Published online www.wanfangdate.com.cn doi:10.3969/j.issn.1006-7108.2014.03.004

·论著·

2 型糖尿病对小鼠骨代谢的影响

徐飞 董永辉 黄鑫 郭风劲 陈安民 黄仕龙*
华中科技大学同济医学院附属同济医院骨科,武汉 430030

中图分类号:R587 **文献标识码**:A **文章编号**:1006-7108(2014)03-0238-04

摘要:**目的** 观察 2 型糖尿病小鼠的骨代谢及骨微结构的特点。**方法** 采用雄性 KK/Upj-Ay/J 小鼠(自发性 2 型糖尿病模型小鼠)10 只作为实验组,同时选用 10 只雄性 C57BL/6 小鼠作为对照组。两组小鼠均给予常规饲料喂养,确认 KK/Upj-Ay/J 小鼠发病,继续饲养 12 周后处死所有小鼠,测定血清骨碱性磷酸酶(BALP)及抗酒石酸酸性磷酸酶(TRAP)活性,并运用 Micro-CT 分析小鼠胫骨微结构定量参数。**结果** 与对照组相比,2 型糖尿病小鼠血清 BALP 活性明显下降(分别为对照组:0.029 ± 0.003 μU/min,T2DM 组:0.014 ± 0.003 μU/min,$P<0.05$),血清 TRAP 活性明显升高(分别为对照组:0.513 ± 0.034 U/L,T2DM 组:0.701 ± 0.054 U/L,$P<0.05$),胫骨平台处骨密度明显下降(对照组:810.000 ± 21.000 mg/cm^3,T2DM 组:709.000 ± 18.000 mg/cm^3,$P<0.05$)。**结论** 2 型糖尿病小鼠的骨吸收加快而骨形成不足,导致其骨量下降及骨折风险增大。

关键词:2 型糖尿病;骨代谢;骨量减少

Effect of type 2 diabetes mellitus on bone metabolism: an in vivo study

XU Fei, DONG Yonghui, HUANG Xin, GUO Fengjin, CHEN Anmin, HUANG Shilong
Department of Orthopedics, Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430030, China
Corresponding author: HUANG Shilong, Email: doctorhsl@gmail.com

Abstract: **Objective** To observe the effect of type 2 diabetes mellitus (T2DM) on bone metabolism and bone microstructure in mice. **Methods** Ten male KK/Upj-Ay/J mice, a mouse model of T2DM, were selected as experimental group, and 10 male C57BL/6 mice were selected as control group. The mice in both groups were fed with routine fodder. After the confirmation of T2DM in KK/Upj-Ay/J mice, all mice were fed for another 12 weeks and then executed. The activity of serum bone alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase (TRAP) were detected. The parameters of the tibia were analyzed using micro-CT. **Results** Compared with those in control group, the activity of serum BALP in T2DM mice decreased significantly (control group: 0.029 ± 0.003 μU/min; T2DM group: 0.014 ± 0.003 μU/min, $P<0.05$), while the activity of serum TRAP increased significantly (control group: 0.513 ± 0.034 U/L; T2DM group: 0.701 ± 0.054 U/L, $P<0.05$). The bone mineral density of the tibial plateau decreased significantly in T2DM group compared with that in control group (control group: 810.000 ± 21.000 mg/cm^3; T2DM group: 709.000 ± 18.000 mg/cm^3, $P<0.05$). **Conclusion** The bone resorption in mice with T2DM accelerates while the bone formation is deficient, resulting in osteopenia and increased risk of bone fracture.

Key words: Type 2 diabetes mellitus; Bone metabolism; Osteopenia

随着社会的发展,2 型糖尿病(type 2 diabetes mellitus,T2DM)的发病率越来越高。2 型糖尿病能影响肾脏,周围神经及周围血管等。同时,骨骼也是糖尿病慢性并发症的受累器官[1]。随着 2 型糖尿病发病率的升高,与之相关的骨质疏松症的发病也在升高,但在 2 型糖尿病是否导致骨流失的问题上,大家有较大争论[2-4]。

我们选用一种自发性 2 型糖尿病模型小鼠(KK/Upj-Ay/J 小鼠),本模型小鼠于 8 周龄开始,自发性发生 2 型糖尿病。为除外雌激素水平对骨代谢的影响,我们选用的所有小鼠均为雄性。通过测定血清学指标与骨参数,我们可以了解 2 型糖尿病对骨代谢的影响。

基金项目:国家自然科学基金资助项目(81070691)
* 通讯作者:黄仕龙,Email:doctorhsl@gmail.com

1 材料与方法

1.1 实验动物

10 只 8 周龄雄性 C57BL/6 小鼠及 10 只 8 周龄 KK/Upj-Ay/J 小鼠(自发性 2 型糖尿病模型小鼠)购于北京华阜康公司。实验动物饲养于华中科技大学动物实验中心,以常规饲养喂养,实行 12 小时昼夜循环。

1.2 实验动物处理

两组小鼠常规饲养 2 周后,利用血糖仪(Bayer, USA)采用剪尾取血法测定小鼠血糖,每 3 天测定一次,连续两次血糖高于 200mg/dl,认为实验组小鼠发生 2 型糖尿病。确认实验组小鼠罹患 2 型糖尿病后,两组小鼠继续饲养 12 周。12 周后,胸腔穿刺采集两组小鼠血液,所取得血液离心后取血清置于-20℃低温冰箱中保存。同时,颈椎脱臼处死所有小鼠,收集小鼠左侧胫骨,于 4% 多聚甲醛溶液中固定保存。

1.3 血清指标

利用 ELISA 法测定两组小鼠血清碱性磷酸酶(Bone-specific alkaline phosphatase, BALP)及血清抗酒石酸酸性磷酸酶(tartrate-resistant acid phosphatase 5b, TRAP5b)水平。BALP 为成骨细胞分泌的一种特异性蛋白,其血清学水平与成骨细胞代谢相关,代表机体的骨形成水平,TRAP 5b 为破骨细胞分泌的一种特异性蛋白,其血清学水平与机体破骨细胞代谢相关,代表机体的骨吸收水平[5]。BALP ELISA 试剂盒购自美国 sigma 公司,TRAP 5b ELISA 试剂盒购自英国 IDS 公司,血清蛋白水平测定方法依照试剂盒的操作方法进行。

1.4 micro-CT 检测

将收集的小鼠胫骨用 micro-CT(μCT-50, Scanco Medical)进行定量骨参数分析。胫骨骨质测定条件为:测定电压 = 70 kV, 电流 = 114 μA, 层厚 = 10 μm, 检测指标为:骨体积, 骨小梁数, 骨小梁厚度及骨密度(bone mineral density, BMD)。

1.5 统计学处理

应用 SPSS12.1 统计软件包进行统计学分析,计量资料用 $\bar{x} \pm s$ 表示,两组间均数比较采用 t 检验,多组间均数比较采用单因素方差分析,$P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 血清学结果

BALP 为成骨细胞分泌的一种特异性蛋白,其血清学水平与成骨细胞代谢相关,代表机体的骨形成水平;TRAP-5b 为破骨细胞分泌的一种特异性蛋白,其血清学水平与机体破骨细胞代谢相关,代表机体的骨吸收水平。我们在确定小鼠患病后 12 周观察小鼠的血清学指标,其结果为:与正常对照组小鼠相比,实验动物(T2DM)组血清 BALP 水平明显下降(分别为对照组:0.029 ± 0.003μU/min, T2DM 组:0.014 ± 0.003 μU/min, $P < 0.05$),血清 TRAP 水平明显升高(分别为对照组:0.513 ± 0.034U/L, T2DM 组:0.701 ± 0.054 U/L, $P < 0.05$)(表 1)。

表 1 血清骨代谢相关指标($\bar{x} \pm s$, $n = 10$)

Table 1 The serum bone metabolism markers ($\bar{x} \pm s$, $n = 10$)

	抗酒石酸酸性磷酸酶 5b(U/L) TRAP5b(U/L)	骨碱性磷酸酶(μU/min) BALP(μU/min)
正常组($n = 10$) normal group($n = 10$)	0.513 ± 0.034	0.029 ± 0.003
2 型糖尿病组($n = 10$) T2DM group($n = 10$)	0.701 ± 0.054[a]	0.014 ± 0.003[a]
P 值 P value	0.005	0.0006

注:TRAP5b:抗酒石酸酸性磷酸酶 5b;BALP:骨碱性磷酸酶;与对照组比较,[a]$P < 0.05$

PS: TRAP5b: tartrate-resistant acid phosphatase 5b; BALP: bone-specific alkaline phosphatase; [a]$P < 0.05$ vs normal group

2.2 定量骨参数

通过 Micro-CT 对小鼠胫骨进行扫描,扫描后进行三维重建,重建后显示 2 型糖尿病组骨小梁较对照组明显减少,见图 1。

与正常组小鼠相比,2 型糖尿病组小鼠胫骨骨小梁数目、厚度均明显降低,即单位体积内骨小梁密码下降。同时,T2DM 组小鼠胫骨骨密度较对照组明显下降(如表 2 所示)。

3 讨论

本研究选用雄性 2 型糖尿病小鼠,排除了雌激素水平对骨代谢的影响,对 2 型糖尿病相关性骨代谢改变的发生机制有重要意义。我们的实验结果显示:2 型糖尿病小鼠体内血清 BALP 水平下降,血清 BALP 为成骨细胞代谢的标志物,其表达水平下降表示小鼠体内成骨代谢水平的降低;同时,血清 TRAP 水平升高,血清 TRAP 为破骨细胞代谢的标志物,其表达水平升高表示小鼠体内溶骨代谢水平增强。从血清学指标的结果,我们可以认为 2 型糖尿

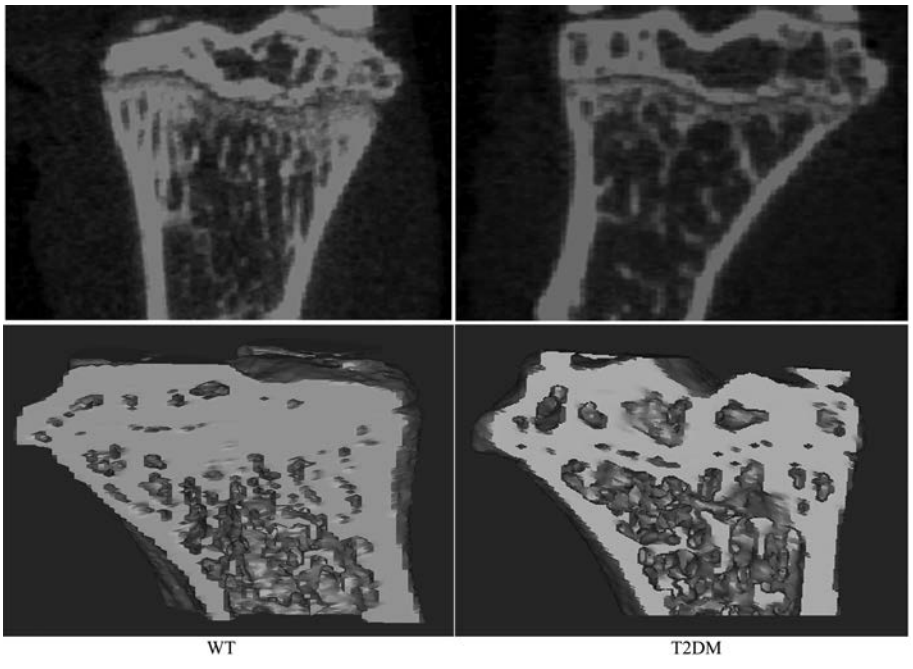

图 1 2 组胫骨平台处 CT 三维重建图像比较

Fig. 1 CT three-dimensional reconstruction images of the tibial plateau in two groups

表 2 胫骨近端骨小梁相关参数($\bar{x} \pm s, n = 10$)

Table 2 The trabecular parameters in the proximal tibia ($\bar{x} \pm s$, $n = 10$)

	骨体积(mm^3) BV(mm^3)	骨小梁数目($1/mm^3$) Tb. N. ($1/mm^3$)	骨小梁厚度(mm) Tb. Th. (mm)	骨密度(mg/cm^3) BMD(mg/cm^3)
正常组($n = 10$) normal group($n = 10$)	0.273 ± 0.040	4.148 ± 0.354	0.028 ± 0.002	810.000 ± 21.000
2 型糖尿病组($n = 10$) T2DM group($n = 10$)	0.241 ± 0.080[a]	3.342 ± 0.613[a]	0.021 ± 0.003[a]	709.000 ± 18.000[a]

注:BV:骨体积;Tb. N.:骨小梁数目;Tb. Th.:骨小梁厚度;BMD:骨密度;与对照组相比,[a]$P < 0.05$

PS: BV: bone volume; Tb. N.: trabecular number; Tb. Th.: trabecular thickness; BMD: bone mineral density; [a]$P < 0.05$ vs normal group

病会导致机体成骨代谢减弱与溶骨作用增加,从而发生骨流失。同时,Micro-CT 的扫描结果显示 2 型糖尿病小鼠的骨密度较对照组小鼠明显下降,符合骨丢失的表现。

正常骨代谢的过程为成骨细胞形成新骨和破骨细胞吸收旧骨的过程,两者处于一种平衡状态,如果这一平衡被破坏就引发了骨代谢的异常,发生骨质硬化或骨质疏松[6]。2 型糖尿病患者的骨代谢异常有多种因素导致,如高糖血症,肥胖等。有学者认为高浓度葡萄糖可以造成成骨细胞增殖减弱[7]和成骨能力减弱[8],同时,高血糖会导致机体渗透性利尿,使钙、磷排泄增加,引起血钙、磷浓度降低,低血钙会刺激甲状旁腺分泌甲状旁腺激素,从而使溶骨作用增强[9]。2 型糖尿病患者有的胰岛素抵抗的发生与肥胖有关,本研究所选的小鼠模型是一种中度肥胖小鼠。肥胖的 T2DM 患者体内有较多的脂肪组织,脂肪组织作为一种内分泌器官能分泌前炎症因子,这些炎症因子会引起骨代谢异常导致骨丢失。从细胞层面上说,脂肪细胞能减弱成骨细胞的成骨作用[10],且通过成骨细胞分泌的骨保护素(OPG)与破骨细胞生成因子(RANKL)水平来增加破骨细胞的分化[11]。

本研究采用的为年轻的 2 型糖尿病小鼠,病程相对较短。所以我们能得出这样的结论:2 型糖尿病在早期会引起骨吸收加快而骨形成不足,导致其骨量下降及骨折风险增大。

【 参 考 文 献 】

[1] Shan P F, Shan P F, Wu X P, et al. Age-related bone mineral density, osteoporosis rate and risk of vertebral fracture in

mainland Chinese women with type 2 diabetes mellitus. J Endocrinol Invest, 2011, 34(3):190-196.
[2] Räkel A, Sheehy O, Rahme E, et al. Osteoporosis among patients with type 1 and type 2 diabetes. Diabetes MeTab, 2008, 34(3):193-205.
[3] Mascitelli L, Pezzetta F. Type 2 diabetes and fracture risk. Lancet, 2006, 368(9537):732.
[4] Yaturu S, Humphrey S, Landry C, et al. Decreased bone mineral density in men with metabolic syndrome alone and with type 2 diabetes. Med Sci Monit, 2009, 15(1):CR5-9.
[5] Halleen JM. Tartrate-resistant acid phosphatase 5B is a specific and sensitive marker of bone resorption. Anticancer Res, 2003, 23(2A):1027-1029.
[6] Reginster JY, Collette J, Neuprez A, et al. Role of biochemical markers of bone turnover as prognostic indicator of successful osteoporosis therapy. Bone, 2008, 42(5):832-836.
[7] Feng Zhengping, Deng Huacong, Du Jia, et al. Lentiviral-mediated RNAi targeting p38MAPK ameliorates high glucose-induced apoptosis in osteoblast MC3T3-E1 cell line. Indian J. Exp. Biol, 2011, 49(2): 94-104.
[8] García-Hernández A, Arzate H, Gil-Chavarría I, et al. High glucose concentrations alter the biomineralization process in human osteoblastic cells. Bone, 2012, 50(1):276-288.
[9] Inaba M, Nishizawa Y, Mita K, et al. Poor glycemic control impairs the response of biochemical parameters of bone formation and resorption to exogenous 1,25-dihydroxyvitamin D3 in patients with type 2 diabetes. Osteoporosis Int, 1999, 9:525-531.
[10] Savopoulos C, C Dokos, Kaiafa G, et al. Adipogenesis and osteoblastogenesis: trans-differentiation in the pathophysiology of bone disorders. Hippokratia, 2011, 15(1): 18-21.
[11] Goto H, M Osaki, Fukushima T, et al. Human bone marrow adipocytes support dexamethasone-induced osteoclast differentiation and function through RANKL expression. Biomed Res, 2011, 32(1): 37-44.

(收稿日期:2013-03-29)

(上接第 237 页)

[4] Pierotti S, Gandini L, Lenzi A, et al. Pre-receptorial regulation of steroid hormones in bone cells: insights on glucocorticoid-induced osteoporosis. J Steroid Biochem Mol Biol, 2008,108(3-5):292-9.
[5] Leclerc N, Luppen CA, Ho VV, et al. Gene expression profiling of glucocorticoid-inhibited osteoblasts. J Mol Endocrinol, 2004, 33(1):175-93.
[6] Maurer T, Zimmermann G, Maurer S, et al. Inhibition of osteoclast generation: a novel function of the bone morphogenetic protein 7/osteogenic protein 1. Mediators Inflamm, 2012,2012: 171209.
[7] Yi SE, Daluiski A, Pederson R, et al. The type I BMP receptor BMPRIB is required for chondrogenesis in the mouse limb. Development, 2000,127(3):621-30.
[8] Wu XB, Li Y, Schneider A, et al. Impaired osteoblastic differentiation, reduced bone formation, and severe osteoporosis in noggin-overexpressing mice. J Clin Invest, 2003,112(6): 924-934.
[9] Sheng Jundong,GU Jiujun,LIU Xingyan, et al. Complex of bone morphogenetic protein 7 and fibrin protein on the healing of osteoporotic vertebral fracture. Chinese Journal of Spine and Spinal Cord, 2010,20(4):291-294.
[10] Blokhuis TJ, Buma P, Verdonschot N, et al. BMP-7 stimulates early diaphyseal fracture healing in estrogen deficient rats . J Orthop Res, 2012,30(5):720-5.
[11] Pountos I, Georgouli T, Henshaw K, et al. The effect of bone morphogenetic protein-2, bone morphogenetic protein-7, parathyroid hormone, and platelet-derived growth factor on the proliferation and osteogenic differentiation of mesenchymal stem cells derived from osteoporotic bone . J Orthop Trauma, 2010, 24(9):552-6.
[12] Zhang Y, Cheng N, Miron R, et al. Delivery of PDGF-B and BMP-7 by mesoporous bioglass/silk fibrin scaffolds for the repair of osteoporotic defects. Biomaterials, 2012, 33(28):6698-708.
[13] Spiro AS, Beil FT, Schinke T, Schilling AF, et al. Short-term application of dexamethasone enhances bone morphogenetic protein-7-induced ectopic bone formation in vivo . J Trauma, 2010,69(6):1473-80.
[14] Wang Xin-wen, He Ai-yong, Cai Yu-zhong, et al. Effect of epimedium and bone morphogenetic protein-2 on fracture healing in rabbits. Journal of Clinical Rehabilitative Tissue Engineering Research, 2011,15(11):2030-2033.
[15] Liu Hai-jiang, Wang Xiao-ping, Lin Juan, et al. The Effect of Icariin and Astragalosid I on the Proliferation and Differentiation of Bone Marrow Stromal Cells. Journal of Chinese Medicinal Materials, 2006,29(10):1062-1065.
[16] He Wei, Li Zi-li, Cui Yuan-lu, et al. Effect of icariin on the mRNA expressions of Cbfα1, BMP-2, BMP-4 in rat osteoblasts. Journal of Peking University (Health Sciences), 2009,41(6): 669-673.

(收稿日期:2013-03-25)

2型糖尿病对小鼠骨代谢的影响

作者： 徐飞， 董永辉， 黄鑫， 郭风劲， 陈安民， 黄仕龙， XU Fei， DONG Yonghui， HUANG Xin， GUO Fengjin， CHEN Anmin， HUANG Shilong

作者单位： 华中科技大学同济医学院附属同济医院骨科,武汉,430030

刊名： 中国骨质疏松杂志 ISTIC

英文刊名： Chinese Journal of Osteoporosis

年，卷(期)： 2014(3)