A POSITIVE DOSE-RESPONSE OF QUERCETIN ON COMPACT BONE MICROSTRUCTURE OF MALE RABBITS

Ramona Babosova1, Radoslav Omelka2, Maria Adamkovichova2, Patrik Sranko2, Michaela Racanska1, Robert Toman1, Marcela Capcarova2, Monika Martiniakova1

Address(es):
1Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Department of Zoology and Anthropology, Nábrežie mládeže 91, 949 74 Nitra, Slovak Republic.
2Masaryk University in Brno, Faculty of Medicine, Department of Anatomy, Kamencík 126/3, 625 00 Brno, Czech Republic.
3Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Resources, Department of Veterinary Sciences, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
4Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Resources, Department of Animal Physiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.

*Corresponding author: rbabosova@ufk.sk

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INTRODUCTION
Quercetin (3,3',4',5,7-pentahydroxylavone) is considered to be one of the best characterized flavonoids present in many common fruits and vegetables, beverages, olive oil, and propolis from the bee hives (Aguierre et al., 2011). Its biological activity has been investigated over the last decades with a great interest. Quercetin has anti-inflammatory, anti-allergic, antibacterial and antiapoptotic effects, as well as it protects against cardiovascular diseases, diabetes and stroke (Wein et al., 2013). However, potentially deleterious effects of the quercetin, related to its cytotoxicity and prooxidant activity have also been reported (Chen et al., 2014). According to Robaszkaiewicz et al. (2007), quercetin-induced antioxidant or prooxidant properties are largely related to its dose given to the biological system.

In general, bone is a rigid organ whose underlying composite structure and chemical composition are responsible for its functionality. Due to very active metabolism, bone microstructure can also be affected by various xenobiotics which either improve or diminish biochemical properties and strength of the bone. According to Notoya et al., 2004, quercetin should have protective effects on metabolism of this organ. Liang et al. (2011) stated a positive impact of quercetin on diabetic osteopenia of rats. Also, it stimulated proliferation and differentiation of osteoblast and MG63 osteoblast-like cells in rats. On the contrary, it decreased osteoclastogenesis of osteoclast precursor 27T-110 (Forte et al., 2016). However, Kanno et al. (2004) mention that quercetin can also induce apoptosis of MC3T3-E1 mouse calvarial osteoblasts. The same findings have also been observed in the research of Nam et al. (2008).

Although quercetin belongs to often analysed flavonoids, its effect on bone microstructure of males has not been sufficiently described yet. Therefore, our study was aimed to determine the impact of quercetin on femoral bone microstructure in 5-month-old male rabbits. Nine rabbits were intramuscularly injected with following doses of quercetin: 10 μg/kg body weight (bw) (E1 group; n=3), 100 μg/kg bw (E2 group; n=3) and 1000 μg/kg bw (E3 group; n=3) for 90 days, 3 times per week. Three rabbits without quercetin administration served as a control group. An accelerated bone resorption at the endosteal surface and intensive periosteal bone apposition were observed in all rabbits administered quercetin.

However, the most evident changes such as a thicker layer of primary vascular longitudinal bone tissue under periosteum and a lower density of secondary osteons were identified in males from the E3 group. Morphometrical evaluations of the compact bone showed significantly decreased sizes of primary osteons’ vascular canals and secondary osteons in rabbits exposed to the highest dose of quercetin. Sizes of secondary osteons were also lower in males from the E2 group. Our results demonstrate that quercetin has a positive dose-response on compact bone microstructure in male rabbits.

Keywords: bone microstructure, histology, quercetin, rabbit

ABSTRACT
Quercetin is one of the most widely distributed flavonoids in plants which should have a broad range of significant health promoting properties. Its effect on bone microstructure of males has not been sufficiently investigated. Therefore, our study was aimed to determine the impact of quercetin on femoral bone microstructure in 5-month-old male rabbits. Nine rabbits were intramuscularly injected with following doses of quercetin: 10 μg/kg body weight (bw) (E1 group; n=3), 100 μg/kg bw (E2 group; n=3) and 1000 μg/kg bw (E3 group; n=3) for 90 days, 3 times per week. Three rabbits without quercetin administration served as a control group. An accelerated bone resorption at the endosteal surface and intensive periosteal bone apposition were observed in all rabbits administered quercetin.

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MATERIAL AND METHODS
Our research was conducted on twelve 5-month-old male rabbits of Californian broiler line, which were obtained from an experimental farm of the Animal Production Research Centre in Nitra (Slovak Republic). Animals with a body weight (bw) of 4.00 ± 0.5 kg were housed individually in flat-deck wire cages under constant temperature (20 - 24 °C), humidity (55 % ± 10 %), and 12/12 h cycle of light and darkness with an access to food (feed mixture) and drinking water ad libitum. Nine adult rabbits were intramuscularly injected with following doses of quercetin (Sigma-Aldrich, Germany): 10 μg/kg bw (E1 group; n=3), 100 μg/kg bw (E2 group; n=3) and 1000 μg/kg bw (E3 group; n=3) for 90 days, 3 times per week. The doses of quercetin reflected the constant exposure of animals to quercetin in rabbit feed. Three rabbits without quercetin application served as the control group (C group). Institutional and national guidelines for the care and use of animals were followed, and all experimental procedures were approved by the State Veterinary and Food Institute of Slovak Republic, no. 3398/11-221/3 and ethics committee.

At the end of experimental period (after 90 days), all rabbits were killed and their femora were used for morphocytological analyses. Thin sections from femora were prepared according to the methodology of Martiniaikova et al. (2008). The qualitative characteristics of the compact bone were determined according to the internationally accepted classification systems of Enlow and Brown (1956) and de Rieqles et al. (1991). The quantitative parameters of the compact bone were assessed using the software Motic Images Plus 2.0 ML (Motic China Group Co., Ltd.). We measured area, perimeter, minimum and...
maximum diameters of 320 primary osteons’ vascular canals, 320 Haversian canals and 320 secondary osteons in all views (anterior, posterior, medialis and lateralis) of the thin sections.

Statistical analysis was performed using SPSS 17.0 software (SPSS Inc.; Chicago, IL, USA). All data were expressed as mean ± standard deviation. The unpaired Games-Howell’s test was used for establishing statistical significance (P<0.05) among all groups of rabbits.

RESULTS AND DISCUSSION

Primary vascular longitudinal bone tissue as a basic structural pattern of femora formed the periostal and endostal surfaces in rabbits from the C group. This type of the bone tissue was created by vascular canals that run in a direction essentially parallel to the long axis of the bone. Additionally, primary vascular radial bone tissue (formed by branching or non-branching vascular canals radiating from the marrow cavity) was found in some areas near endostal surfaces. The middle part of the compact bone consisted of irregular and/or dense Haversian bone tissues. Irregular Haversian bone tissue was characterized by an occurrence of isolated and scattered secondary osteons. For dense Haversian bone tissue, a large density of secondary osteons was typical (Figure 1a).

We identified differences in compact bone microstructure of rabbits from the E1, E2 and E3 groups (Figure 1b, c, d). Primary vascular longitudinal bone tissue was completely resorbed in some areas (mainly in anterior and posterior views) near endostal surfaces. In the other views (medialis and lateralis), a thinner layer of this tissue type was observed. Also, an intensive apposition of primary vascular longitudinal bone tissue under periostum was identified in all quercetin-exposed rabbits. However, the thicker layer of this bone tissue was found in rabbits from the E3 group (Figure 1d) which also had fewer secondary osteons when compared to the other groups.

Our results from qualitative histological analysis of male rabbits are consistent with those of several authors (Enlow and Brown, 1958; Martiniakova et al., 2006). Primary vascular longitudinal, primary vascular radial, dense Haversian and/or irregular Haversian bone tissues were found in all groups of male rabbits. However, the exposure to quercetin leads to dose-dependent changes in these characteristics which were associated with accelerated bone resorption at the endostal surface and increased periostal bone apposition. According to Ahlborg et al. (2003), excessive endostal resorption results in increased mechanical stress in the bone tissue, which consequently stimulates periostal bone formation. Deposition of bone tissue onto the periostal surface is considered to be a compensative response of the bone against endostal resorption in an effort to increase bone resistance to loss of bone strength which would lead to microfracture creation (Seeman, 2007). Generally, reactive oxygen species (ROS) are physiologically involved in the process of bone resorption. During this process, osteoclasts produce large amounts of ROS, and their excessive accumulation inhibits bone formation and stimulates further bone resorption (Back et al., 2010). However, the effect of ROS on bone cells depends on their concentration in the bone tissue (Brzoska et al., 2011). According to Rahman et al. (1992), high doses of quercetin contribute to the generation of ROS. Therefore, the thinner layer of primary vascular longitudinal bone tissue near endostal surfaces, the thicker layer of this tissue type under periostum and a lower density of secondary osteons in male rabbits from the E3 group could be associated with these aspects.

On the contrary, opposite results have been observed in female rabbits which were administered to identical doses of quercetin (Babosova et al., 2016). The most evident changes in qualitative histological characteristics of compact bone tissue were found in female rabbits exposed to the lowest dose of quercetin. It indicates that quercetin had a negative dose-response on these characteristics in females. We suppose that these variations may be associated with different growth and modeling of the femur through influence by sex-specific steroids, genetic factors and a multitude of other sources. In humans, histological qualitative bone morphology expresses a low sex-related variation. Together with a strong genetic component of qualitative traits they can be used for taxonomic identification of various species (Martiniakova et al., 2008). Remodeling of bones seems to be lower in females, since estrogens slow down bone remodeling and protect against bone loss (Martiniakova et al., 2003). Sex-specific steroids (estrogens in females and androgens in males) play an important role in bone remodeling (Bord et al., 2006; Manolagas et al., 2013). It is known that hormones do not act on bone tissue in isolation (Hogler et al., 2008). Bone tissue must integrate a variety of signals that result from mechanical loads, hormones, and a multitude of other sources. The deficit of these hormones causes changes in the production of regulatory cytokines in the bone marrow (Aydin et al., 2013), which can lead to the increased bone resorption near endostal surfaces and also increased bone formation near periostal area (Fehler et al., 2010). The results by Miodini et al. (1999) demonstrated an antagonistic effect of quercetin on the function of estrogen receptor (ER) in breast cancer cell lines MCF-7.
Krazeisen et al. (2001) described an inhibitory effect of quercetin on the 17β-hydroxysteroid dehydrogenase type 5 production which is involved in estrogen and androgen metabolisms. In connection with these facts, Santini et al. (2009) indicated that quercetin had been found to exhibit both estrogenic and anti-estrogenic actions in vitro depending on the dose. Similarly, Yuan et al. (2004) reported the dose-dependent inhibitory effect of quercetin on the expression and function of the androgen receptor (induction of enzyme c-Jun) in mice. Besides these facts, the lower density of secondary osteons in the middle part of substantia compacta of male rabbits from the E3 group leads to weakness of biomechanical properties of their bones. The results of morphometrical analysis of compact bone tissue in male rabbits from all groups are summarized in Table 1. Measured variables (area, perimeter and minimum diameter) of the primary osteons’ vascular canals were significantly decreased in males from the E3 group compared to the C group. Significant differences were also found between E1 and E3 groups (area, perimeter). Haversian canals’ values did not differ significantly among all analysed groups. On the other hand, sizes of the secondary osteons (except for maximum diameter) were significantly lower in males from the E2 and E3 groups compared to the C group.

Our results revealed significantly decreased sizes of primary osteons’ vascular canals in rabbits from the E3 group. Primary osteons’ vascular canals contain blood vessels which provide nutrition for the bone (Greenlee and Dunnell, 2010). Pries et al. (2005) found different results in female rabbits exposed to the same doses of quercetin. Significantly decreased sizes of the primary osteons’ vascular canals were observed in individuals from E1 and E2 groups. Secondary osteons were significantly decreased in all quercetin administered females. These results indicate that quercetin had not only a positive dose-response on qualitative and quantitative histological characteristics of the compact bone in female rabbits as it would be expected. A positive dose response of quercetin has been identified for sizes of primary osteons’ vascular canals and secondary osteons. On the contrary, quercetin had a negative dose-response on qualitative histological characteristics of the compact bone.

In our study, a positive dose-response of quercetin on compact bone tissue microstructure of male rabbits was demonstrated. Therefore, it can be concluded that intramuscular application of quercetin at the doses used in our studies had sex-dependent and also dose-dependent effects on compact bone microstructure in adult rabbits.

### Table 1 Morphometrical results of compact and trabecular bone tissues in male rabbits

<table>
<thead>
<tr>
<th>Measured structures</th>
<th>Group</th>
<th>N</th>
<th>Area (µm²)</th>
<th>Perimeter (µm)</th>
<th>Max. diameter (µm)</th>
<th>Min. diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vascular canals of primary osteons</td>
<td>E1 (1)</td>
<td>80</td>
<td>340.70 ± 54.93</td>
<td>66.06 ± 5.38</td>
<td>11.48 ± 1.08</td>
<td>9.48 ± 0.99</td>
</tr>
<tr>
<td></td>
<td>E2 (2)</td>
<td>80</td>
<td>331.47 ± 54.19</td>
<td>65.21 ± 5.21</td>
<td>11.33 ± 1.03</td>
<td>9.35 ± 1.07</td>
</tr>
<tr>
<td></td>
<td>E3 (3)</td>
<td>80</td>
<td>317.35 ± 51.82</td>
<td>63.80 ± 5.11</td>
<td>11.10 ± 1.00</td>
<td>9.13 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>C (4)</td>
<td>80</td>
<td>344.68 ± 50.07</td>
<td>66.43 ± 4.78</td>
<td>11.48 ± 0.99</td>
<td>9.60 ± 0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3*; 3.4*</td>
<td>1.3*; 3.4*</td>
<td>NS</td>
</tr>
<tr>
<td>Haversian canals</td>
<td>E1 (1)</td>
<td>80</td>
<td>300.42 ± 63.05</td>
<td>61.94 ± 6.63</td>
<td>10.75 ± 1.38</td>
<td>8.90 ± 1.04</td>
</tr>
<tr>
<td></td>
<td>E2 (2)</td>
<td>80</td>
<td>280.99 ± 48.66</td>
<td>60.04 ± 5.36</td>
<td>10.43 ± 1.21</td>
<td>8.63 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>E3 (3)</td>
<td>80</td>
<td>301.32 ± 56.49</td>
<td>62.20 ± 6.06</td>
<td>10.82 ± 1.37</td>
<td>8.90 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>C (4)</td>
<td>80</td>
<td>322.15 ± 65.07</td>
<td>64.25 ± 6.53</td>
<td>11.13 ± 1.35</td>
<td>9.20 ± 1.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.3*; 2.4*; 3*; 3.4*</td>
<td>1.3*; 2.4*; 3*; 3.4*</td>
<td>NS</td>
</tr>
<tr>
<td>Secondary osteons</td>
<td>E1 (1)</td>
<td>80</td>
<td>5079.12 ± 1619.61</td>
<td>259.19 ± 44.09</td>
<td>46.74 ± 10.18</td>
<td>34.28 ± 6.29</td>
</tr>
<tr>
<td></td>
<td>E2 (2)</td>
<td>80</td>
<td>4518.80 ± 2169.25</td>
<td>239.66 ± 52.11</td>
<td>43.07 ± 9.65</td>
<td>32.12 ± 8.25</td>
</tr>
<tr>
<td></td>
<td>E3 (3)</td>
<td>80</td>
<td>4629.72 ± 1888.92</td>
<td>244.67 ± 45.93</td>
<td>43.81 ± 8.79</td>
<td>32.86 ± 8.00</td>
</tr>
<tr>
<td></td>
<td>C (4)</td>
<td>80</td>
<td>5979.63 ± 2816.19</td>
<td>273.19 ± 60.51</td>
<td>47.97 ± 11.30</td>
<td>38.12 ± 9.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.4*; 3.4*</td>
<td>2.4*; 3.4*</td>
<td>NS</td>
</tr>
</tbody>
</table>

Legend: N - number of measurements; E1 group: 10 µg/kg bw; E2 group: 100 µg/kg bw; E3 group: 1000 µg/kg bw; NS - non-significant differences; P<0.05 (*);
P<0.01 (**);
P<0.001 (***).

### CONCLUSION

The results indicate that subchronic intramuscular administration of quercetin has a positive dose-response on compact bone microstructure in male rabbits. The most evident changes in both qualitative and quantitative histological characteristics were observed in males exposed to the highest dose of quercetin. On the contrary, quercetin had not only a positive dose-response on these characteristics in female rabbits as it was documented in our previous study. These results could extend our knowledge related to quercetin’s impact on bone microstructure in experimental animals of both genders.

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### REFERENCES


expression and excessive TFAM expression in irradiated murine bone marrow. Toxcolgy Reports. 1(2014), 450-458. https://doi.org/10.1016/j.toxrep.2014.07.014


Miodini, P., Fioravanti, L., Dr Fronzo, G. & Cappelletti, V. (1999). The two phyto-oestrogens genistein and quercetin exert different effects on osteoblast receptor function. British Journal of Cancer. 80(8), 1150-1155


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