

# 1 EFFECT OF EXERCISE ON ADMA LEVEL IN TYPE 2 DIABETES MELLITUS

## 2 ADMA LEVEL IN EXERCISING TYPE 2 DIABETICS

### 4 ABSTRACT

5 **Objectives:** The increase in symmetric (SDMA) and asymmetric dimethyl arginine (ADMA) is  
6 associated with endothelial dysfunction. The aim of the this study was to evaluate the effect of  
7 exercise on ADMA and SDMA levels and related ratios in type 2 DM.

8 **Study design:** Thirty-six women with type 2 DM and 44 healthy women were recruited to the  
9 study. Fourteen and 19 subjects, respectively, attended a four-weeks of moderate intensity  
10 exercise. Plasma SDMA, ADMA, and L-arginine levels were measured by HPLC method at the  
11 initial, after the first exercise session, and after one month.

12 **Results:** All measurements were within normal range in both groups and did not change  
13 significantly immediately after exercise and after the first month. In exercising healthy group, L-  
14 arginine/ADMA and L-arginine/SDMA ratios after one month were significantly higher than the  
15 initial values ( $p<0.022$  and  $p=0.017$ , respectively). After one month of exercise L-  
16 arginine/ADMA and L-arginine/SDMA ratios increased in diabetics and decreased in healthy  
17 subjects when compared to those after first exercise session ( $p=0.026$  and  $p=0.037$ , respectively).

18 **Conclusion:** We suggest that L-arginine/ADMA and L-arginine/SDMA ratios should be used in  
19 spite of absolute values. These molecules have a substantial role in beneficial effects of exercise  
20 in healthy subjects while their role is scarce in diabetics.

21 **Key words:** ADMA, arginine, exercise, diabetes mellitus type 2

### 22 INTRODUCTION

23 Cardiovascular disease is the most leading cause of death in diabetes mellitus (DM) and  
24 regular exercise reduces the risk, irrespective of weight loss and glucose control [1,2].

25 Systemic inflammation decreases and endothelial vasodilation recovers during exercise.  
26 One of the systems involved is nitric oxide (NO) pathway. The most anti-atherogenic substance  
27 secreted by endothelium is NO. It is derived from a semi-essential amino acid, L-arginine [3].  
28 NO regulates vascular smooth muscle tone and inhibits many proatherogenic process such as  
29 monocyte and platelet adhesion, low-density lipoprotein oxidation, and synthesis of  
30 inflammatory cytokines. Besides NO increases insulin sensitivity [3].

31 An L-arginine metabolite, ADMA, is the major endogenous NOS inhibitor [4]. L-  
32 arginine/ADMA ratio shows NO synthesis [5,6]. ADMA is degraded by the enzyme  
33 dimethylarginine dimethylaminohydrolase (DDAH). ADMA demolishes endothelium derived

34 flow response substantially [5]. Increased ADMA impairs endothelium derived NO mediated  
35 vasodilation, enhances monocyte adhesion, and causes platelet hyperaggregation [7].

36 Plasma ADMA levels increase in many instances related to endothelial dysfunction such  
37 as hypercholesterolemia, atherosclerosis, hypertension, chronic renal insufficiency, chronic heart  
38 failure, and smoking [8,9]. Furthermore ADMA increases 2-3 fold in type 2 DM [4,10,11,12]. L-  
39 arginine levels hover in normal range in these clinical situations where ADMA increases.  
40 Thereby L-arginine/ADMA ratio decreases [3].

41 Another dimethylarginine (DMA) compound, SDMA competes with cellular uptake of  
42 L-arginine and cannot inhibit NOS. There are reports showing that SDMA as well as ADMA is  
43 related to vascular diseases [3]. SDMA is more closely related to renal insufficiency compared to  
44 ADMA [6]. ADMA/SDMA ratio reflects DDAH activity [10]. L-arginine/SDMA ratio is an  
45 indicator of renal insufficiency [13]. SDMA, which competes with L-arginine for reabsorption, is  
46 responsible for changing ratios in renal insufficiency.

47 The only research about ADMA level in regularly exercising diabetics was conducted in  
48 type 1 DM. It was shown that ADMA decreased during regular exercise but the beneficial effect  
49 dissipated months after cessation of exercise [14]. ADMA status is obscure in exercising type 2  
50 diabetics. In this study we aimed to evaluate ADMA, SDMA, and L-arginine levels in type 2  
51 DM and their relation to exercise.

## 52 **MATERIALS AND METHODS**

### 53 **Subjects**

54 Thirty-six women diagnosed with type 2 DM [15] and 44 healthy women without any  
55 known systemic disease (control group) were enrolled in the study.

56 None of the diabetic subjects were on steroid, insulin, nebivolol, and nitrate therapy. The  
57 patients with a history of hypertension and those with blood pressure of  $\geq 140$  mmHg systolic and  
58  $\geq 90$  mmHg diastolic were recorded as hypertensive. Those who had blood pressure above  
59 160/90 mmHg, proliferative diabetic retinopathy, muscle-skeletal disease, chronic renal disease  
60 (creatinine clearance calculated in 24 hour urine less than 30 mL/minute), or liver failure were  
61 not included to the study.

62 The control group had a fasting glucose level below 100 mg/dL and 2-hour glucose level  
63 less than 140 mg/dL after 75 g glucose load. They had a normal blood pressure of less than 140  
64 mmHg systolic and less than 90 mmHg diastolic.

65 Height, weight, and waist and hip circumference were measured. Body mass index (BMI)  
66 was calculated by dividing weight in kilograms by square of height in meters ( $\text{kg}/\text{m}^2$ ). Waist/hip

67 ratio were recorded. The subjects with a BMI of 25-29 kg/m<sup>2</sup> were classified as overweight and  
68 BMI of  $\geq 30$  kg/m<sup>2</sup> as obese [16].

69 TSH and serum insulin was measured by chemiluminescence immunoassay method  
70 (BIO-DPC, Immulite, USA). HDL and total cholesterol, triglyceride levels were measured by  
71 spectrophotometry (Beckman Coulter). LDL cholesterol was calculated according to Friedewald  
72 formula [total cholesterol-(triglyceride/5+HDL)]. Creatinine was evaluated by  
73 spectrophotometer autoanalyser (MEGA 600, Merck, Germany) using Diasis (Germany) kits.  
74 Insulin resistance was calculated using the homeostasis model assessment (HOMA-R) formula  
75 [17]. Hemoglobin A<sub>1c</sub> was measured by immunoassay method.

76 The study was approved by ethics committee. Written informed consent was taken from  
77 the enrollees.

### 78 **Exercise Protocol**

79 None of the subjects had participated in regular exercise programme during the  
80 preceding three months. Fourteen diabetic and 19 healthy subjects attended an exercise  
81 programme that consisted 12 sessions. Daily activities of the subjects who refused to contribute  
82 to the exercise programme were released free. The subjects who did not regularly attend to the  
83 programme were dropped off the exercise group.

84 Fat percentage was measured before the exercise programme (Tanita Model 300, Tokyo,  
85 Japan). In order to determine the intensity of exercise, a submaximal work test was performed  
86 and maximal oxygen uptake (VO<sub>2max</sub>) was calculated. Exercise programme was tailored  
87 individually with an intensity of 40% VO<sub>2max</sub>, 40 minutes per day, 3 days per week for 4  
88 successive weeks. Exercise intensity was increased in subjects who adhered to the programme.  
89 Therefore moderate intensity exercise was considered to be appropriate.

90 Submaximal work test (Astrand Cycle Ergometer Test) was performed using a  
91 computerized ergometer (Monark 894-E, Monark Exercise AB, Sweden). A suitable load is  
92 chosen as 300 kpm/min (1 kp and 50 pedal turns) or 600 kpm/min (2 kp and 50 pedal turns).  
93 Ergometer pedal was ridden for 6 minutes and the heart rate was recorded every minute until the  
94 same heart rate was provided in the successive 2 minutes or difference between last two heart  
95 rates was four in maximum. The heart rate was monitored with a chest belt telemetry system  
96 (Polar 610i, Monark Exercise AB, Sweden). The values were evaluated in the Modified Astrand-  
97 Ryhming nomogram and VO<sub>2max</sub> was calculated [18]. The test was discontinued when heart rate  
98 of the subjects aging over 40 years exceeded 150 rpm. Load was not let to exceed 600 kpm.

### 99 **ADMA, SDMA and L-arginine Measurement**

100 Blood samples drawn at 08:30-09:00 am after one night fasting were collected to ethylene  
101 diamine tetra acetic acid (EDTA) containing tubes at the beginning of the study. The aliquots  
102 were centrifuged at 5000 rpm for 5 minutes within 30 minutes of collection. The supernatant was  
103 stored at -80 °C until analysis. The exercise attendees were sampled again within 15 minutes  
104 after the first and last session of exercise. Non-exercise subjects were also sampled after one  
105 month.

106 ADMA was determined using the established HPLC method described by Teerlink [19].

### 107 **Statistical Analysis**

108 Descriptive statistics of the variables and normal distribution was tested by one-sample  
109 Kolmogorov Simirnov test. Clinical variables were analyzed by Fisher exact test.

110 Student's t testi was used for variables showing normal distribution to evaluate difference  
111 between two groups. ADMA, SDMA, and L-arginine values and L-arginine/ADMA, L-  
112 arginine/SDMA, and ADMA/SDMA ratios at the beginning, after the first and last session of  
113 exercise, and after one month were compared by Bonferroni multiple comparison test within  
114 each group (diabetic and control group). Analysis of covariance was used for comparison of the  
115 groups.

116 The values were expressed as mean  $\pm$  standard deviation. A p value lower than 0.05 was  
117 accepted as statistical significance. Statistica 7.0 (Installer Code: 31N6YUCV38) programme  
118 was used for statistical analysis.

### 119 **RESULTS**

120 Two (9%) from diabetic non-exercise group (n=22) and 5 (35%) diabetic exercise group  
121 (n=14) had hypertension. One (4%) subject from diabetic non-exercise group and 2 (14%)  
122 subjects from diabetic exercise group had non-proliferative retinopathy. Other clinical features  
123 and laboratory values are shown in Table 1 and Table 2.

124 Among exercising subjects 8 diabetic patient and 5 healthy control were unable to  
125 complete the programme. One exercising diabetic had positive exercise test on Bruce protocol  
126 and underwent coronary angiography. None of the subjects could exercise beyond stage 3 of the  
127 Bruce protocol. The most common symptom for discontinuation was tiredness. Angina and  
128 hypotension did not occur.

129 There was no correlation between ADMA and age in contrast to the previous data [20].  
130 There was a positive correlation between SDMA and age (beta=0.226, p=0.044). These relations  
131 did not affect L-arginine/ADMA, L-arginine/SDMA, and ADMA/SDMA ratios.

132 Creatinine clearance was measured only in DM group. There was a negative correlation  
133 between creatinine clearance and SDMA (beta=-0.477, p=0.003) and between creatinine

134 clearance and ADMA (beta=-0.482, p=0.003). This correlation reflected to ADMA/SDMA ratio  
135 as a positive correlation (beta=0.348) (p=0.037). Even though L-arginine was not correlated with  
136 creatinine clearance, there was a positive correlation between creatinine clearance and L-  
137 arginine/SDMA ratio (beta=-0.343, p=0.04). There was no correlation between creatinine  
138 clearance and L-arginine/ADMA ratio.

139 Initial mean ADMA, L-arginine, and SDMA levels and ADMA/SDMA L-  
140 arginine/ADMA and L-arginine/SDMA ratios did not differ significantly in diabetic and healthy  
141 subgroups (Table 2). After the first exercise session, these values did not change significantly  
142 compared with the initial values. At the end of one month ADMA, L-arginine, and SDMA levels  
143 and ADMA/SDMA ratio did not change significantly either.

144 The only change was observed in exercising control group at the end of one month. L-  
145 arginine/ADMA ratio increased significantly from the initial value in comparison to the other 3  
146 groups [non-exercising control, (p=0.004); non-exercising diabetics (p=0.022); exercising  
147 diabetics (p=0.001)]. On the contrary, L-arginine/ADMA ratio decreased in exercising control  
148 group while increased in exercising diabetic group compared to the value obtained at the end of  
149 first exercise session (p=0.026).

150 After the first month L-arginine/SDMA ratio was significantly higher from the initial  
151 value in exercising control group only in comparison to non-exercising control group (p=0.017).  
152 When compared to the values obtained at the end of first exercise session, L-arginine/SDMA  
153 ratios decreased in exercising control group while increased in exercising diabetic group  
154 (p=0.037).

## 155 **DISCUSSION**

156 It is known that ADMA plays a role in decreased NO production in DM, but the levels  
157 vary a great deal according to the method and sample used. Serum ADMA levels are 60-70%  
158 higher than plasma levels. Results with ELISA are higher than the golden standard HPLC  
159 method [3]. Therefore there are contradictory data about ADMA levels. Most of the studies  
160 showed higher ADMA levels in type 2 DM [11,21], while others yielded normal levels  
161 [4,20,24,25]. Inhibition of DDAH by hyperglycemia and advanced glycosylated products was  
162 blamed [23,24]. In our study ADMA and SDMA levels did not differ between healthy control  
163 and diabetic subjects. We did not observe 2-3 fold increases as described in previous reports  
164 [3,10].

165 ADMA decreases as hyperglycemia is controlled [26] and is in negative relation with  
166 HbA1c level [20]. Our HbA1c results were lower than these studies. We did not find any

167 correlation between ADMA and SDMA levels and HbA<sub>1c</sub>. Not any degree but a certain level of  
168 glycemia may effect ADMA level.

169 ADMA increases in renal failure due to decreased excretion and decreased DDAH  
170 activity [7,24]. ADMA is higher in type 2 diabetics with hyperfiltration than non-diabetic  
171 subjects [20]. In our study all diabetic patients had creatinine clearance over 30 mL/min.  
172 Creatinine clearance was negatively correlated with ADMA and SDMA levels. This negative  
173 correlation reflected to ADMA/SDMA ratio as a positive correlation (beta=0.348, p=0.037). In  
174 another words since the decrease in SDMA was to a greater extent than the increase in ADMA, a  
175 positive correlation was detected between their ratio and creatinine clearance. Therefore it may  
176 be concluded that SDMA increases more than ADMA and is a better indicator of renal clearance.  
177 This result is compatible with the relation detected between renal clearance and ADMA and  
178 SDMA levels in the literature [3,12,20,24].

179 Since ADMA levels might overlap in healthy people and patients, it is advised that L-  
180 arginine/ADMA ratio should be used instead [3,5]. In our study mean L-arginine levels and L-  
181 arginine/ADMA ratio of healthy control and diabetic subjects were compatible with the  
182 literature.

183 There are three reports in the literature about how regular exercise effects ADMA.  
184 Mittermayer et a [14] studied type 1 DM patients and found that ADMA decreased after regular  
185 exercise and therefore L-arginine/ADMA ratio increased similar to our study. The effect  
186 continued months after cessation of exercise. There was no healthy control group in that study.

187 In the study done by Gomes et al [27], 18 patients with metabolic syndrome attended a 3  
188 month exercise programme. Age and gender matched healthy control subjects did not participate  
189 in exercise programme. The groups did not differ in terms of ADMA level. After 3 months  
190 ADMA increased in the non-exercise group, while decreased significantly in the exercise group.  
191 Exercise may induce NO production via increased blood flow and vascular stress. The increased  
192 NO level was proposed to be responsible for the decrease of ADMA after exercise. OGTT was  
193 not done to exclude DM and plasma glucose levels of the exercise group were at the range of  
194 prediabetes. Mean age of the enrollees were higher than our subjects. ADMA levels as high as  
195 1.5 µM even in healthy group were above the expected normal levels. L-arginine/ADMA ratio  
196 was not stated. This may be due to ELISA method used.

197 In a study done by Niebauer et al [28], after an 8 week exercise programme ADMA and  
198 L-arginine measured by HPLC increased, while SDMA and L-arginine/ADMA ratio decreased  
199 in patients with chronic heart failure and increased in the healthy group. These changes were not  
200 statistically significant. It was suggested that ADMA compensatorily increased due to enhanced

201 NOS enzyme activity in heart failure. Mean age of the enrollees were higher than ours. There is  
202 no data in that report about glucose metabolism status (fasting plasma glucose or OGTT).

203 In our study ADMA decreased in the exercising healthy control group. The decrease was  
204 more prominent after first exercise session. The decrease after one month of regular exercise was  
205 less. On the contrary ADMA increased after first exercise session in the exercising DM group.  
206 ADMA decreased after one month of regular exercise although remained higher than the initial  
207 levels. SDMA decreased to a greater extent in healthy control group than in DM group after one  
208 month of regular exercise. L-arginine increased in the exercising healthy control group, while it  
209 increased in the exercising DM group. Although changes in ADMA, SDMA, and L-arginine  
210 levels did not reach statistical significance, the ratios between them (L-arginine/ADMA and L-  
211 arginine/SDMA) differed significantly. The most prominent increase observed in exercising  
212 healthy control subjects suggested that healthy control subjects benefited exercise the most. In  
213 DM, there may be other protective pathways besides NOS system components (ADMA, SDMA,  
214 and L-arginine) that are operational in the effect of exercise on hypertension, weight, body fat  
215 composition, and glycemic control.

216 Our exercising subjects did not experience significant weight loss at the end of one month  
217 exercise programme. Similarly in a meta-analysis of 12 studies done by Boulé et al [2] revealed  
218 no significant weight loss in type 2 DM patients admitted to an aerobic exercise programme  
219 more than 8 weeks of duration. Boulé proposed that exercise was not done at enough frequency  
220 and for duration to provide weight loss. The author also suggested that body composition was  
221 not recorded in these studies and fat mass lost with exercise might be compensated by fat free  
222 mass. In another meta-analysis of aerobic exercise studies [29], fat mass was measured by  
223 magnetic resonance imaging and abdominal visceral fat mass decreased by 48% and abdominal  
224 subcutaneous fat mass by 18%. In our study the number of diabetic patients completing exercise  
225 programme was not large enough to conclude about the change in body composition.

226 Our study is the first one that evaluated the effect of regular exercise on ADMA, SDMA,  
227 and L-arginine levels in type 2 DM. We suggest that L-arginine/ADMA and L-arginine/SDMA  
228 ratios should be used to evaluate NOS system instead of their absolute levels. Although the  
229 components of the NOS system plays a master role in regressing atherosclerosis in exercising  
230 healthy people, its importance decreases in DM. Exercise programmes of longer duration and a  
231 larger number of diabetic patients are needed to reach a final decision.

232

## 233 REFERENCES

234 1. American Diabetes Association. Nutrition recommendations and interventions for diabetes: a

- 235 position statement of the American Diabetes Association. *Diabetes Care* 2007;31:48-65.
- 236 2. Boulé NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic  
237 control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical  
238 trials. *JAMA* 2001;286(10):1218–27.
- 239 3. Bode-Böger SM, Scalera F, Ignarro LJ. The l-arginine paradox: Importance of the l-  
240 arginine/asymmetrical dimethylarginine ratio. *Pharmacol Ther* 2007;114(3):295-306.
- 241 4. Kawata T, Daimon M, Hasegawa R, Teramoto K, Toyoda T, Sekine T et al. Effect of  
242 angiotensin-converting enzyme inhibitor on serum asymmetric dimethylarginine and  
243 coronary circulation in patients with type 2 diabetes mellitus. *Int J Cardiol.* 2009 Feb  
244 20;132(2):286-8.
- 245 5. Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous  
246 inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992;339(8793):572–5.
- 247 6. Bode-Böger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M et  
248 al. Symmetrical dimethylarginine: a new combined parameter for renal function and extent of  
249 coronary artery disease. *J Am Soc Nephrol* 2006;17:1128–34.
- 250 7. Blardi P, de Lalla A, Pieragalli D, de Franco V, Meini S, Ceccatelli L et al. Effect of iloprost  
251 on plasma asymmetric dimethylarginine and plasma and platelet serotonin in patients with  
252 peripheral arterial occlusive disease. *Prostaglandins Other Lipid Mediat* 2006;80(3-4):175–  
253 82.
- 254 8. Böger RH. Asymmetric dimethylarginine (ADMA) and cardiovascular disease: insights from  
255 prospective clinical trials. *Vascular Medicine* 2005;10(2)19-25.
- 256 9. Oguz A, Uzunlulu M, Yorulmaz E, Yalçın Y, Hekim N, Fici F. Effect of nebivolol and  
257 metoprolol treatments on serum asymmetric dimethylarginine levels in hypertensive patients  
258 with type 2 diabetes mellitus. *Anadolu Kardiyol Derg* 2007;7(4):383-7.
- 259 10. Abbasi F, Asagmi T, Cooke JP, Lamendola C, McLaughlin T, Reaven GM et al. Plasma  
260 concentrations of asymmetric dimethylarginine are increased in patients with type 2 diabetes  
261 mellitus. *Am J Cardiol* 2001;88(10):1201-3.
- 262 11. Sugai M, Ohta A, Ogata Y, Nakanishi M, Ueno S, Kawata T et al. Asymmetric  
263 dimethylarginine (ADMA) in the aqueous humor of diabetic patients. *Endocrine Journal*

- 264 2007;54(2):303-9.
- 265 12. Malecki MT, Undas A, Cyganek K, Mirkiewicz-Sieradzka B, Wolkow P, Osmenda G et al.  
266 Plasma asymmetric dimethylarginine (ADMA) is associated with retinopathy in type 2  
267 diabetes mellitus. *Diabetes Care* 2007;30(11):2899-901.
- 268 13. Fleck C, Schweitzer F, Karge E, Busch M, Stein G. Serum concentrations of asymmetric  
269 (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases.  
270 *Clin Chim Acta* 2003;336(1-2):1-12.
- 271 14. Mittermayer F, Pleiner J, Krzyzanowska K, Wiesinger GF, Francesconi M, Wolzt M. Regular  
272 physical exercise normalizes elevated asymmetrical dimethylarginine concentrations in  
273 patients with type 1 diabetes mellitus. *Wien Klin Wochenschr* 2005;117(23-24):816-20.
- 274 15. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes*  
275 *Care* 2008;31(1):55-60.
- 276 16. World Health Organization. Physical status: the use and interpretation of anthropometry:  
277 report of a WHO expert committee. *World Health Organ Tech Rep Ser* 1995;854:1-452.
- 278 17. Matthews DR, Hosker JR, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis  
279 model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and  
280 insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.
- 281 18. Teräslinna P, Ismail AH, MacLeod DF. Nomogram by Astrand and Ryhming as a predictor  
282 of maximum oxygen intake. *Journal of Applied Physiology* 1966;21:513-5.
- 283 19. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PA. Determination of arginine, asymmetric  
284 dimethylarginine, and symmetric dimethylarginine in human plasma and other biological  
285 samples by high-performance liquid chromatography. *Anal Biochem* 2002;303:131-7.
- 286 20. Päivä H, Lehtimäki T, Laakso J, Ruukonen I, Rantalaiho V, Wirta O et al. Plasma  
287 concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic  
288 control and glomerular filtration rate but not with risk factors of vasculopathy. *Metabolism*  
289 2003;52(3):303-7.
- 290 21. Yamagishi S, Ueda S, Nakamura K, Matsui T, Okuda S. Role of asymmetric  
291 dimethylarginine (ADMA) in diabetic vascular complications. *Curr Pharm Des*  
292 2008;14(25):2613-8.

- 293 22. Surdacki A, Stochmal E, Szurkowska M, Bode-Böger SM, Martens-Lobenhoffer J, Stochmal  
294 A et al. Nontraditional atherosclerotic risk factors and extent of coronary atherosclerosis in  
295 patients with combined impaired fasting glucose and impaired glucose tolerance. *Metabolism*  
296 2007;56(1):77–86.
- 297 23. Yamagishi S, Ueda S, Okuda S. A possible involvement of crosstalk between advanced  
298 glycation end products (AGEs) and asymmetric dimethylarginine (ADMA), an endogenous  
299 nitric oxide synthase inhibitor in accelerated atherosclerosis in diabetes. *Med Hypotheses*  
300 2007;69(4):922-4.
- 301 24. Krzyzanowska K, Mittermayer F, Shnawa N, Hofer M, Schnabler J, Etmüller Y et al.  
302 Asymmetrical dimethylarginine is related to renal function, chronic inflammation and  
303 macroangiopathy in patients with Type 2 diabetes and albuminuria. *Diabet Med*  
304 2007;24(1):81-6.
- 305 25. Makino H, Doi K, Hiuge A, Nagumo A, Okada S, Miyamoto Y et al. Impaired flow-  
306 mediated vasodilatation and insulin resistance in type 2 diabetic patients with albuminuria.  
307 *Diabetes Research and Clinical Practice* 2008;79(1):177–82.
- 308 26. Yasuda S, Miyazaki S, Kanda M, Goto Y, Suzuki M, Harano Y et al. Intensive treatment of  
309 risk factors in patients with type-2 diabetes mellitus is associated with improvement of  
310 endothelial function coupled with a reduction in the levels of plasma asymmetric  
311 dimethylarginine and endogenous inhibitor of nitric oxide synthase. *Eur Heart J*  
312 2006;27(10):1159–65.
- 313 27. Gomes VA, Casella-Filho A, Chagas ACP, Tanus-Santos JE. Enhanced concentrations of  
314 relevant markers of nitric oxide formation after exercise training in patients with metabolic  
315 syndrome. *Nitric Oxide* 2008;19(4):345-50.
- 316 28. Niebauer J, Clark AL, Webb-Peploe KM, Böger R, Coats AJS. Home-based exercise training  
317 modulates pro-oxidant substrates in patients with chronic heart failure. *Eur J Heart Fail*  
318 2005;7(2):183–8.
- 319 29. Boulé NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of  
320 structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus.  
321 *Diabetologia* 2003;46(8):1071–81.



324 **Table 1. Clinical features and laboratory values of the attendees.**

325

	Non-exercising controls (N=25)		Exercising controls (N=19)		Non-exercising diabetics (N=22)		Exercising diabetics (N=14)		P
Age*	40.8±9.3		30.7±5.7		50.5±7.9		47.3±10.7		<0.0001
Smoking	5		7		6		3		0.61
Menopause status	7		1		15		8		0.50
TSH (mIU/L)	1.90±1.05		2.13±1.32		2.01±1.2		2.25±1.76		0.72
Total cholesterol (mg/dL)	192±42		175±28		199±45		186±38		0.11
TG* (mg/dL)	115±41		107±46		142±85		127±45		0.03
LDL (mg/dL)	119±40		105±28		128±38		110±30		0.31
HDL* (mg/dL)	51±12		49±13		42±8		48±10		0.02
HbA1c (%)					7.58±1.53		7.67±1.18		0.42
Ccr (mL/min)					85.59±30.96		92.82±16.63		0.54
Ccr group**					1:n=14, 2:n=5, 3:n=6		1:n=5, 2:n=6, 3:n=3		0.43
MAU					9 (%40)		2 (%14)		0.09
VO <sub>2max</sub> * (mL/kg/min)			34.4±8.9				17.4±3.4		<0.0001
Fat free mass (kg)			48.5±5.1				47.1±5.9		0.46
Fat mass* (%)			25.6±7.8				41.0±6.2		<0.0001
	<b>Initial</b>	<b>1<sup>st</sup> month</b>	<b>Initial</b>	<b>1<sup>st</sup> month</b>	<b>Initial</b>	<b>1<sup>st</sup> month</b>	<b>Initial</b>	<b>1<sup>st</sup> month</b>	
Waist* (cm)	95.8±10.9	96.05±11.7	88.5±11.5	87.7±10.7	99.5±8.5	100.3±10.0	109.0±10.8	112.6±12.0	<0.0001,0.001
WHR	0.90±0.05	0.91±0.05	0.90±0.07	0.90±0.06	0.92±0.07	0.93±0.07	0.95±0.07	0.95±0.06	0.09,0.09
BMI* (kg/m <sup>2</sup> )	29.9±5.1	29.8±11.7	25.6±4.7	25.01±4.4	33.0±4.9	32.1±4.3	35.4±6.3	38.3±8.3	<0.0001, <0.0001
HOMAR					4.5±4.2	4.7±1.8	3.9±1,1	8.6±9.9	0.002, 0.007

326 **WHR:** Waist/hip ratio, **TG:** Triglyceride, **Ccr:** Creatinine clearance, **MAU:** Microalbuminuria, **HbA1c:** Hemoglobin A1c, **HOMAR:** Homeostatic model assessment of  
327 resistance. \* p<0.05, DM vs control group. \*\*Ccr group: 1=>90 mL/min, 2=60-90 mL/min, 3=30-60 mL/min.

328 **Table 2. Measurements at three time-points. ADMA, SDMA, and L-arginine values are**  
 329 **expressed in micromolar units ( $\mu\text{M}$ ).**  
 330

	<b>Non-exercising controls</b>	<b>Exercising controls</b>	<b>Non-exercising Diabetics</b>	<b>Exercising diabetics</b>
<b>Initial</b>				
ADMA	0.51 $\pm$ 0.18	0.52 $\pm$ 0.14	0.56 $\pm$ 0.20	0.56 $\pm$ 0.12
SDMA	0.40 $\pm$ 0.17	0.40 $\pm$ 0.09	0.42 $\pm$ 0.15	0.41 $\pm$ 0.20
Arginine	65.1 $\pm$ 23.6	68.8 $\pm$ 29.7	70.0 $\pm$ 30.2	82.3 $\pm$ 53.0
Arginine/ADMA	137.9 $\pm$ 56.7	128.9 $\pm$ 36.0	128.9 $\pm$ 43.5	139.1 $\pm$ 63.5
Arginine/SDMA	199.8 $\pm$ 155.3	168.2 $\pm$ 54.3	182.7 $\pm$ 84.2	158.8 $\pm$ 32.4
ADMA/SDMA	1.38 $\pm$ 0.49	1.31 $\pm$ 0.25	1.40 $\pm$ 0.36	1.30 $\pm$ 0.50
<b>First exercise session</b>				
ADMA	-	0.47 $\pm$ 0.23	-	0.58 $\pm$ 0.18
SDMA	-	0.37 $\pm$ 0.09	-	0.37 $\pm$ 0.18
Arginine	-	83.3 $\pm$ 40.6	-	87.2 $\pm$ 34.5
Arginine/ADMA	-	171.7 $\pm$ 59.7	-	152.8 $\pm$ 49.9
Arginine/SDMA	-	221.2 $\pm$ 91.4	-	238.2 $\pm$ 129.4
ADMA/SDMA	-	1.28 $\pm$ 0.23	-	1.48 $\pm$ 0.37
<b>First month</b>				
ADMA	0.55 $\pm$ 0.12	0.46 $\pm$ 0.11	0.53 $\pm$ 0.18	0.57 $\pm$ 0.10
SDMA	0.43 $\pm$ 0.14	0.36 $\pm$ 0.11	0.41 $\pm$ 0.12	0.50 $\pm$ 0.20
Arginine	72.9 $\pm$ 26.5	92.9 $\pm$ 49.3	65.6 $\pm$ 31.3	69.0 $\pm$ 24.4
Arginine/ADMA*	136.3 $\pm$ 51.4	<b>187.9<math>\pm</math>58.1</b>	122.1 $\pm$ 49.6	119.0 $\pm$ 35.8
Arginine/SDMA**	181.0 $\pm$ 70.9	<b>248.4<math>\pm</math>91.6</b>	166.1 $\pm$ 71.0	156.9 $\pm$ 86.9
ADMA/SDMA	1.33 $\pm$ 0.24	1.31 $\pm$ 0.25	1.34 $\pm$ 0.36	1.26 $\pm$ 0.40

331 \*First month vs initial values:  
 332 Exercising controls vs. Non-exercising controls, p= 0.004  
 333 Exercising controls vs. Non-exercising diabetics, p=0.022  
 334 Exercising controls vs. exercising diabetics, p=0.001  
 335 \*\*First month vs initial values:  
 336 Exercising controls vs. Non-exercising controls p=0.017  
 337