

L-arginine enriched diet protects bb/ok rats from developing type 1 diabetes

Abstract

L-arginine is the source of all forms of nitric oxide (NO), which can be an extremely relevant factor in the treatment and reversal of important diseases. This observation prompted us to use L-arginine in BB/OK (Bio Breeding/Ottawa Karlsburg) rats developing insulin-dependent type 1 diabetes to evaluate the effect of L-arginine on the prevention of this disease.

BB/OK rats were given L-arginine in drinking water (2%) during pregnancy and to the progeny (group 1), to newborn (group 2) and not given (group 3) up to an age of 30 weeks. Diabetes frequency and age at onset of diabetes were recorded in all BB/OK rats. The mRNA expression of genes (*Nfkb2*, *Il10*, *Il1b*, *Rarres 2*, *Pparg*, *Adipoq*, *Lep* and *Slc2a4*) was measured in subcutaneous and visceral adipose tissue in BB/OK rats which did not develop diabetes up to an age of 30 weeks.

Diabetes frequency was reduced in the L-arginine supplemented BB/OK compared to the untreated BB/OK rats (group 1: $p < 0.001$ and group 2: $p < 0.05$ vs. control group 3). Group 2 showed gender specific differences, because more females than males developed diabetes (94/59%; $p < 0.05$). Gene expression in subcutaneous and visceral adipose tissue was reduced in the L-arginine drinking BB/OK rats compared to the control group.

L-arginine in drinking water can protect from type 1 diabetes development in a sex specific manner. Because L-arginine is a precursor of NO, it may be concluded that this manipulation normalized NO activity in β cells, partially preventing type 1 diabetes development.

Keywords: type 1 diabetes, l-arginine, adipose tissue, gene expression

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Abbreviations: BB/OK, bio breeding/ottawa karlsburg; NO, nitric oxide; T1D, type 1 diabetes; *Nfkb2*, nuclear factor of kappa light polypeptide gene enhancer in β -cells; *Il10*, interleukin 10; *Il1b*, interleukin 1 beta; *Rarres 2*, retinoic acid receptor responder protein 2; *Pparg*, peroxisome proliferator-activated receptor gamma; *Adipoq*, adiponectin; *Lep*, leptin; *Slc2a4*, insulin-regulated facilitative glucose transporter member 4

Introduction

Many improved effects of dietary supplementation of L-arginine are known in diverse diseases. Long-term oral L-arginine supplementation in humans improves coronary small-vessel endothelial function and a decrease in plasma endothelin concentrations.¹ Other data indicate that L-arginine supplementation improves wound healing in diabetic animals and alters the spectrum of tumour-infiltrating lymphocytes in human colorectal cancers in vivo.^{2,3} In addition, dietary arginine supplementation reduced the mass of adipose tissue in ZDF rats.⁴

Type 1 diabetes (T1D) is a disease that reflects an interplay of genetic, environmental (e.g. dietary constituents) and immunological factors.⁵ Spontaneous diabetes in BB/OK rats shares many common features with human insulin-dependent T1D.⁶ In our study, BB/OK rats were treated with L-arginine to evaluate the effect of L-arginine on the prevention of T1D. Diet during pregnancy can affect perinatal outcomes through direct physiological effects that permanently affect phenotype. A number of studies showed that dietary supplements induce various effects on the progeny during the pregnancy.⁷⁻¹⁰ Therefore, in this study; dietary supplementation with L-arginine

during pregnancy was conducted in order to determine possible effects on the diabetes frequency.

A few days before BB/OK rats become diabetic, their body seems to be flabby, which may be attributed to loss of subcutaneous adipose tissue. It is well-known that the lack of insulin leads to a marked increase in the rate of adipose tissue lipolysis. This “flabby body” phenomenon is not observed in BB/OK rats which do not become diabetic. A recent study on BB/OK rats grafted with adipose tissue showed that T1D development was prevented in about 50% of these rats.¹¹ These findings indicated that altered lipid metabolism may play an important role in the pathogenesis of T1D. Therefore, gene expression levels were recorded after the administration of L-arginine in subcutaneous and visceral adipose tissues of rats which were non-diabetic after 30 weeks of observation.

Materials and methods

Animals

All rats were bred and kept in our own animal facility. They were kept under strict hygienic conditions and had free access to food and acidulated water. All experiments were performed in accordance with the regulations for animal care of the Ministry of Nutrition, Agriculture and Forestry of the Government of Mecklenburg-Vorpommern (Germany).

BB/OK rats were randomly divided into three groups. Rats were given L-arginine in drinking water (2%) during pregnancy. The progeny of these treated rats were also given 2% L-arginine in drinking

water up to an age of 30 weeks and constituted group 1 (n=38). Group 2 (n=48) was only treated with L-arginine from weaning up to an age of 30 weeks. Group 3 (n=39) consisted of untreated BB/OK rats and served as controls for the observation period of 30 weeks.

Phenotypic characterization

Diabetes frequency and age at onset of diabetes were recorded in all BB/OK rats up to an age of 30 weeks. Diabetes onset was defined by blood glucose levels exceeding 200 mg% on 2 consecutive days. Blood was taken by an incision of the tail vein and circulating glucose concentration was measured with a glucose analyzer (ESAT 6660-2, Medingen, Germany).

Gene expression analysis

At the end of observation of 30 weeks, 9 males and 8 females of group 1 and 13 males of group 2 (no female) were sacrificed with an overdose of anesthesia (Sevofluran, Abbott, Germany). As controls served 6 male and 12 non-diabetic BB/OK rats at an age of 30 weeks which were untreated with L-arginine. From these rats, subcutaneous and visceral adipose tissues were removed and immediately frozen in liquid nitrogen to determine the mRNA expression levels of the following immuno and fat-regulated genes: Nuclear factor of kappa

light polypeptide gene enhancer in β -cells (*Nfkb2*), Interleukin 10 (*Il10*), Interleukin 1 beta (*Il1b*), Retinoic acid receptor responder protein 2 (*Rarres 2*), Peroxisome proliferator-activated receptor gamma (*Pparg*), Adiponectin (*Adipoq*), Leptin (*Lep*) as well as an insulin-regulated facilitative glucose transporter member 4 (*Slc2a4*). Total RNA of subcutaneous and visceral adipose tissue was isolated and mRNA expression levels were measured by real-time qRT-PCR as described previously.^{12,13}

Statistical analysis

Data are given as mean \pm SD. Statistical significance was assessed using statistical computing and graphics of graph pad prism. The Wilcoxon rank sum test was used to test the equality of the means and the diabetes frequency was compared using the Chi-square test. Differences were considered to be statistically significant at a level of $p < 0.05$.

Results and discussion

The age at onset of diabetes and diabetes frequency are listed in (Table 1). A significant difference was located between group 1 and the control group ($p = 0.046$). In group 1, a significant sex difference showed that males became diabetic earlier than females ($p = 0.016$).

Table 1 Diabetes frequency and age at onset of diabetes (days) in BB/OK rats of groups 1-3

Trait	Sex	Group 1*	P value 1 vs.3	Group 2	P-value 2 vs. 3	Group 3(Control)
Diabetes Frequency	Males	44% *(7/16)	0.0056	59%(19/32)	0.0257	94%(16/17)
	Females	60%(13/22)	0.036	94%(15/16)	0.773	91%(20/22)
	Total	53%(20/38)	0.0003	71%(34/48)	0.0251	92%(36/39)
Age at onset of diabetes (Days)	Males	98 \pm 24	0.333	114 \pm 32	0.804	107 \pm 23
	Females	143 \pm 38	0.003	114 \pm 17	0.068	105 \pm 20
	Total	127 \pm 40	0.046	114 \pm 26	0.191	106 \pm 21

*Significant sex differences in group 1 ($p = 0.016$)

The total diabetes frequency was significantly lower in treated groups than in the untreated control group (p_{group1 vs. group3} < 0.001, p_{group2 vs. group3} < 0.05). Twenty out of 38 (53%) from group 1 and 34 out of 48 (71%) BB/OK rats from group 2 treated with L-arginine developed type 1 diabetes. In the controls (group 3), 36 out of 39 (92%) BB/OK rats became diabetic up to an age of 30 weeks. In addition, significantly more treated females (94%) than males (59%) developed diabetes up to an age of 30 weeks ($p < 0.05$) in group 2. In contrast, BB/OK rats showed no significant differences between the genders in groups 1 (males 44%, females 60%) and 3 (males 94%, females 91%). Several studies found a beneficial effect of L-arginine, e.g., L-arginine can prevent the development of diabetes on chemically induced diabetes.¹⁴ The results of the L-arginine treatment in the present study also positively influenced the diabetes frequency, that is, it decreased.

As shown in Figure 1 & Figure 2 the relative gene expression was

significantly decreased in treated groups versus the untreated controls (group 3) in subcutaneous and visceral adipose tissues in male and female rats. In terms of relative gene expression, the L-arginine treatment resulted in significantly reduced mRNA expression of all examined genes in adipose tissue in treated groups versus the control group. L-arginine is the substrate for the synthesis of nitric oxide (NO). In recent years, intensive research has shown that NO is not only a very important bioactive signaling messenger in the cardiac circulatory system, but it also plays a role in the immune defense against infections and controlling the nervous system.¹⁵⁻¹⁸ It can be assumed that L-arginine normalizes the NO level, influencing gene expression. The fall in the relative gene expression suggests that the Langerhans islets were beneficially affected, inhibiting the autoimmune destruction of β cells. However, this should be confirmed by the determination of NO in β cells of Langerhans islets after L-arginine treatment.

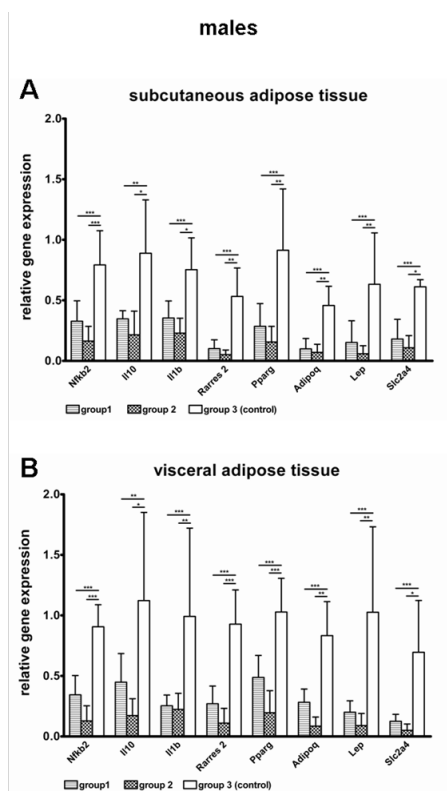


Figure 1 Relative gene expression in subcutaneous (A) and visceral (B) adipose tissue of male BB/OK rats. Values are expressed as means \pm SD. Significant differences are indicated: * p <0.05, ** p <0.01, *** p <0.001.

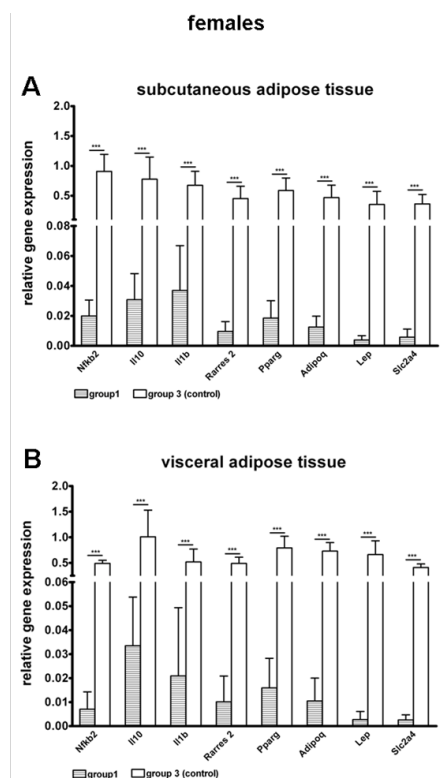


Figure 2 Relative gene expressions in subcutaneous (A) and visceral (B) adipose tissue of female BB/OK rats. Values are expressed as means \pm SD. Significant differences are indicated: * p <0.05, ** p <0.01, *** p <0.001.

Conclusion

This study shows that supplementing L-arginine protects BB/OK rats from developing type 1 diabetes in a sex-specific manner. Significantly fewer males developed diabetes. The reduced diabetes frequency was only observed in the females which were given L-arginine during pregnancy. Additionally, the age at onset of diabetes was later in females than in males. Furthermore, the expression of genes is influenced in adipose tissue by L-arginine. Because L-arginine is a precursor of NO, it is reasonable to suggest that this manipulation normalized NO activity, inhibiting the autoimmune destruction of β cells.

A recent study with BB/OK rats fed a high fat diet during pregnancy showed that the overall diabetes frequency and the relative gene expression in adipose tissue was reduced in comparison with a normal diet.¹⁹ Moreover, this study confirmed that males were more protected from the development of T1D than females through a high fat diet. Further investigations are needed to clarify the sex-specific mechanism.

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Conflict of interest

Author declares that there is no conflict of interest.

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