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Naringin prevents bone loss in a rat model of type 1 Diabetes mellitus

M. Rivoira[a,1], V. Rodríguez[a,1], G. Picotto[a], R. Battaglino[b], N. Tolosa de Talamoni[a,*]

[a] Laboratorio "Dr. Cañas", Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, INICSA (CONICET-Universidad Nacional de Córdoba), Córdoba, Argentina

[b] Department of Physical Medicine and Rehabilitation, School of Medicine, University of Colorado, Aurora, USA

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ABSTRACT

The aim of this work was to know whether naringin (NA) could prevent the bone complications in a model of streptozotocin (STZ) induced diabetes. Rats were divided in: 1) controls, 2) STZ-rats, 3) STZ-rats treated with 40 mg NA/kg, and 4) STZ-rats treated with 80 mg NA/kg. BMD and BMC were performed by DEXA. Bone histomorphometry and histology as well as TRAP staining were done in tibia. Osteocalcin (OCN) was determined in bone and serum. Glutathione content and SOD and catalase activities were assayed in bone marrow from femur. The data showed that NA80 increased the BMD and BMC from the long bones of STZ-rats. Both NA40 and NA80 normalized the trabecular number and the trabecular separations. An increase in the number of adipocytes and TRAP(+) cells in tibia from STZ-rats was blocked by NA. NA40 treatment increased the number of OCN(+) cells, but only the NA80 treatment allowed to reach the control values. NA normalized the SOD and catalase activities in bone marrow of femur from STZ-rats. In conclusion, NA avoids alterations in the physical properties and microstructure of bone from STZ-rats probably by stimulation of osteoblastogenesis, inhibition of the osteoclastogenesis and adipogenesis *via* blocking the oxidative stress.

1. Introduction

The impact of Diabetes mellitus (D. m.) on mineral metabolism and bone fragility are not yet totally appreciated [1]. Despite the link between type 1 D.m. and osteoporosis was detected decades ago, this issue has gained attention in recent years [2], maybe in part due to the recognition that bone disorders alter significantly life quality. The risk of fragility fractures is augmented either in type 1 D.m. or type 2 D.m. patients [3]. However, the low bone mineral density (BMD), a risk factor of fragility fracture, is detected in type 1 D.m., whereas in type 2 D.m. the BMD has been found to be normal, low or even high as compared to that of healthy people [4,5]. Insulin treatment is another risk factor for falls and fractures in diabetic patients, as a consequence of increased rate of hypoglycaemic episodes [4]. Certain other medications for the D.m. such as thiazolidinediones and SGTL2 inhibitors [6–8] could also contribute to deteriorating the diabetic bones because of their direct impact on bone and mineral metabolism.

Since histomorphometry evaluates accurately bone turnover, which is rarely undertaken in humans because requires a bone biopsy, animal models become necessary [9]. Streptozotocin (STZ) induced diabetes in mice and rats constitute classical models of type 1 D.m., in which lots of data were obtained increasing the knowledge about the pathophysiology of this disease. Although there is no a conclusive model of bone fragility in D.m., some cellular and molecular mechanisms have been observed. Among them, it has been detected that D.m. is associated with low bone turnover and alteration in bone material properties and in the microstructure, mainly when there are microvascular complications [10]. A decrease in the number and activity of osteoblasts (OB), in the percentage of osteoid area and in the rate of mineral apposition was demonstrated [11]. D.m. also induces lipid accumulation in the bone marrow of long bones, leading to expansion of the bone marrow cavity and reduction in the bone cortical region [12]. With regard to osteoclasts (OC), different studies have reported that their activities in diabetic animals are normal [13], decrease [14] or increase [15]. Maycas et al. [16] have recently found increased apoptosis of osteocytes and expression of the osteocyte-derived bone formation inhibitor Sost/sclerostin in mice with type 1 D.m. The proposed pathophysiological mechanisms of bone disease in D.m. include hyperglycaemia, oxidative

Abbreviations: D.m, Diabetes mellitus; BMD, bone mineral density; STZ, streptozotocin; OB, osteoblasts; OC, osteoclasts; NA, naringin; BMC, bone mineral content; DXA, dual energy X-ray absorptiometry; HE, hematoxylin-eosin; TRAP, tartrate-resistant acid phosphatase; OCN, osteocalcin; GSH, glutathione; CAT, catalase; ECLIA, electro-chemiluminescence; SOD, superoxide dismutase

* Corresponding author. Cátedra de Bioquímica y Biología Molecular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Pabellón Argentina, 2do. Piso, Ciudad Universitaria, 5000, Córdoba, Argentina.

E-mail addresses: ntolosatalamoni@yahoo.com.ar, ntolosa@biomed.fcm.unc.edu.ar (N. Tolosa de Talamoni).

[1] Have equally contributed.

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Table 1
General characteristics from control, diabetic (STZ) and diabetic rats treated with naringin 40 (N40) and 80 (N80) mg/kg b.w.

	C	STZ	STZ + N40	STZ + N80
Body weight (g)	266.80 ± 7.00	179.80 ± 7.90*	179.60 ± 14.10*	185.70 ± 15.10*
Serum glucose (mg/dL)	157.00 ± 4.70	444.60 ± 31.40*	449.00 ± 35.50*	411.70 ± 19.80*
HbA_{1c} (%)	8.00 ± 0.30	11.40 ± 0.60*	11.70 ± 0.30*	11.40 ± 0.40*
Insulin (ng/mL)	1.59 ± 0.03	0.40 ± 0.03*	0.45 ± 0.02*	0.44 ± 0.02*
Serum Ca (mg/dL)	9.92 ± 0.56	9.96 ± 0.44	10.12 ± 0.26	9.55 ± 0.23
Serum P (mg/dL)	5.33 ± 0.22	5.47 ± 0.23	5.59 ± 0.31	5.51 ± 0.24
$25(OH)D_3$ (ng/mL)	10.08 ± 0.76	4.21 ± 0.53*	3.80 ± 0.26*	3.20 ± 0.07*
Osteocalcin (ng/mL)	21.28 ± 1.28	3.78 ± 0.31‡	5.65 ± 0.26‡	17.43 ± 1.33

Values are expressed as means ± S.E. from 8 rats for each experimental condition. C: control; STZ: streptozotocin induced diabetic rats (60 mg/kg b.w.), STZ + N40 and STZ + N80: diabetic rats treated with naringin (40 mg/kg and 80 mg/kg b.w. respectively). Naringin was subcutaneously administered from the third day until the 30th day after STZ injection. *$p < 0.001$ *vs* control; ‡$p < 0.001$ *vs* control and STZ + N80.

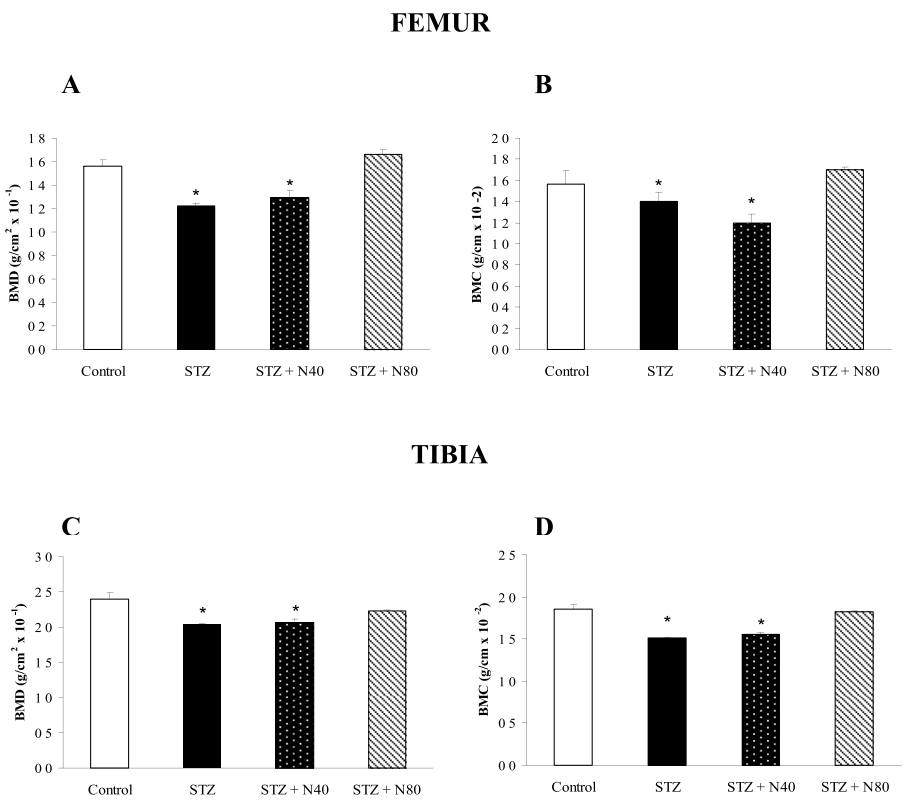

Fig. 1. Bone mineral density (BMD) (**A, C**) and bone mineral content (BMC) (**B, D**) of rat distal femur (**A, B**) and proximal tibia (**C, D**). Values are expressed as means ± S.E. from 7 rats for each experimental condition. STZ: streptozotocin induced diabetic rats (60 mg/kg b.w.), STZ + N40 and STZ + N80: diabetic rats treated with naringin (40 mg/kg and 80 mg/kg b.w. respectively). *$p < 0.001$ *vs* control and STZ + N80.

Table 2
Histomorphometric parameters of proximal tibia from control, diabetic (STZ) and diabetic rats treated with naringin 40 (STZ + N40) and 80 (STZ + N80) mg/kg b.w.

	C	STZ	STZ + N40	STZ + N80
BV/TV (%)	25.02 ± 0.86	12.70 ± 1.28*	20.80 ± 0.76#	18.03 ± 0.72#
Tb. Th (μm)	45.26 ± 1.92	36.59 ± 0.90#	38.54 ± 0.64#	38.64 ± 0.71#
Tb. N (1/mm. 10^{-3})	5.50 ± 0.17	3.30 ± 0.25*	5.40 ± 0.15	5.00 ± 0.09
Tb. Sp (μm)	134.06 ± 5.16	280.20 ± 16.34*	151.31 ± 4.67	169.63 ± 4.88

Values are expressed as means ± S.E. from 8 rats for each experimental condition. C: control; STZ: streptozotocin induced diabetic rats (60 mg/kg b.w.), STZ + N40 and STZ + N80: diabetic rats treated with naringin (40 mg/kg and 80 mg/kg b.w. respectively). BV/TV (%): bone volume, Tb. Th (μm): trabecular thickness, Tb. N (1/mm): trabecular number, Tb. Sp (μm): trabecular separation. *$p < 0.05$ *vs* control, STZ + N40 and STZ + N80; #$p < 0.05$ *vs* control.

stress, increase in advanced glycation endproducts that alter collagen properties and in the marrow adipogenesis, and release of inflammatory factors, among others [4].

In the last years a great interest has aroused on hypoglycemic agents isolated from natural sources such as bioflavonoids because they are considered to be less toxic, with fewer side effects than those from synthetic sources [17]. Naringin (NA, (4′,5,7-trihydroxy flavonone-7-rhamnoglucoside) is an abundant flavonoid present in citrus fruits with interesting biological and/or pharmacological actions due to its antioxidant, antiapoptotic and anti-inflammatory properties [18]. A protective effect of NA on the activities of the antioxidant enzymes in diabetic animals has been reported [19]. It has also been observed that NA improves ketoacidosis and lipid peroxidation [20] and ameliorates cardiac hypertrophy by reducing oxidative stress and inactivating c-Jun nuclear kinase-1 protein in type 1 Diabetes rat model [21]. So far, there is no information about the effect of NA on bone metabolism in diabetic