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1-Methylnicotinamide (MNA) prevents endothelial dysfunction in hypertriglyceridemic and diabetic rats

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Abstract:
For many years, 1-methylnicotinamide (MNA), a primary metabolite of nicotinamide, has been considered inactive. Recently however, it has been discovered that MNA possesses anti-thrombotic and anti-inflammatory activity. In the present study we investigated whether chronic administration of MNA to hypertriglyceridemic or diabetic rats would reverse endothelial dysfunction characterized by the impairment of nitric oxide (NO)-dependent vasodilatation.
Hypertriglyceridemia in rats was induced by fructose-rich (60%) diet, while diabetes was induced by streptozotocin injection (70 mg/kg). After eight weeks, in hypertriglyceridemic or diabetic rats treated or non-treated with MNA (100 mg/kg), we analyzed the magnitude of endothelium-dependent or endothelium-independent vasodilatation in aorta induced by acetylcholine or S-nitroso-N-acetyl-penicilamine (SNAP), respectively, as well as plasma concentration of: cholesterol, triglycerides, glucose, HbA_{1c}, fructosamine, peptide C, endogenous MNA and its metabolites (M2PY, M4PY).
In diabetic rats plasma concentration of glucose, HbA_{1c} and fructosamine was elevated (402.08 ± 19.01 *vs*. 82.06 ± 5.41 mg/dl, $p < 0.001$; 9.55 ± 0.56 *vs*. 4.93 ± 0.24%, $p = 0.052$ and 2.53 ± 0.10 *vs*. 1.14 ± 0.06 mmol DTF/mg protein, $p < 0.001$ in diabetic and control rats, respectively). In hypertriglyceridemic rats plasma concentration of triglycerides was elevated (4.25 ± 0.27 *vs*. 1.55 ± 0.12 mmol/l, $p < 0.001$ in hypertriglyceridemic and control rats, respectively). In both models the NO-dependent vasodilatation in aorta induced by acetylcholine was significantly impaired as compared to control rats, while the response to SNAP was largely preserved. In hypertriglyceridemic rats, 4 weeks of treatment with MNA (100 mg/kg, *po*) resulted in a three to six-fold increase in endogenous levels of MNA and its metabolites (M2PY and M4PY), the fall in triglycerides concentration in plasma (from 4.25 ± 0.27 to 2.22 ± 0.14 mmol/l, $p < 0.001$), and the preservation of the NO-dependent vasodilatation. In diabetic rats chronic treatment with MNA also prevented the impairment of NO-dependent vasodilatation, while it displayed only a mild effect on hyperglycemia and did not lower triglycerides concentration.
In summary, MNA treatment decreased plasma triglycerides concentration in hypertriglyceridemic, but not in diabetic rats, while it prevented the development of endothelial dysfunction in aorta in both of these models. Accordingly, the ability of MNA to reverse endothelial dysfunction seems to be independent of its hypolipemic activity.

Key words:
1-methylnicotinamide, hypertriglyceridemia, diabetes, endothelium, NO, endothelial dysfunction

Abbrevations: ACE – angiotensin converting enzyme, Ach – acetylcholine, Hist – histamine, L-NAME – N^G-nitro-L-arginine methyl ester, M2PY – 1-methyl-2-pyridone-5-carboxamide, M4PY – 1-methyl-4-pyridone-5-carboxamide, MNA – 1-Methylnicotinamide, NA – nicotinamide, PGI_2 – prostacyclin, Phe – phenylephrine, SNAP – S-nitroso-N-acetyl-penicilamine, STZ – streptozotocin

Introduction

1-Methylnicotinamide (MNA) is a major metabolite of nicotinamide (NA). It is formed in the liver by nicotinamide N-methyltransferase (NNMT) and is further metabolized to 1-methyl-2-pyridone-5-carboxamide (M2PY) or 1-methyl-4-pyridone-5-carboxamide (M4PY) by aldehyde oxidase [1, 44]. Nicotinamide, is known to be an essential nutrient (vitamin B3), as it is the precursor of nicotinamide adenine dinucleotide (NAD) that participates in a wide range of biological processes including energy production, cellular resistance to injury as well as longevity [41, 48]. In addition, NA is an inhibitor of several enzymes that use NAD as a substrate, e.g. sirtuins [50].

In contrast to NA, MNA has for a long time been considered as a biologically inactive molecule and the endogenous level of MNA was merely considered to be a biomarker of the NA-degradation pathways in various circumstances including niacin deficiency [47], alteration in renal tubular excretion [31] or peroxisome proliferation in the liver [19]. Furthermore, alterations in the endogenous levels of MNA have been reported in various pathological states including liver cirrhosis [37], Parkinson disease [1], affective disorders [13] and burns in children [5]. It was also found that urine excretion of MNA displayed a day-night cycle [33] and declined with age [1]. Interestingly, increased activity of NNMT in cancer patients was shown to have favorable prognostic significance [42].

Recently the biological activity of MNA has been discovered putting a novel perspective on the biological role of the NA-MNA pathway. The anti-inflammatory efficacy of MNA after its topical application was demonstrated in patients with skin diseases [22, 49] followed by experimental studies in animal models *in vivo* demonstrating the anti-thrombotic and anti-inflammatory activities of MNA [12, 15]. Interestingly, the anti-thrombotic and anti-inflammatory actions of MNA were both mediated by a prostacyclin (PGI_2)-dependent mechanism suggesting that modulation of endothelial function may be a major target for MNA biological activity.

It is increasingly appreciated that NO-dependent function is a surrogate end-point of cardiovascular health. Indeed, the impairment of NO-dependent function in conduit vessels has diagnostic and prognostic significance in cardiovascular diseases [10, 14]. Moreover, the impairment of NO-dependent vasodilatation in conduit vessels represents an important therapeutic target. In fact, the amelioration of NO-dependent endothelial function with a variety of interventions, such as physical exercise, angiotensin converting enzyme (ACE) inhibitors or statins seems to be associated with the inhibition of the progression of atherosclerosis and better prognosis of cardiovascular diseases [7, 14, 23].

Accordingly, the aim of the present study was to investigate whether chronic administration of MNA in animal models of hypertriglyceridemia and diabetes would reverse the endothelial dysfunction characterized by the impairment of NO-dependent vasodilatation. For that purpose Sprague-Dawley rats were rendered diabetic by a streptozotocin (STZ) injection and Wistar rats were rendered hypertriglyceridemic by a diet rich in fructose (60%). After 8 weeks of hypertriglyceridemia or diabetes, the magnitude of the endothelium-dependent and endothelium-independent vasodilatation in aorta *ex vivo,* as well as various biochemical indices in plasma, were measured in untreated rats or rats chronically treated with MNA (100 mg/kg). Our data demonstrated that chronic treatment of diabetic or hypertriglyceridemic rats with MNA prevented the impairment of NO-dependent endothelial function.

Materials and Methods

Animals

All animal procedures conform with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and the Guidelines for Animal Care and Treatment of the European Community. The experimental procedures used in the present

study were approved by the local Jagiellonian University Ethical Committee on Animal Experiments.

Hypertriglyceridemic rats

Male Wistar [Krf:(WI)WU; n = 40] rats (3 month-old), weighing 350–550 g were obtained from the Animal House of the Jagiellonian University. The rats were housed during the experimental period of 8 weeks in an isolated room under conditions of controlled temperature, humidity and a 12 h light-dark cycle. The animals were acclimated to these conditions for 1 week and given free access to water and the basal semi-purified AIN-93G diet. After the adaptation period, the Wistar rats were divided into two groups. The control group (n = 10) was fed for 8 weeks with a control diet (basal AIN-93G). The hypertriglyceridemic group was fed for 8 weeks with AIN-93G diet in which fructose (60%) was substituted for maize starch and sucrose. The final composition of the diets is shown in Table 1. Animals were kept untreated or were treated with MNA (100 mg/kg, *po* in drinking water) for the final 4 weeks of the hypertriglyceridemic diet (n = 15, for each group of rats, treated or untreated with MNA). MNA chloride salt was synthesized by alkylation of NA with methyl chloride in methanol solution as described previously [15]. MNA used in the experiments was of high purity (> 99.8%) and NA was identified as a major impurity (< 0.2%).

Tab. 1. The composition of the control and hypertriglyceridemic diets (in g/kg diet) based on the AIN 93 standard diet [39]

Ingredient	Control AIN93G	Hypertriglyceridemic AIN93G
Maize starch	532.486	–
Casein (85%)	200.00	200.00
Sucrose	100.00	–
Soyabean oil	70.00	70.00
Fibre	50.00	50.00
Mineral mix	35.00	35.00
Vitamin mix	10.00	10.00
Choline bitartrate	2.50	2.50
Tert-butylhydroquinone	0.014	0.014
Fructose	–	632.486

Diabetic rats

Male Sprague-Dawley rats (SPRD/Mol/Lod) weighing 200–250 g were obtained from the Animal House of Polish Mother's Research Institute-Hospital in Łódź, Poland. Similarly to hypertriglyceridemic rats, they were housed in an isolated room under conditions of controlled temperature, humidity and a 12 h light-dark cycle and were given free access to water and feed. After a 1 week of adaptation, the Sprague-Dawley rats were randomly divided into three groups fed a standard diet for 8 weeks: control (n = 8), diabetic (n = 8) and MNA-treated diabetic rats (n = 8). Rats were rendered diabetic by a single intraperitoneal (*ip*) injection of STZ (70 mg/kg) dissolved in citrate buffer (0.01 M solution, pH 4.6). Age-matched control rats were injected with the buffer solution alone. One week after STZ injection, blood samples were obtained to verify the development of excessive hyperglycemia (> 200 mg/dl). Randomly selected rats injected with STZ that developed hyperglycemia were allocated to the untreated group or the group receiving MNA (100 mg/kg, *po* in drinking water). MNA treatment was initiated one week after STZ injection and continued for 7 weeks.

Assessment of the development of hypertriglyceridemia and diabetes

After 4 and 8 weeks of fructose-rich diet (hypertriglyceridemia) or 8 weeks after injection of STZ (diabetes), the rats were anesthetized with an *ip* injection of thiopental (120–150 mg/kg) and killed by cervical translocation. Blood samples were taken 2 h after fasting from the left ventricle of the heart. To obtain plasma, blood samples were collected into test tubes with anticoagulant (sodium citrate 3.2%; 1:9 v/v) and subsequently centrifuged for 10 min (4000 × g). The lipid profile was analyzed using commercially-available kits for high density lipoprotein (HDL) – cholesterol, low density lipoprotein (LDL) – cholesterol (Olympus Diagnostica GmbH, Hamburg, Germany), total cholesterol and triglycerides (CORMAY, Lublin, Poland) and expressed in mmol/l. The blood glucose concentration was measured in the drop of full blood with a One-Touch™ II Blood Glucose Me-

ter (Lifescan INC, Johnson & Johnson company, Milpitas, CA, USA). The level of glycosylated hemoglobin in the sample of full blood (HbA_{1c}) was measured by automatic analyzer DS5 (Drew Scientific, Cumbria, Great Britain) using commercially available tests (Pink 300 Test reagent Kit, Drew Scientific).

Assessment of the concentration of MNA and its metabolites in plasma

Blood samples from rats, taken 2 h after fasting from the left ventricle of the heart were collected into test tubes with anticoagulant (sodium citrate 3.2%; 1:9 v/v) and centrifuged for 10 min (4000 × g) to obtain plasma. The concentration of endogenous MNA and its metabolites in plasma samples was measured using liquid chromatography mass spectrometry method (LC/MS) as described previously [42]. Briefly, chromatographic separation was performed using 3 μm Hypersil C18-BDS 150 × 2.0 mm column. Buffer A was 10 mM nonafluoropentanoic acid (NFPA) in water and buffer B was 100% acetonitrile. The mobile phase was run at 0.2 ml/min in a gradient from 0% to 60% B in 12 min. The mass detector (Thermo-Finnigan LCQ Advantage, Waltham, MA, USA) with electrospray ion (ESI) source was operating in a positive single ion monitoring (SIM) mode for detection of $[M+H]^+$ species of NA, MNA, M2PY, M4PY, with the collision energy setting at 25%. Internal standard (2-chloroadenosine) signal was extracted from full MS mode. Electrospray cone voltage was set at 4.5 kV and heated capillary temperature was 275°C. Sheath gas flow was set at 35 arbitrary units. Ion optics were optimized using standard instrument procedures during infusion of nicotinamide. Rat plasma was deproteinized using 10% trichloroacetic acid followed by ether extraction. Recovery of M2PY, M4PY and NA added to the samples with known concentration was 75–95%. The coefficient of variation was below 10% for repeated injections on the same day. However, much larger (>20%) variation was observed for repeated injections between days [42].

Assessment of NO-dependent vasodilatation in the isolated aorta

Thoracic aorta from anesthetized (thiopental, *ip* 120–150 mg/kg) rat was removed and carefully dissected free from the surrounding tissue. Isolated aorta was placed in Krebs-Hanseleit solution, cleaned of the connective and fat tissue and cut into rings. The rings were washed out with Krebs-Hanseleit solution mounted between 2 hooks attached to an isometric force transducer (Biegastab K30 type 351; Hugo Sachs March-Fr, Germany) with continuous recording of tension (Graphtec WR3320, UK). After mounting of the rings, the resting tension was increased in a step-wise fashion to reach final 4g, after which the rings were incubated to equilibrate for 30 min. Six circular segments (3–5-mm in length) of the artery were simultaneously used for an experiment. Aortic rings were kept in 5 ml organ baths containing prewarmed (37°C) Krebs-Hanseleit that was continuously bubbled with 5% CO_2 in O_2 to maintain a pH 7.4. Krebs-Hanseleit solution was of the following composition (in mM): 118.0 NaCl, 4.7 KCl, 2.25 $CaCl_2$, 1.64 $MgSO_4$, 1.18 KH_2PO_4, 24.88 $NaHCO_3$, 10.0 glucose, 2.2 $C_3H_3O_3Na$ and 0.5 EDTA.

After stretching and 60 min of further equilibration, the experiment was initiated by obtaining maximum contraction in response to KCl (60–90 mM). Then, the aortic rings were contracted with increasing concentrations of phenylephrine (Phe 0.01–10 μM) to determine a concentration of Phe that gives 60–80% of maximum KCl-induced contraction. Endothelial function was assessed by a cumulative concentration-dependent response to acetylcholine (Ach) (0.01–10 μM) or histamine (Hist) (0.01–300 μM) in Phe – preconstricted vessels. The endothelium-independent function was tested through a response evoked by S-

Tab. 2. Effect of chronic treatment with MNA (100 mg/kg) on plasma concentration (mmol/l) of triglycerides (TG), total-, LDL-, HDL-cholesterol in hypertriglicerydemic rats. Data represent mean ± SEM of control (n = 12–44), non-treated hypertriglyceridemic rats (n = 7–19) or MNA-treated hypertriglyceridemic rats (n = 5–22). * and ** indicates $p < 0.05$ and $p < 0.001$, respectively, *vs.* control rats. ## indicates $p < 0.001$ between non-treated and MNA-treated hypertriglyceridemic rats

	TG	Total-Cholesterol	LDL-Cholesterol	HDL-Cholesterol
Control	1.55 ± 0.12	1.58 ± 0.06	0.88 ± 0.15	0.92 ± 0.08
Hypertriglyceridemia	4.25 ± 0.27 **	1.61 ± 0.06	0.89 ± 0.12	0.87 ± 0.17
Hypertriglyceridemia+MNA	2.22 ± 0.14 *##	1.49 ± 0.03	0.70 ± 0.18	1.33 ± 0.06

nitroso-N-acetyl-penicilamine (SNAP from 0.001 to 10 μM). Subsequently the cumulative concentration-dependent curve for Ach was repeated in the presence of the inhibitor of nitric oxide synthesis-N^G-nitro-L-arginine methyl ester (L-NAME; 300 μM). L-NAME was incubated for at least 15 min before eliciting a response to Ach or Hist.

Statistical analysis

Results are presented as means ± SEM. As the results did not follow a normal distribution pattern, the differences between experimental groups were evaluated by Kruskal-Wallis test and Mann-Whitney test with Bonferroni correction for multiple comparisons. $p < 0.05$ was considered significant. For statistical analysis GraphPad Prism 5 software (San Diego, CA, USA) was used.

Results

Effects of MNA on the development of hypertriglyceridemia

A hypertriglyceridemic diet fed to rats led to the increase in plasma triglycerides concentration (from 1.55 ± 0.12 to 4.25 ± 0.27 mmol/l, $p < 0.001$), while the concentration of total-cholesterol, LDL-cholesterol and HDL-cholesterol remained mostly unchanged (Tab. 2). As shown in Figure 1, the chronic treatment (4 weeks) of hypertriglyceridemic rats with MNA with a dose of 100 mg/kg resulted in a three- to six-fold increase of the plasma concentration of endogenous MNA and its metabolites (M2PY and M4PY). In contrast, treatment (4 weeks) of hypertriglyceridemic rats with MNA with a dose of 10 mg/kg did not increase the plasma concentration of endogenous MNA, M2PY and M4PY (data not shown).

MNA given at a dose of 100 mg/kg for 4 weeks to hypertriglyceridemic rats significantly lowered plasma triglyceride concentration (from 4.25 ± 0.27 to 2.22 ± 0.14 mmol/l, $p < 0.001$), without a significant effect on the plasma concentration of total cholesterol, LDL-cholesterol and HDL-cholesterol (Tab. 2).

Effects of MNA on the NO-dependent vasodilatation in hypertriglyceridemic rats

Endothelium-dependent vasodilatation induced by Ach in the aorta from hypertriglyceridemic rats was impaired already 4 weeks into the hypertriglyceridemic diet (81.44 ± 3.90% *vs.* 66.90 ± 4.54% for 1 μM Ach for control and hypertriglyceridemic rats, respectively, $p = 0.069$). After 8 weeks of hypertriglyceridemia, the impairment of endothelium-dependent relaxation induced by Ach was even more pronounced and significant (52.41 ± 7.89% for 1 μM Ach, $p < 0.05$ *vs.* control, Fig. 2A). Similar degree of

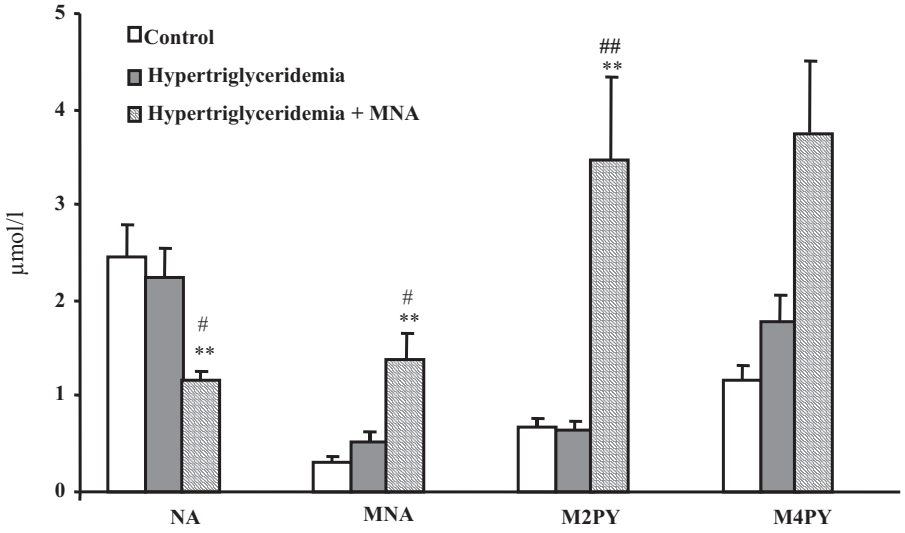

Fig. 1. Levels of endogenous MNA and its metabolites in control (n = 13), hypertriglyceridemic rat: untreated (n = 13) or treated with MNA 100 mg/kg (n = 14). Data represent means ± SEM ** indicates $p < 0.001$ *vs.* control rats, # and ## indicates $p < 0.05$ and $p < 0.001$ *vs.* hypertriglyceridemic rats, respectively

the impairment of NO-dependent vasodilatation by hypertriglyceridemic diet was detected when Hist was used instead of Ach (data not shown).

As shown in Figure 2A, when hypertriglyceridemic rats were treated with MNA for 4 weeks (100 mg/kg), the endothelium-dependent vasodilatation evoked by Ach in aorta was preserved (79.24 ± 5.37% *vs.* 52.41 ± 7.89% for 1 μM Ach, for hypertriglyceridemic rats treated and non-treated with MNA, respectively, $p < 0.05$). In the presence of L-NAME (300 μM) Ach-induced vasodilatation in the aorta was substantially inhibited by more then 90% in control rats (by 90.69 ± 0.81%) as well as in hypertriglyceridemic rats treated (by 95.01 ± 0.45%) or untreated with MNA (by 94.85 ± 0.49%). In turn, endothelium-independent relaxation induced by SNAP was in general not impaired by hypertriglyceridemia and not affected by MNA treatment (Fig. 2B).

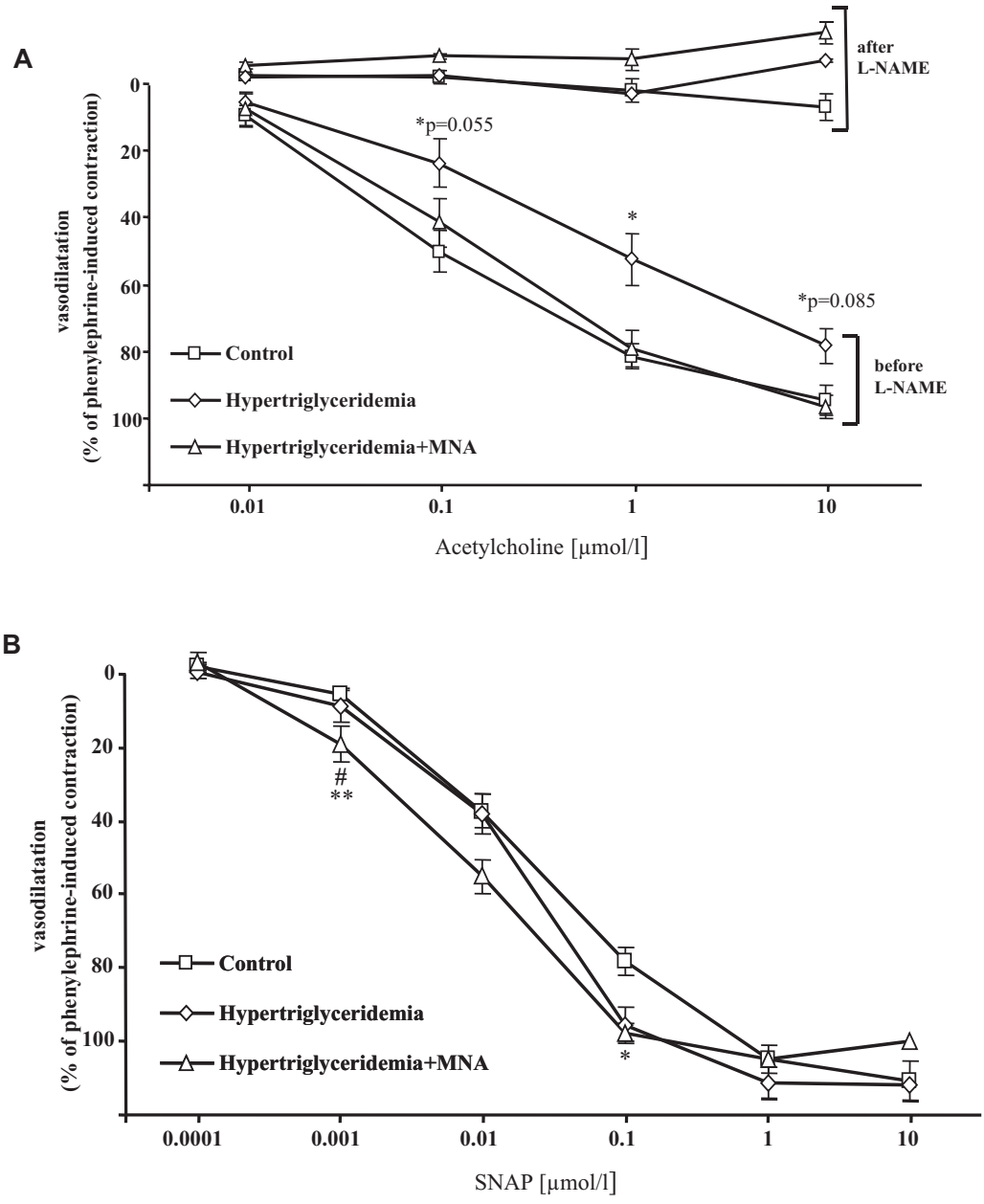

Fig. 2. Effects of chronic treatment with MNA (100 mg/kg) on the endothelium-dependent relaxation to acetylcholine (**A**) and on endothelium-independent relaxation to SNAP (**B**) in aorta in hypertriglyceridemic rats. Data represent mean ± SEM, of n = 5–27 experiments. * and ** indicates $p < 0.05$ and $p < 0.001$, respectively, *vs.* control rats, # indicates $p < 0.05$ between non-treated or MNA-treated hypertriglyceridemic rats

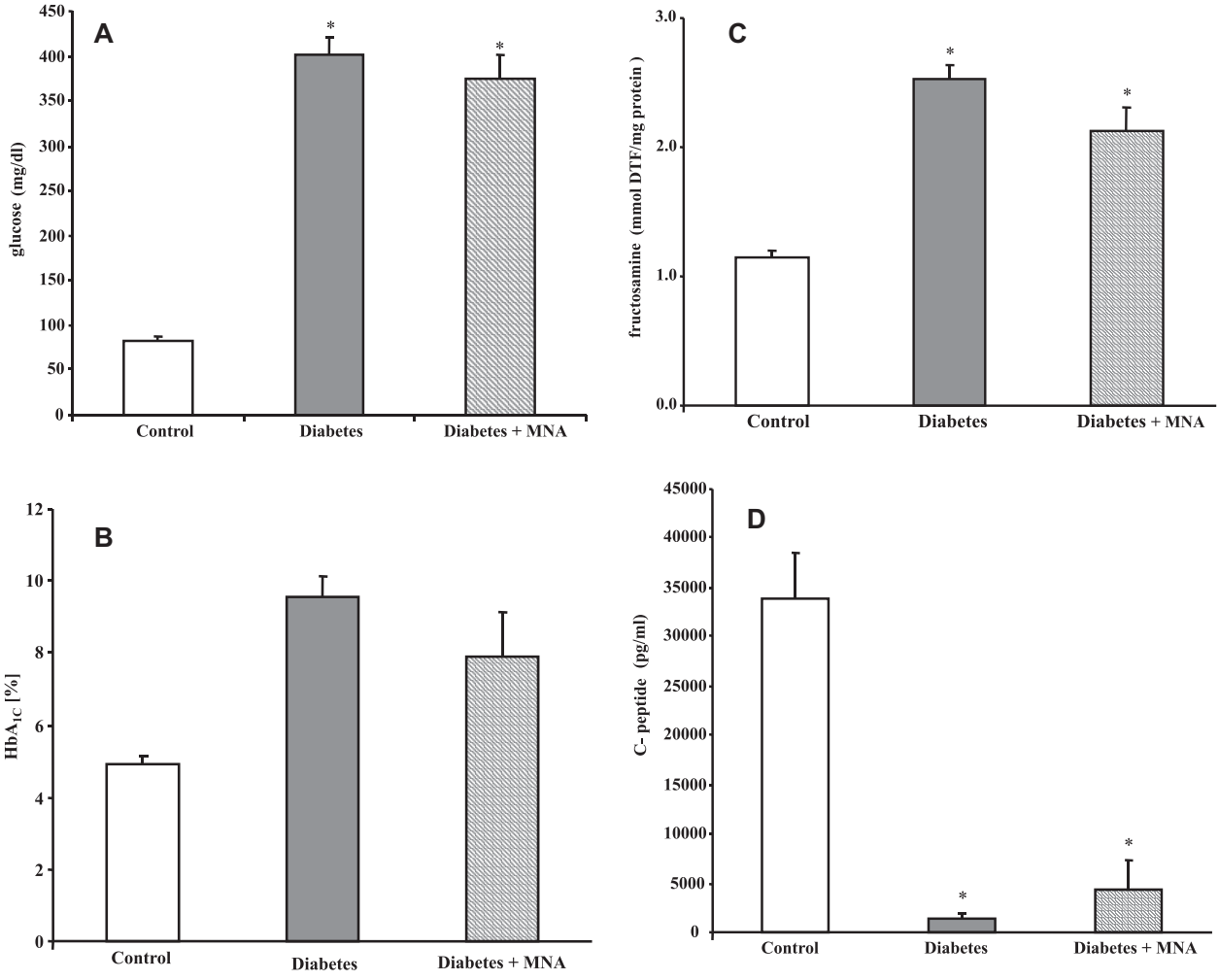

Fig. 3. Effects of chronic treatment with MNA (100 mg/kg) on plasma concentration of glucose (**A**), glycosylated hemoglobin (HbA_{1C})(**B**), fructosamine (**C**) and C-peptide (**D**) in diabetic rats. Data represent mean ± SEM of control (n = 3–17), non-treated diabetic rats (n = 4–24), diabetic rat treated with MNA (n = 3–8). * indicates $p < 0.05$ *vs.* control rats

Effects of MNA on the biochemical parameters of diabetes

As shown in Figure 3, the blood concentration of glucose, fructosamine and HbA_{1c} was increased in diabetic rats, while the concentration of peptide C was significantly decreased as compared to the control rats. Diabetic rats displayed also a mild hypertriglyceridemia (from 1.05 ± 0.12 to 1.31 ± 0.08 mmol/l, $p < 0.05$), while levels of total-cholesterol, LDL-cholesterol and HDL-cholesterol remained unchanged (Tab. 3). Chronic treatment of diabetic rats with MNA (100 mg/kg, *po* for 7 weeks) elevated the concentration of endogenous MNA and its metabolites in plasma to a similar degree as in hypertriglyceridemic rats (data not shown).

In diabetic rats chronically treated with MNA (100 mg/kg) there was a modest but significant increase in plasma triglyceride concentration (from 1.31 ± 0.08 to 1.66 ± 0.14 mmol/l, $p < 0.05$), while the concentration of total cholesterol, LDL- and HDL-cholesterol remained unchanged (Tab. 3). Treatment with MNA had a minor effect on the biochemical parameters of diabetes as it slightly decreased the blood concentration of glucose, HbA_{1c} and fructosamine although this effect was not of statistical significance. The effect of MNA on the concentration of peptide C was also not significant (Fig. 3).

Tab. 3. Effects of chronic treatment with MNA (100 mg/kg) on plasma concentration (mmol/l) of triglycerides (TG), total-, LDL-, HDL-cholesterol in diabetic rats. Data represent mean ± SEM of control (n = 18–30) non-treated diabetic rats (n = 26–30) and diabetic rat treated with MNA (n = 12–22). * and ** indicates $p < 0.05$ and $p < 0.001$, respectively, *vs.* control rats, # indicates $p < 0.05$ between non-treated or MNA-treated diabetic rats

	TG	Total-Cholesterol	LDL-Cholesterol	HDL-Cholesterol
Control	1.05 ± 0.12	1.51 ± 0.07	0.49 ± 0.07	1.16 ± 0.09
Diabetes	1.29 ± 0.08*	1.28 ± 0.07	0.48 ± 0.04	1.10 ± 0.08
Diabetes + MNA	1.79 ± 0.15**#	1.25 ± 0.15	0.49 ± 0.14	1.03 ± 0.20

Effects of MNA on the NO-dependent vasodilatation in aorta in diabetic rats

Endothelium-dependent vasodilatation induced by Ach in the aorta from diabetic rats (8 weeks after STZ injection) was impaired (50.51 ± 3.19% *vs.* 70.95 ± 3.73% for 1 μM Ach, $p < 0.001$, for diabetic and control rats, respectively, Fig. 4).

When diabetic rats were treated with MNA (100 mg /kg, *po* for 7 weeks), the endothelium-dependent response evoked by Ach (Fig. 4A) or Hist (Fig. 4B) was preserved and not significantly different from the respective response in the control rats. In the presence of L-NAME (300 μM), Ach-induced or Hist-induced vasodilatation in the aorta was profoundly inhibited in all experimental groups (Fig. 4).

Endothelium-independent relaxation induced by SNAP (only for SNAP at the concentration of 0.1 μM and 1 μM) was impaired in diabetic rats. In diabetic rats treated with MNA the response to SNAP was not significantly different as compared to control rats (Fig. 4C).

Discussion

In the present work we demonstrated that in hypertriglyceridemic and diabetic rats chronic treatment with MNA, a major metabolite of NA, reversed endothelial dysfunction in aorta characterized by the impairment of NO-dependent vasodilatation. We also demonstrated that chronic treatment with MNA decreased plasma triglyceride concentration in hypertriglyceridemic, but not in diabetic rats. Although, we did not investigate the mechanism involved, our data shows for the first time that MNA prevents endothelial dysfunction and lowers triglycerides, and that these effects seem to be independent.

The ability of MNA to reverse endothelial dysfunction was studied in two rat models of endothelial dysfunction – high-fructose diet induced-hypertriglyceridemia and STZ-induced diabetes. Both models are widely used and have been extensively characterized [4, 6, 18]. High-fructose diet in rats leads to insulin resistance, hyperinsulinemia, hypertiglyceridemia with a mild elevation of blood pressure, the set of symptoms that resembles metabolic syndrome in humans [8]. STZ injection selectively destroys insulin producing cells of the pancreas, rendering the rat diabetic within 24 h of the injection and then leading to the typical long-term complications of diabetes [9, 43]. Importantly, endothelial dysfunction and the impairment of NO-dependent vasodilatation in aorta is a common denominator of these two pathologies [6, 17]. Accordingly, as the hypercholesterolemic rats – whether normotensive or hypertensive – are resistant to develop endothelial dysfunction [30, 36], hypertriglyceridemic and diabetic rats seem more relevant animal models for the experimental pharmacology of endothelial dysfunction. Importantly, both hypertriglyceridemia and diabetes are considered important risk factors of atherosclerosis [2, 24, 35] and are associated with endothelial dysfunction also in humans [20, 34].

In the present work we confirmed the impairment of NO-dependent vasodilatation in hypertriglyceridemic and diabetic rats using two endothelium-dependent vasodilators – Ach and Hist, while endothelium-independent relaxation induced by SNAP was largely preserved. Interestingly, in the presence of L-NAME, Ach- and Hist-induced vasodilatation in the aorta in both models was nearly abrogated, suggesting that in hypertriglyceridemia and diabetes the impairment of NO-dependent vasodilatation was not associated with the up-regulation of the EDHF pathway [16]. Furthermore, in a number of experimental models the impairment of NO-dependent function was associated with

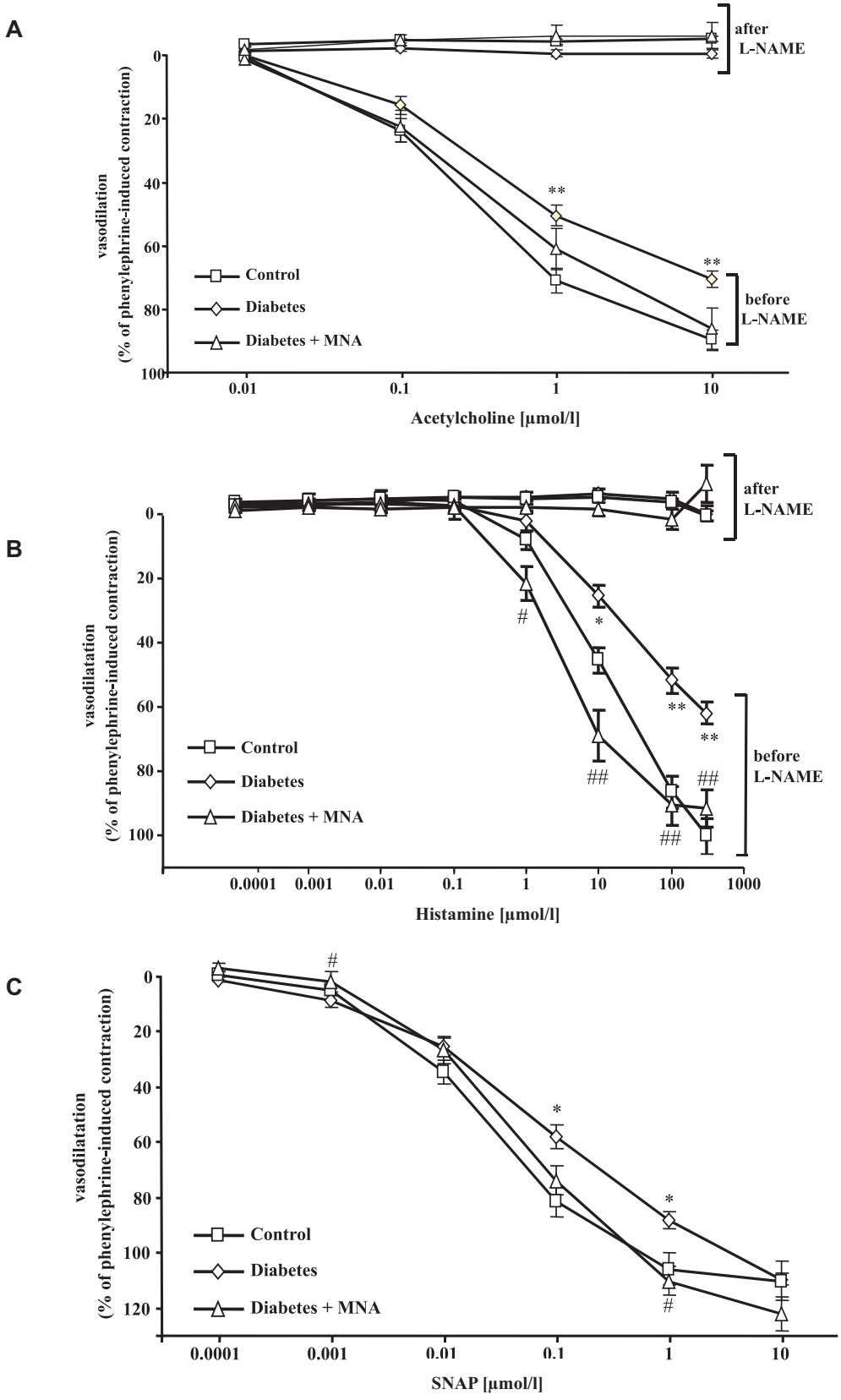

Fig. 4. Effects of chronic treatment with MNA (100 mg/kg) on endothelium-dependent relaxation to acetylcholine (**A**) and histamine (**B**) and endothelium-independent relaxation to SNAP (**C**) in aortic rings of diabetic rats. Data represent mean ± SEM of n = 6–22 experiments. * and ** indicates $p < 0.05$ and $p < 0.001$ respectively, *vs.* control rats, # and ## indicates $p < 0.05$ and $p < 0.001$, respectively *vs.* diabetic rats

the impairment of cyclooxygenase (COX)-1 derived PGI_2 production [21, 40]. Indomethacin did not modify the magnitude of endothelium-dependent vasodilatation induced by Ach or Hist in control, hypertriglyceridemic or diabetic rats (data not shown), excluding the involvement of COX-derived products in the endothelium-dependent vasodilatation in this experimental setting [30]. Yet the alterations in the functional activity of PGI_2 pathways may be present in hypertriglyceridemia [38] and diabetes [17] and they need to be analyzed in more detail.

The impaired NO-dependent vasodilatation detected in the aorta from hypertriglyceridemic or diabetic rats was most likely associated with increased reactive oxygen species (ROS) production [20, 51] and various pro-thrombotic and pro-inflammatory alterations in endothelial function [25, 34]. In the present work we did not analyze comprehensively the complex phenotype of endothelial dysfunction in these models and the mechanisms involved. Our aim was to use NO-dependent vasodilatation in the aorta as a surrogate end-point of endothelial function in order to examine the ability of MNA to prevent the development of endothelial dysfunction. This approach stems from the wealth of clinical evidence suggesting that impaired NO-dependent vasodilatation in the conduit vessel has diagnostic, prognostic and therapeutic significance in cardiovascular diseases [10, 14] and may be regarded as a specific barometer of endothelial dysfunction [46]. In view of the above, our results demonstrating that supplementation of MNA leading to the increase of endogenous levels of MNA by three- to six-fold prevented the development of endothelial dysfunction in hypertriglyceridemia and diabetes suggest important vasoprotective activity of MNA that warrants further experimental and clinical studies. Furthermore, our results suggest that exogenous MNA being a stable and non-toxic molecule can be viewed as a good candidate for a drug to treat endothelial dysfunction in various diseases associated with endothelial dysfunction. On the other hand, our results may implicate that MNA formed in the liver by nicotinamide N-methyltrasferase can be an endogenous regulator of the endothelial function in conduit vessels.

We have recently discovered the anti-thrombotic action of MNA [15]. Interestingly, MNA administered *iv* limited platelet-dependent experimental thrombosis by a mechanism dependent on the COX-2/PGI_2 pathway [15]. Anti-thrombotic activity of MNA was later shown to be associated with the decreased production of plasminogen activator inhibitor-1 [32] that could also be linked to PGI_2 [11]. In our most recent work, using the mouse model of contact hypersensitivity, we demonstrated that MNA afforded anti-inflammatory action mediated by PGI_2 [12]. Accordingly, it is tempting to speculate that the reversal of endothelial dysfunction by MNA could also involve a PGI_2-mediated mechanism [3, 26, 29]. This possibility needs to be addressed in further studies.

It is worth adding that MNA prevented the development of endothelial dysfunction in hypertriglyceridemia and diabetes, while it lowered triglyceride levels primarily in hypertriglyceridemia. It is of note, however, that the concentration of triglycerides was highly elevated in hypertriglyceridemia but not in diabetes. Nevertheless, it seems that the ability of MNA to reverse endothelial dysfunction is independent on its hypolipemic activity. We did not investigate here a possible mechanism of the hypolipemic activity of MNA. There are several possibilities, including the activation of endothelial lipoprotein lipase (LPL). Indeed, the impaired activity of LPL may lead to hypertriglyceridemia [27] suggesting important involvement of endothelial LPL in the regulation of plasma triglyceride levels. Of note, this enzyme was reported to be inhibited by glycation in diabetes [28, 45] that could offer a plausible explanation of the absence of the MNA lowering effect on triglycerides in diabetes, if LPL was to be its target.

Interestingly, MNA displayed a mild effect on the biochemical parameters of diabetes. In our experiments these effects did not have statistical significance, which may be due to the small size of the experimental groups. In another study, performed in larger animal cohorts, MNA reduced significantly glycated haemoglobin and fasting 8-week blood glucose (Kazmierczak and Watala, manuscript submitted).

Summing up, we demonstrate here for the first time that MNA prevented endothelial dysfunction in hypertriglyceridemic and diabetic rats. MNA treatment also decreased plasma triglyceride concentration in hypertriglyceridemic rats, but this effect was not manifested in diabetic rats. Altogether our results suggest that the ability of MNA to reverse endothelial dysfunction is independent of its hypolipemic activity. Although mechanisms of endothelial and hypolipemic actions of MNA have not been explained, our findings of novel biological activities of MNA extends our pre-

vious studies [12, 15] and may have important physiological as well as therapeutic implications.

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