Effects of Fructose-induced Metabolic Syndrome and Metformin in the Skeleton of Wistar Rats

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INTRODUCTION

- Metabolic Syndrome (MS) is a heterogeneous disorder produced by different combinations of dyslipidemia, elevated blood pressure, abdominal obesity and glucose intolerance.
- MS has been associated with a reduction in bone strength and increased non-vertebral osteoporotic fractures.
- The insulin-sensitizer metformin (MET) is one of the most widely-used agents to treat metabolic disturbances related with MS. However, the effects of MET on bone metabolism are not well established.

HYPOTHESIS

MS-induced decrease in the osteogenic potential of bone marrow mesenchymal stromal cells (MSC), could alter the structure, composition and metabolism of bone tissue. Additionally, that these alterations could be prevented by oral treatment with MET.

GOALS

To investigate the effect of experimental MS and treatment with MET, on MSC osteogenic commitment, bone micro-architecture and biomechanics.

METHODS

- 38 male 12-wk-old Wistar rats were randomized into four groups (n=8-10): 1) autoclaved drinking water (**DW**) control (**Veh**), 2) Veh + oral MET (100 mg/kg BW/day) (**MET**), 3) DW with 20% fructose (**F**), and 4) DW with simultaneous F + MET treatments (**F+MET**). (**Figure 1**).
- MSC were isolated from humeri to determine their osteogenic potential *ex vivo*.
- Tibiae were excised for pQCT, and static and dynamic histomorphometric analyses.
- Femora were excised for biomechanical analyses.
- Maxillae were histologically processed to assess alveolar bone loss (ABL) at interdental molars (M)1 M2 and M2M3 .

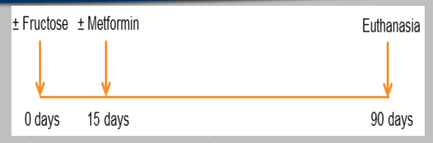

Figure 1. Experimental design

Grupo	Glucose (g/l)	Triglycerides (g/l)
Veh	2,10 +/- 0,06	1,12 +/- 0,26
MET	2,41 +/- 0,11	1,14 +/- 0,22
F	3,15 +/- 0,36 (**)	2,44 +/- 0,43 (**)
F+MET	2,37 +/- 0,13 (#)	1,48 +/- 0,22

Table 1. Metabolic results

Group	ALP (% of Veh at 0 days)	COL (% of Veh at 0 days)	MIN (% of Veh at 0 days)
Veh	381 +/- 23	259 +/- 11	100 +/- 9
MET	231 +/- 22 (***)	187 +/- 5 (**,$)	147 +/- 11 (*)
F	300 +/- 25	171 +/- 8 (**,$$)	51 +/- 4 (**,###,$$$)
F+MET	384 +/- 32 (##)	234 +/- 12	108 +/- 9

Table 2. Results for MSC osteogenic induction

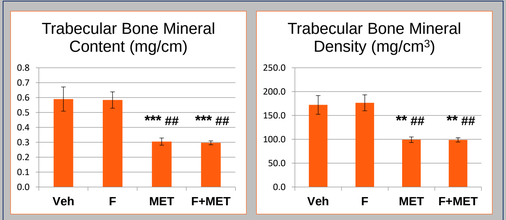

Figure 2. pQCT analysis of tibiae

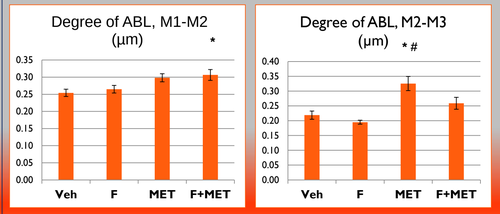

Figure 5. Measurement of vertical ABL

* p<0.05, ** p<0.01, *** p<0.001 vs Veh; # p<0.05, ## p<0.01, ### p<0.001 vs MET; $ p<0.05, $$ p<0.01, $$$ p<0.001 vs F+MET

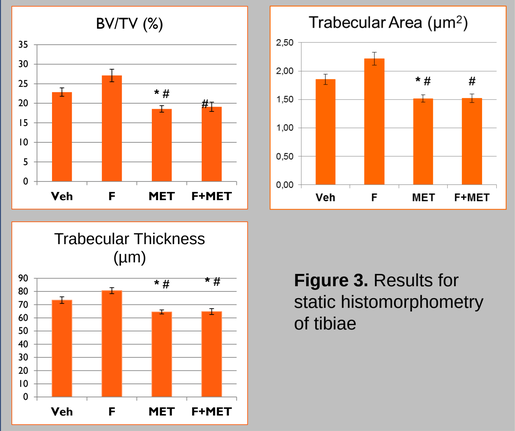

Figure 3. Results for static histomorphometry of tibiae

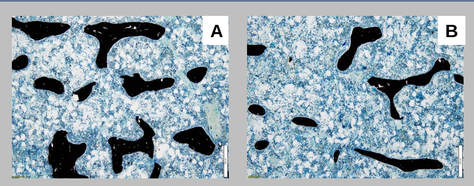

Figure 4. Proximal tibial metaphysis of Veh (A) and MET (B) groups (Von Kossa stain and Tetrachrome counterstain)

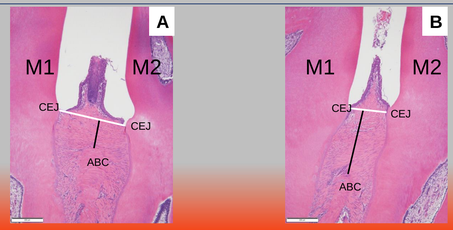

Figure 6. Alveolar bone loss (ABL) at maxillary interdental molar (M)1M2 of a Veh (A) and a MET (B) rats (H&E). ABL was calculated by measuring the distance between the cemento-enamel junction (CEJ) of 2 adjacent M and the alveolar bone crest (ABC). H&E.

SUMMARY OF RESULTS

- At 12 weeks of **F** treatment, rats had metabolic abnormalities compatible with MS that were reverted by co-treatment with **MET** (**Table 1**).
- MSC derived from **F**-treated rats had lower levels of type 1 collagen synthesis (COL), and decreased formation of mineral nodules (MIN) after 15 days of osteogenic cultures.
- **MET**-derived MSC displayed a reduction in alkaline phosphatase activity (ALP) and COL syntheses compared to **Veh**-derived MSCs (**Table 2**).
- pQCT and bone histomorphometric analyses revealed no differences in cortical and cancellous bone histomorphometirc parameters between **F**-treated and **Veh**-treated rats.
- Decreased tibial trabecular bone mineral content and density in **MET** rats compared to **Veh** rats (**Figure 2**).
- Reduced tibial BV/TV, Tb.Th and Tb area in MET-treated rats (groups **MET** and **F+MET**) compared to **Veh** rats (**Fig. 3 and 4**).
- No differences between groups were observed for biomechanical measurements, nor for cortical static and dynamic bone histomorphometric parameters.
- **F-MET** rats had greater ABL at M1M2 compared to **Veh** rats, and **MET** rats had greater ABL at M2M3 compared to **Veh** and **F**, respectively (**Fig. 5 and 6**).

CONCLUSIONS

12 weeks of **F** treatment in Wistar rats induced metabolic abnormalities characteristic of MS, but not skeletal changes. Furthermore, 10 weeks of **MET** treatment reverted the MS metabolic disturbances but, rather than protecting the skeleton, induced detrimental effects in some of the structural parameters of cancellous bone but not of cortical bone and accelerate ABL.

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