arginase expression, decreasing L-arg availability and consequently NO production. The uncoupled eNOS produces superoxide, instead of NO, resulting in the production of pro-inflammatory factors that leads to the expression of adhesion molecules, promoting inflammation (Michell et al., 2011) So, L-arg supplementation could reverse this cascade, attenuating the inflammatory process.

Also, it is important to highlight that the difference observed on gene expression of iNOS and eNOS between CO and CA groups proved that the L-arg treatment was able to up regulate the NOS isoforms, which led us to the conclusion that the protection observed must be, at least in part, due to higher NO availability.

Experiments with aortic rings of genetic diabetic-prone rats, demonstrated that there was a limitation in the utilization of L-arg, either by compartmentalization or limitation in a cofactor for NO synthase, contributing to decreased NO production in the endothelium. Thus, the mechanism of the L-arg effect on impaired endothelium-dependent relaxation in diabetic blood vessels could be due to NO reduction (Pieper et al., 1997). Our results suggest that these mechanisms could be involved in our model, as we observed less inflammation in treated animals.

In conclusion, we can suggest that activation of inflammatory pathways is present at early age in the kidney of DO animals and that L-arg can attenuate these markers of tissue inflammation. Moreover, the DO rats can represent a suitable model of

hypertension with kidney involvement, similar to SHR and Dahl rats. Our results also reinforce the concept that intrauterine environmental factors can define the metabolic characteristics of the offspring, being a fundamental determinant in the development of metabolic and vascular diseases later in life.

Acknowledgments

The authors would like to thank Francis Ball for proofreading the article and providing help with the English language.

References

Ahmed, M., Gaffen, S.L., 2010. IL-17 in obesity and adipogenesis. Cytokine Growth Factor Rev. 21, 449–453.
Anders, H.J., Ryu, M., 2011. Renal microenvironments and macrophage phenotypes determine progression or resolution of renal inflammation and fibrosis. Kidney Int. 80, 915–925.
Angele, M.K., Smail, N., Ayala, A., Cioffi, W.G., Bland, K.I., Chaudry, I.H., 1999. L-arginine: a unique amino acid for restoring the depressed macrophage functions after trauma-hemorrhage. J. Trauma 46, 34–41.
Boloker, J., Gertz, S.J., Simmons, R.A., 2002. Gestational diabetes leads to the development of diabetes in adulthood in the rat. Diabetes 51, 1499–1506.
Brennan, A.M., Mantzoros, C.S., 2006. Drug insight: the role of leptin in human physiology and pathophysiology—emerging clinical applications. Nat. Clin. Pract. 2, 318–327.
Caldefie-Chezet, F., Poulin, A., Vasson, M.P., 2003. Leptin regulates functional capacities of polymorphonuclear neutrophils. Free Radic. Res. 37, 809–814.
Carlsson, S., Persson, P.G., Alvarsson, M., Efendic, S., Norman, A., Svanstrom, L., Ostenson, C.G., Grill, V., 1999. Low birth weight, family history of diabetes, and glucose intolerance in Swedish middle-aged men. Diab. Care 22, 1043–1047.
Cavanal Mde, F., Gomes, G.N., Forti, A.L., Rocha, S.O., Franco Mdo, C., Fortes, Z.B., Gil, F.Z., 2007. The influence of L-arginine on blood pressure, vascular nitric oxide and renal morphometry in the offspring from diabetic mothers. Pediatr. Res. 62, 145–150.
DeLano, F.A., Balete, R., Schmid-Schonbein, G.W., 2005. Control of oxidative stress in microcirculation of spontaneously hypertensive rats. Am. J. Physiol. 288, H805–H812.
Delano, F.A., Zhang, H., Tran, E.E., Zhang, C., Schmid-Schonbein, G.W., 2010. A new hypothesis for insulin resistance in hypertension due to receptor cleavage. Expert Rev. Endocrinol. Metab. 5, 149–158.
Ehses, J.A., Lacraz, G., Giroix, M.H., Schmidlin, F., Coulaud, J., Kassis, N., Irminger, J.C., Kergoat, M., Portha, B., Homo-Delarche, F., Donath, M.Y., 2009. IL-1 antagonism reduces hyperglycemia and tissue inflammation in the type 2 diabetic GK rat. Proc. Natl. Acad. Sci. U S A 106, 13998–14003.
Emanuelli, B., Peraldi, P., Filloux, C., Chavey, C., Freidinger, K., Hilton, D.J., Hotamisligil, G.S., Van Obberghen, E., 2001. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. J. Biol. Chem. 276, 47944–47949.
Hojna, S., Kunes, J., Zicha, J., 2010. Alterations of NO synthase isoforms in brain and kidney of rats with genetic and salt hypertension. Physiol. Res. 59, 997–1009.
Holemans, K., Gerber, R.T., Meurrens, K., De Clerck, F., Poston, L., Van Assche, F.A., 1999. Streptozotocin diabetes in the pregnant rat induces cardiovascular dysfunction in adult offspring. Diabetologia 42, 81–89.
Kitching, A.R., Holdsworth, S.R., 2011. The emergence of TH17 cells as effectors of renal injury. J. Am. Soc. Nephrol. 22, 235–238.
Koh, K.K., Quon, M.J., Han, S.H., Chung, W.J., Ahn, J.Y., Kim, J.A., Lee, Y., Shin, E.K., 2006. Additive beneficial effects of fenofibrate combined with candesartan in the treatment of hypertriglyceridemic hypertensive patients. Diab. Care 29, 195–201.
Kroncke, K.D., Fehsel, K., Suschek, C., Kolb-Bachofen, V., 2001. Inducible nitric oxide synthase-derived nitric oxide in gene regulation, cell death and cell survival. Int. Immunopharmacol. 1, 1407–1420.
Langrehr, J.M., White, D.A., Hoffman, R.A., Simmons, R.L., 1993. Macrophages produce nitric oxide at allograft sites. Ann. Surg. 218, 159–166.
Loffreda, S., Yang, S.Q., Lin, H.Z., Karp, C.L., Brengman, M.L., Wang, D.J., Klein, A.S., Bulkley, G.B., Bao, C., Noble, P.W., Lane, M.D., Diehl, A.M., 1998. Leptin regulates proinflammatory immune responses. FASEB J. 12, 57–65.
Lynch, S.A., Wright, C., 1997. Sirenomelia, limb reduction defects, cardiovascular malformation, renal agenesis in an infant born to a diabetic mother. Clin. Dysmorphol. 6, 75–80.
Magaton, A., Gil, F.Z., Casarini, D.E., Cavanal Mde, F., Gomes, G.N., 2007. Maternal diabetes mellitus—early consequences for the offspring. Pediatr. Nephrol. (Berlin, Germany) 22, 37–43.
Manderson, J.G., Mullan, B., Patterson, C.C., Hadden, D.R., Traub, A.I., McCance, D.R., 2002. Cardiovascular and metabolic abnormalities in the offspring of diabetic pregnancy. Diabetologia 45, 991–996.
Martinez-Frias, M.L., 1994. Epidemiological analysis of outcomes of pregnancy in diabetic mothers: identification of the most characteristic and most frequent congenital anomalies. Am. J. Med. Genet. 51, 108–113.
Matarese, G., Moschos, S., Mantzoros, C.S., 2005. Leptin in immunology. J. Immunol. 174, 3137–3142.
Matavelli, L.C., Huang, J., Siragy, H.M., 2010. (Pro)renin receptor contributes to diabetic nephropathy by enhancing renal inflammation. Clin. Exp. Pharmacol. Physiol. 37, 277–282.
Meldrum, D.R., McIntyre, R.C., Sheridan, B.C., Cleveland Jr., J.C., Fullerton, D.A., Harken, A.H., 1997. L-arginine decreases alveolar macrophage proinflammatory monokine production during acute lung injury by a nitric oxide synthase-dependent mechanism. J. Trauma 43, 888–893.
Messmer, U.K., Briner, V.A., Pfeilschifter, J., 1999. Tumor necrosis factor-alpha and lipopolysaccharide induce apoptotic cell death in bovine glomerular endothelial cells. Kidney Int. 55, 2322–2337.
Messmer, U.K., Briner, V.A., Pfeilschifter, J., 2000. Basic fibroblast growth factor selectively enhances TNF-alpha-induced apoptotic cell death in glomerular endothelial cells: effects on apoptotic signaling pathways. J. Am. Soc. Nephrol. 11, 2199–2211.
Michell, D.L., Andrews, K.L., Chin-Dusting, J.P., 2011. Endothelial dysfunction in hypertension: the role of arginase. Front. Biosci. (Scholar Ed.) 3, 946–960.
Monroy, A., Kamath, S., Chavez, A.O., Centonze, V.E., Veerasamy, M., Barrentine, A., Wewer, J.J., Coletta, D.K., Jenkinson, C., Jhingan, R.M., Smokler, D., Reyna, S., Musi, N., Khokka, R., Federici, M., Tripathy, D., DeFronzo, R.A., Folli, F., 2009. Impaired regulation of the TNF-alpha converting enzyme/tissue inhibitor of metalloproteinase 3 proteolytic system in skeletal muscle of obese type 2 diabetic patients: a new mechanism of insulin resistance in humans. Diabetologia 52, 2169–2181.
Monti, L.D., Galluccio, E., Lucotti, P., Setola, E., Costa, S., Fontana, B., Oldani, M., Merante, D., Di Blasi, P., Bosi, E., Piatti, P.M., 2008. Beneficial role of L-arginine in cardiac matrix remodelling in insulin resistant rats. Eur. J. Clin. Invest. 38, 849–856.
Muniyappa, R., Montagnani, M., Koh, K.K., Quon, M.J., 2007. Cardiovascular actions of insulin. Endocr. Rev. 28, 463–491.
Navarro-Gonzalez, J.F., Mora-Fernandez, C., 2008. The role of inflammatory cytokines in diabetic nephropathy. J. Am. Soc. Nephrol. 19, 433–442.
Nieto-Vazquez, I., Fernandez-Veledo, S., Kramer, D.K., Vila-Bedmar, R., Garcia-Guerra, L., Lorenzo, M., 2008. Insulin resistance associated to obesity: the link TNF-alpha. Arch. Physiol. Biochem. 114, 183–194.
Nold, J.L., Georgieff, M.K., 2004. Infants of diabetic mothers. Pediatr. Clin. North Am. 51, 619–637. (viii).
Pettitt, D.J., Baird, H.R., Aleck, K.A., Bennett, P.H., Knowler, W.C., 1983. Excessive obesity in offspring of Pima Indian women with diabetes during pregnancy. N. Engl. J. Med. 308, 242–245.
Pieper, G.M., Siebeneich, W., Moore-Hilton, G., Roza, A.M., 1997. Reversal by L-arginine of a dysfunctional arginine/nitric oxide pathway in the endothelium of the genetic diabetic BB rat. Diabetologia 40, 910–915.
Pini, M., Fantuzzi, G., 2010. Enhanced production of IL-17A during zymosan-induced peritonitis in obese mice. J. Leukoc. Biol. 87, 51–58.
Potenza, M.A., Marasciulo, F.L., Chieppa, D.M., Brigiani, G.S., Formoso, G., Quon, M.J., Montagnani, M., 2005. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. Am. J. Physiol. 289, H813–H822.
Reckelhoff, J.F., Kellum Jr., J.A., Racusen, L.C., Hildebrandt, D.A., 1997. Long-term dietary supplementation with L-arginine prevents age-related reduction in renal function. Am. J. Physiol. 272, R1768–R1774.
Rocco, L., Gil, F.Z., da Fonseca Pletiskaitz, T.M., de Fatima Cavanal, M., Gomes, G.N., 2008. Effect of sodium overload on renal function of offspring from diabetic mothers. Pediatr. Nephrol. (Berlin, Germany) 23, 2053–2060.
Rocha, S.O., Gomes, G.N., Forti, A.L., do Carmo Pinho Franco, M., Fortes, Z.B., de Fatima Cavanal, M., Gil, F.Z., 2005. Long-term effects of maternal diabetes on vascular reactivity and renal function in rat male offspring. Pediatr. Res. 58, 1274–1279.
Shin, J.H., Shin, D.W., Noh, M., 2009. Interleukin-17A inhibits adipocyte differentiation in human mesenchymal stem cells and regulates pro-inflammatory responses in adipocytes. Biochem. Pharmacol. 77, 1835–1844.
Suzuki, H., Tokuriki, T., Kamita, H., Oota, C., Takasu, M., Saito, K., Suzuki, K., 2006. Age-related pathophysiological changes in rat oligomeganephronic hypoplastic kidney. Pediatr. Nephrol. (Berlin, Germany) 21, 637–642.
Swei, A., Lacy, F., Delano, F.A., Parks, D.A., Schmid-Schonbein, G.W., 1999. A mechanism of oxygen free radical production in the Dahl hypertensive rat. Microcirculation 6, 179–187.
Tousoulis, D., Boger, R.H., Antoniades, C., Siasos, G., Stefanadi, E., Stefanadis, C., 2007. Mechanisms of disease: L-arginine in coronary atherosclerosis—a clinical perspective. Nat. Clin. Pract. Cardiovasc. Med. 4, 274–283.
Turner, J.E., Paust, H.J., Steinmetz, O.M., Panzer, U., 2010. The Th17 immune response in renal inflammation. Kidney Int. 77, 1070–1075.
Winer, S., Paltser, G., Chan, Y., Tsui, H., Engleman, E., Winer, D., Dosch, H.M., 2009. Obesity predisposes to Th17 bias. Eur. J. Immunol. 39, 2629–2635.
Zhou, M.S., Schulman, I.H., Raij, L., 2010. Vascular inflammation, insulin resistance, and endothelial dysfunction in salt-sensitive hypertension: role of nuclear factor kappa B activation. J. Hypertens. 28, 527–535.