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# The Effects of Endurance Running Training on Young Adult Bone: Densitometry vs. Biomaterial Properties

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## 1. Introduction

Densitometric measurement of bone mineral parameters has been developed in recent decades. Since bone strength is associated with bone mineral density (BMD) and/or bone mineral content (BMC), densitometric measurement is widely accepted and used as one golden standard in clinical settings to determine bone health. Based on this concept, some human studies have suggested that endurance training, such as long distance running, provides no benefit and may even be harmful to bone health or bone mineral accretion during development, since long distance runners often have low BMD and/or BMC and may even exhibit conditions associated with bone loss or osteopenia.<sup>1, 2</sup> Conversely, serum bone marker assays in healthy distance runners show normal or positive bone metabolism status.<sup>3, 4</sup> Therefore, the definite role of endurance running training (ERT) on bone health remains a controversial issue. It would be valuable to further clarify whether ERT benefits bone health through a pathway other than absolutely increasing BMD or BMC.

Clinical observations of human subjects require further basic studies to investigate possible mechanisms. Animal studies can provide unique ways not feasible in studies using human subjects of assessing the effects of endurance running on bone. Generally, previous animal studies further verified benefits of ERT to bone health. However, the limitations of animal studies must be clarified before applying their findings to human beings.

The present article reviews the phenomena shown in bone of adolescent or young adult distance runners. Moreover, previous animal studies which adopted growing and young adult rats as subjects are reviewed, and the applicability of the findings to humans is also discussed.

## 2. The effects of endurance training on human bone: results and limitations

Conventionally, the extrinsic parameters of bone, such as BMD, BMC, and size-related measurements (*e.g.*, bone dimension, bone geometry), have been widely accepted as indicators of bone strength as well as predictors of fracture risk. Unfortunately, endurance running is usually considered an exercise mode that confers no benefit in terms of bone mineral accretion.<sup>1, 2</sup> Moreover, distance runners reportedly have low BMD and are often candidates for osteoporosis or stress fracture.<sup>1, 2, 5-8</sup> This section reviews studies on the effects of distance running training on both BMD and bone metabolism.

### 2.1 Results of human studies

#### 2.1.1 BMD and BMC in distance runners

For references to human studies, the NIH website (<http://www.ncbi.nlm.nih.gov/sites/entrez/>) was searched by subject (adolescent, young adult runners) and research type (cross-sectional studies). Additionally, the major purpose of this article is to describe the long-term effects of ERT on bone in runners without concomitant health problems. Hence, reports describing energy deficiencies and/or serious menstrual cycle disorders in runners were excluded. The summary of previous cross-sectional studies in Table 1 indicates that distance runners usually reveal lower BMD and BMC values than those who engage in higher impact sports.<sup>9</sup> According to Frost's theory,<sup>10</sup> the slenderer body dimensions (Body Mass Index = 20 ~ 22) of runners who have a relatively lower body weight (BW) might partially contribute to a lower BMD and BMC. However, when compared to body-size matched control groups or another non-weight bearing exercise group, runners still do not seem to have much advantage on whole body, lumbar spine or regional cortical bone BMD.<sup>11-18</sup> Although oligomenorrhea or amenorrhea has been considered the cause of low BMD in female runners, even healthy female runners with normal menstrual cycles had lower BMD when compared to their size-matched control subjects.<sup>12-14</sup> Thus, ERT is usually concluded to be profitless for bone mineral accretion and bone health as well. However, if the analysis is limited to weight-bearing sites, runners do reveal higher site-specific regional BMD and/or BMC (*e.g.*, femoral neck, distal tibia, calcaneus) than do controls.<sup>15, 19-21</sup> Therefore, ERT is not entirely non-beneficial for bone mineral accretion when considering BMD and/or BMC as the major predictors of bone health.

Table 1 shows the findings of several studies indicating that distance runners have absolutely higher BMD values than do control groups.<sup>3, 22-24</sup> In the research publicized by Brahm and associates,<sup>3</sup> the runners showed only a slightly higher total-body BMD (3.6% higher,  $p=0.03$ ), and no significant difference from the control group in total-body BMC was revealed. Interestingly, this study found that runners had distinctly higher BMD values in the legs and in the proximal femora. Regarding subject specificity, the training level of subjects or the normality of control subjects would be a major concern. Compared to elite distance runners, high school or club level runners may be trained at a more moderate intensity. Thus, these subjects did not really have typical body dimensions (*e.g.* slender body shape, low BMI) of elite distance runners.<sup>22, 24</sup> On the other hand, the BMI of 20.7 in the control group recruited in Kemmler *et al.* may have been too low for a normal control group.<sup>23</sup>

Authors	Subjects (gender, age, BMI, training status)	Results	Vs. control group
Grimston et al. <sup>12</sup>	♀, age (C:32.9, NR:32.2, LR:30.3), BMI (C: 20.6, NR:20.5, LR: 21.7), Training (NR:55.6km/wk for 7.7yr, LR: 53.0km/wk, 9.2yr) <sup>a</sup>	Most subjects reveal normal menstrual cycle (11-13 cycle/yr), LR group showed lower BMD in L2-L4, femoral neck, and tibia than C group	↓
Robinson et al. <sup>13</sup>	♀, collegiate gymnasts (n = 21, age 16.2±1.7 years) runners (n = 20, 14.4±1.7 years), and nonathletic college women (n = 19, 13.0±1.2 years), No BMI data.	Lumbar spine BMD was lower in runners compared with both gymnasts and controls. Whole body BMD was lower in runners compared with gymnasts and controls.	↓
Taaffe et al. <sup>14</sup>	♀, 19.7±1.2yr, 19.5, 4-5d/wk, college level runners	Runners showed significantly lower BMD in femoral neck, lumbar spine but not in whole body BMD.	↓
Mudd et al. (2007) <sup>9</sup>	♀, 20.2±1.3yr, 21.0±1.6, college level athletes	Runners had lowest lumbar spine and pelvis BMD when compared to other athletes	↓
Emslander et al. <sup>17</sup>	♀, 20.3±0.6 yr, 21.9, 40mile/wk for 3yr	No significant difference was shown in total body, spine and proximal femoral BMD among runners, swimmers and control groups.	=
Duncan et al. <sup>18</sup>	♀, 17.8±1.4 yr, 21.3±1.6, 8.4±1.2 h/wk for 6.2±1.7 yr, high school level athletes	Areal BMD estimation was performed on mid-third femur. Runners had higher BMD only than cyclist. No difference was shown among groups of runners, swimmers, triathlete and controls.	=
Moen et al. <sup>25</sup>	♀, 15.1-18.8 yr, No BMI data, 58.1km/wk for >1.5yrs	There is no significant difference among amenorrheic runners, eumenorrheic runners, and controls in lumbar BMD.	=
Greene et al. <sup>16</sup>	♂, 16.8±0.6yr, 20.68±1.6, 6hr/wk for 2yr	After adjusting for lean tissue mass per kg of body weight, no difference in BMC was detected.	=
Jürimäe et al. <sup>11</sup>	♀, 22.6±4.3 yr, 20.6±1.6, 6h/wk for > 5yr	Endurance trained group showed no difference with normal-weight control group in BMD, but was lower than over-weight control group.	=
MacDougall et al. <sup>15</sup>	♂, 22-45yr, runners were divided into five groups per their training mile/wk <sup>b</sup>	Runners with running mileage 15-20mile/wk showed the highest BMD values in legs but not in total body and spine. Runners with higher	SS ↑

		training mileage did not show difference in BMD as compared to control group.	
Greene et al. <sup>20</sup>	♀, 16±1.7, 18.7±1.5, 6hr/wk for >2yr	Runner showed higher BMD and BMC in distal tibia, densitometric measurement performed only in distal tibia.	SS ↑
Egan et al. <sup>19</sup>	♀, 21.5±2.6 yr, 20.23, 8.4±3.4h/wk for 6.0±2.1yr	All sports groups had higher BMD values than did the controls. Runners showed a higher BMD only in legs and proximal femur, but lower than rugby athletes.	SS ↑
Fredericson et al. <sup>21</sup>	♂, 24.2±3.2 yr, 20.3±1.3, 70mile/wk for at least 1yr	Soccer player was higher in BMD of the skeleton at all sites measured. Runners only showed higher BMD in calcaneus than control group.	SS ↑
Brahm et al. <sup>3</sup>	♀&♂, 32yr, 22, 7h/wk for 12yr	Runners were significantly higher in total body, legs, femoral neck, trochanter wards triangle and calcaneus BMD than control group.	↑
Stewart & Hannan <sup>24</sup>	♂, 27.6±6.1yr, 21.9±1.3, 8.7±2.7h/week, club level runners	Runners showed higher total body and legs BMD.	↑
Duncan et al. <sup>22</sup>	♀, 17.6±1.4 yr, 21.3±1.6, 8.4±1.2 h/wk for 6.2±1.7 yr, high school level athlete	Runner were significantly higher in total body, lumbar spine, femoral neck and leg BMD as compared to BMI-matched control group	↑
Kemmler et al. <sup>23</sup>	♂, 26.6±5.5yr, 20.9, 9.25±2h/wk for 8.9 yr	Runners were higher in total body BMD, legs, pelvis, femoral neck, calcaneus BMD as compared to the BMI (20.7) matched control group	↑

Note: <sup>a</sup>, subjects were divided into three groups (NR, runners with normal BMD; LR, runners with low BMD, C, control group); <sup>b</sup>, runners were divided into five groups according to their training mileage per week, BMI value was not matched among groups that control group showed the highest value; ↓, runners were comprehensively lower than control group in total body and local bone; =, no significant difference was shown between runners and control group; SS ↑, runners showed site-specific increment in BMD; ↑, runners showed higher BMD in total body as well as in local bone.

Table 1. Summary of cross-sectional studies of BMD in adolescent or young adult distance runners

### 2.1.2 Results of human studies: Bone metabolism status in distance runners

As mentioned above, ERT conferred no clear benefits to bone health. An important inquiry is whether endurance running influences the physiology (*e.g.* exercise-induced acidosis) or causes related abnormalities in hormonal homeostasis (*e.g.* menstrual disorders in females or lower testosterone in males) that negatively affect the bone.

It's well known that patients with pathological acidosis suffer negative bone turn-over, which causes a net bone mineral loss.<sup>26</sup> Endurance exercise may induce acidosis, which negatively affects bone metabolism. However, transient acidosis caused by exercise is buffered by  $\text{HCO}_3^-$  and disappears within hours after exercise.<sup>27</sup> In addition, it has been suggested that acid buffer capacity is enhanced after a period of exercise training.<sup>28</sup> At the cellular level, a single bout of intense exercise induces transient increases in serum and urine calcium levels without showing cellular osteoclastical activities.<sup>29,30</sup>

With respect to the impact from abnormalities of hormone regulation, oligomenorrhea and amenorrhea related to bone loss are often reported in female runners undergoing intensive training. However, recent investigations suggest that endurance running does not directly cause menstrual disorders and the subsequent bone loss.<sup>31-33</sup> Menstrual disorders in endurance runners are more likely due to either energy or nutrition deficiencies. Therefore, dietary adjustment is usually more effective than hormone replacement therapy for restoring menstrual cycles and bone metabolism.<sup>34</sup> In males, ERT is known to lower testosterone, but lower testosterone does not necessarily correlate with lower BMD.<sup>35</sup> In addition, runners with different training mileage (from 5 to 75 mile/week) do not significantly differ in serum testosterone levels.<sup>15</sup>

Regarding studies of bone metabolism status, healthy distance runners at rest usually exhibit normal bone metabolism, and some studies even show a positive bone metabolism status, as revealed by serum bone markers.<sup>3, 4</sup> Brahm *et al.*, in a study of bone metabolism in runners using various serum markers, found that runners had lower bone formation as well as lower bone resorption activities.<sup>3</sup> Moreover, triathletes reveal no difference in bone metabolism during the intense competitive season as compared to their non-training period.<sup>4</sup>

## 2.2 Limitation of human studies

To summarize the above, distance runners do not seem to acquire much benefit from their training when densitometric measurements are used to determine the bone health. However, as shown by serum bone marker assays, distance runners did not reveal an inadequate bone metabolism status. Actually, over the past decades, an increasing number of reports suggest that BMD does not accurately predict bone health or bone strength. Patients with fractures also show normal BMD values.<sup>36</sup> The BMD and BMC measures apparently correlate strongly with body mass and body size.<sup>11, 37-39</sup>

Today, "bone quality" is used as a new term to represent bone health, which is composed of various parameters, including tissue architecture, turnover, microfracture and mineralization, of a healthy bone.<sup>40</sup> Further, the organization of the bone matrix may also play an important role in bone strength.<sup>41</sup> Unfortunately, many of the bone quality measurements are too invasive to be feasible in human subjects. Thus, animal studies have been frequently used for further clarifying related issues.

## 3. Comprehensive results of animal studies: BMD and BMC

In biomedical science, animal experiments are performed to establish models that mimic human physiological phenomena. Either estimation methods or experimental designs, which may not be feasible in humans, can then be performed to further investigate possible mechanisms. Rodent models of treadmill activity are commonly used to investigate the effects and mechanisms of exercise on bone metabolism. This section reviews the findings of

previous animal studies. Briefly, the results of animal studies using rodents as subjects showed gender differences, which might affect their further applicability in human subjects. As mentioned above, intensity-trained runners usually have a lower body mass and often have equal or even lower bone mass than non-athletes. Therefore, an animal model of ERT would be expected to reveal the same phenomena. Animal studies reviewed in the present article were selected according to training type (typically endurance treadmill training) and the age of animals (growing or young adult rats).

### **3.1 Rodents adapted to endurance exercise showed gender differences**

#### **3.1.1 Male rodent studies**

Table 2 summarizes the outcomes of studies using male rats as subjects. The studies were reviewed and classified into two categories. The first category includes those using diet control or adjustment to achieve equivalent BW gains between exercise and control groups.<sup>42-47</sup> These studies demonstrated that trained animals have a higher BMD.<sup>43-45</sup> Tissue mechanical properties were not available in every study, and only one of them shows a higher load-withstanding capacity in the femoral diaphysis.<sup>43</sup> However, it must be mentioned that diet prohibition for the purpose of equalizing body weights among groups might cause an additional negative effect on tissue mechanical properties. Diet prohibition impairs the tissue levels (intrinsic) and mechanical properties of bone, suggesting that dietary manipulation of a control group might not be appropriate.<sup>48, 49</sup>

The second category of studies included animals fed *ad libitum*. In these studies, the exercise groups revealed significantly less BW gain after a programmed ERT.<sup>50-55</sup> With lower BW, exercise trained animals showed no difference or lower BMD values as compared to the sedentary control group. As in human subjects, male rats undergoing intense ERT exhibit lower BW gain and no benefits to bone health when considering BMD or BMC as a predictor. However, the higher load-withstanding and energy-absorption capacity of the bones in training rats introduced new research into how endurance exercise benefits bone quality (see section 4).

#### **3.1.2 Female rodent studies**

Compared with the treadmill training results for male rats, those for female rats are inconsistent with human subjects, and the data are somewhat controversial. Table 3 summarizes the results of ERT in growing or young adult female rats. Most studies indicate that female growing or young adult rats exhibit no change in BW after a period of ERT.<sup>56-64</sup> One study even reported increased BW in female rats after training.<sup>58</sup> Of the studies performing BMD analysis in female rats, many report positive effects from endurance running not only in site-specific increments but also in whole bone. Although densitometric measurements demonstrate this advantage, female rats rarely show improved biomechanical properties and may even reveal adverse effects after an intense training program (see Table 3). Therefore, female rats acclimate to ERT differently than do male rats. In human beings, distance runners are also expected to exhibit gender differences in physiological response to similar ERT. However, it seems inappropriate to use the gender difference found in rats to explain the one found in humans, since a period of programmed ERT would commonly reduce BW either in women or men. Thus, the phenomena observed in female rats may not be applicable to female humans. Based on the idea that animal models should mimic the phenomena shown in human subjects, studies using female rats may not be applicable to female humans, since female rats and women have been shown to respond differently to ERT. Possible reasons are discussed in the next section.

Author	strain, age	Protocol	BW control	BW or BW gain (g)	BMD or BMC	Biomechanical testing
Nordsletten et al. <sup>53</sup>	Wistar, 11 wk	27m/min, 60min/d, 10% inclination, 5d/wk, 4 wk	—	EXE<CON N*	N/A	Ultimate bending moment (N·m×10 <sup>-2</sup> ) EXE > CON
Bourrin et al. <sup>50</sup>	Wistar, 10wk	30m/min, 1.5h/d, 5d/wk, 5wk	—	EXE<CON N*	N/A	No data
Horcajada-Molteni et al. <sup>51</sup>	Wistar, 8wk	20m/min to 30m/min, 60min/d, 6d/wk, 90d	—	EXE<CON N*	Femoral BMD: NS	Femoral failure load (N): EXE > CON*
Huang et al. <sup>54</sup>	Wistar, 5wk	24m/min, 60min/d, 5 d/wk, 10wk	—	EXE<CON N*	Tibiae BMD (g/cm <sup>2</sup> ) :NS	No data
Huang et al. <sup>52</sup>	Wistar, 7 wk	22m/min, 60min/d, 5d/wk, 8wk	—	EXE < CON*	Tibia and femur BMD: NS	Three-point bending load (N), energy (mJ), stress (MPa), toughness (mJ/mm <sup>3</sup> ): EXE > CON*
Huang et al. <sup>55</sup>	Wistar, 7wk	Two groups: 22m/min, 60min/day vs. 30m/min, 5d/wk, 8wk	—	EXE < CON*	EXE < CON* in total femur (p=0.04)	Femoral midshaft bending energy and toughness (mJ & mJ/mm <sup>3</sup> ): EXE > CON
Joo et al. <sup>43</sup>	Wistar, 4 wk	30 m/min, 60 min/d, 5 d/wk, 10wk	+	NS	Femurs BMD (g/cm <sup>2</sup> ) EXE > CON	Femoral mid-diaphysis Bending stress (N/mm <sup>2</sup> ): NS Maximum load (N): EXE > CON
Kiuchi, et al. <sup>44</sup>	Wistar, 4 wk	35m/min, 5° inclination, 60min/d, 5d/wk, 10 wks	+	NS	BMC: EXE > CON*	No data
Notomi et al. <sup>46</sup>	S.D., 4 wk	24m/min, 60min/d, every other d, 4 wk	+	NS	NS	Vertebra and femoral maximal load (N): NS
Sakamoto & Grunewald <sup>47</sup>	Wistar, 4 wk	24m/min, 75min/d, 5d/wk, 8 wk	+	NS	N/A	Tibia breaking strength (kg): NS

Newhall et al. <sup>45</sup>	S.D., 47 d	10.2km/d, for 6 wks (voluntary running)	+	EXE > CON	Femur BMD & BMC: EXE > CON*	N/A
Ferreira et al. <sup>42</sup>	Wistar, 10 wk	12m/min, 1h/d, 10 wks.	+	NS	NS	N/A

Note: Protocol, training protocols were presented serially by the final training intensity (m or cm per minute), training time per day (minute or hour per day), training frequency (times per week), and training periods (day or week); d, day; wk, week; min, minute; NS, none significant difference; N/A, none available.

Table 2. Studies of endurance running training vs. growing or young adult male rats

Author	Strain and age	Protocol	BW control	BW or BW gain and tissue measurement	BMD or BMC	Biomechanical testing
Iwamoto et al. <sup>56</sup>	S.D., 4wk	24m/min, 60min/d, 5 d/wk, 8wk or 12wk	–	BW: NS Muscle weight & Femoral length (12wk): EXE > CON*	Femoral and L6 vertebral BMD (mg/ $\mu$ L): NS Femoral and L6 vertebral bone volume ( $\mu$ L), wet weight (mg): EXE > CON*	N/A
Iwamoto et al. <sup>57</sup>	Wistar, 4wk	24m/min, 60min/d, 5 d/wk, 7wk or 11wk	–	BW: NS Femoral length: EXE > CON*	Tibial BMC (g): EXE > CON* Tibial BMD (g/cm <sup>2</sup> ): NS	N/A
Hagihara et al. <sup>58</sup>	Wistar rats, 8wk	A group: control group B-E group: 4~7d/wk, running at 15m/min, 30min/d, 8wk	–	BW gain: B > A*	Tibial trabecular BMD (mg/cm <sup>3</sup> ): B, C, D, E > A group*. But, NS in tibial cortical BMD.	N/A
Wheeler et al. <sup>59</sup>	S.D., 120 d	55%, 65%, 75% $\dot{V}O_{2max}$ , 30min/d, 60min/d, 90min/d, 4d/wk, 10wk	–	BW: NS	Tibial BMD (g/cm <sup>2</sup> ): EXE > CON	Group trained at 75% $\dot{V}O_{2max}$ and 90min/d showed higher stiffness but lower energy to

						withstand torsion test.
Raab et al. <sup>60</sup>	Fischer	36m/min, 344, 2.5 month	grade15%, 60min/day, 5days/wk for 10wk	BW: NS	N/A	Femur ultimate force (kg/mm): NS
Hou et al. <sup>61</sup>	S.D., 8 wk	49cm/s, 12% grade, 60min/d, 5d/wk, 10 wk (~75-80% of maximum oxygen capacity)	—	BW: NS	N/A	Femoral neck maximum load (N): NS Energy to maximum load (mJ): NS
Shimamura et al. <sup>62</sup>	Wistar, 6 wk	25m/min, 60min/d, 5d/wk, for 7 or 11 wk	—	BW: NS	BMC in Total tibia (mg): EXE > CON*	N/A
van der Wiel et al. <sup>63</sup>	Wistar, 5 month	20m/min, 30 min/d, 5° inclination, 5d/wk, 17wk	—	BW: NS	Total body BMC (g): NS CON : 9.3±1.1 EXE : 9.9±1.0	Femoral neck maximal load (N): NS Femoral shaft maximal load (N): NS
Salem et al. <sup>64</sup>	S.D., 8 wk	45 cm/s, 5% grade, 60 min/d, 3d/wk, 10wk	—	BW: NS	N/A	Femoral neck maximal load (N):NS

Note: Protocol, training protocols were presented serially by the final training intensity (m or cm per minute), training time per day (minute or hour per day), training frequency (times per week), and training periods (day or week); d, day; wk, week; min, minute; NS, none significant difference; N/A, none available.

Table 3. Studies of endurance running training vs. growing or young adult female rats

As mentioned above, male and female rats adapt differently to endurance treadmill training, especially in densitometric measurements. The reasons for this gender difference in rodents have been comprehensively investigated elsewhere. According to the theory of Frost,<sup>10</sup> this difference may partially contribute to different adaptations in BW gain. Female rats usually exhibit a similar or sometimes higher body mass after training; they therefore may acquire a greater advantage from local mechanical loading than male rats with lower BW gain after forced endurance treadmill training or voluntary running.<sup>65-68</sup> The mechanisms of this gender difference in BW gain associated with ERT are unknown. A possible explanation is the involvement of gonadal hormones in BW regulation. Endurance exercise reportedly lowers plasma testosterone levels in male rats.<sup>69</sup> The down regulation of this anabolic

hormone in growing male rats may account for the significantly lower protein mass gain and BW gain. In female rats, however, estrogens would suppress body mass, food consumption and fat deposition.<sup>70, 71</sup> Progesterone, on the other hand, has been verified to increase body fat and body mass.<sup>72, 73</sup> Moreover, a previous study suggests that regular treadmill training results in extended periods of progesterone secretion, which was associated with significant weight gain.<sup>74</sup> Women may respond to ERT with similar regulation in progesterone. However, the up-regulation of progesterone may be more pronounced in rats than in women, since female rats reveal no decrease in BW even under vigorous ERT.<sup>60</sup>

### 3.2 Studies of male rats mimic human practice

An analysis of gender differences observed in the above animal studies reveals that ERT increases BMD and BMC in female rats but not in male rats. However, the physiological response (e.g. BW gain) of female rats to ERT differs from that in female humans. Given that animal studies are intended to clarify the mechanisms of biological phenomena in humans, female rats may not be a suitable model for investigating the effects of ERT on developing or young adult bone. On the other hand, the changes in BW and densitometric parameters associated with ERT in male rats were similar to those in humans, indicating that male rats are a suitable model for investigating the effects of endurance running.

## 4. Effects of endurance training on bone biomaterial properties

Aside from BMD and BMC, biomaterial related analysis will provide more valuable information to predict the capacity that bone tissue can withstand extra mechanical loading generated by daily physical activity or accidents (e.g. fall), and thus, prevent bone from loading-induced damage.

Generally, biomaterial properties of bone tissue can be analyzed at a structural level and a tissue level. Structure biomaterial properties are size-dependent, that is, tissues bigger in size tend to be stronger than smaller ones. Conversely, tissue-level properties are analyzed under size-independent conditions using mathematic methods (e.g. normalized tissue size by cross-sectional moment of inertia) or mechanical methods (e.g. a specimen with consistent size is sectioned from a whole tissue).<sup>75</sup>

### 4.1 Effects on structural (whole bone) properties

Structural properties are calculated from original biomechanical testing raw data without any normalization. Related parameters are load (Nt), displacement (mm), energy (mini joule, mJ) and stiffness (Nt/mm) etc. As shown in previous studies, results of rodents' whole tissue biomechanical properties after a period of endurance running training were controversial. Some studies show that exercise groups were higher in load-withstanding capacity,<sup>51-53</sup> while others revealed a higher energy absorption capacity.<sup>55</sup> One possible explanation for these discrepancies may be differences in training protocol. Animals trained at a higher intensity tended to show higher bone strength (e.g. higher bending load or moment),<sup>51, 53</sup> suggesting that higher mechanical loading generated by intensively running may benefit to bone strength. Moreover, the specific testing conditions would also affect testing results; for instance, Nordsletten and colleagues measured bone strength *in vivo*, at which time bone strength may be affected by muscle strength gains achieved through training.<sup>53</sup>

However, in considering the applicability of exercise, a training program with moderate exercise intensity would be expected to show a higher compliance and therefore be more appropriate for the general population. As shown in Table 2, animals trained at a relatively moderate intensity (20-24m/min), which corresponds to 70%  $\dot{V}O_2$  max,<sup>76</sup> also had lower body masses and slightly lower (~5% lower) total BMD ( $p = 0.04$ ), but were not found to have enhanced structural bone strength.<sup>55</sup> The authors' previous study used body mass as a covariate to equalize raw data, which then revealed a comprehensively stronger bone tissue either in structural or tissue-level biomaterial properties.<sup>52</sup> With lower body mass, the data of the exercise group would be adjusted to a higher level, and the effects of ERT seemed to become "good" for animal bones. However, it would be a more relevant and natural study if animals were fed ad libitum and data were not adjusted

In aspects of biological efficiency, an athlete at her/his optimal physiological status will not necessarily be absolutely higher in every physiological parameter. Therefore, a smaller muscle mass or skeleton size seems to be a benefit, rather than a weakness, for a distance runner or an endurance athlete. With such a smaller bone size, moderate ERT rats did not show absolutely enhanced structural bending load values but, interestingly, they showed better energy absorption capacity in long bone tissue that ERT rats were found to have a four-fold increase in energy absorption after long bone tissue reached the yield-point (post-yield energy).<sup>55</sup> As mentioned in previous studies,<sup>41</sup> post-yield behaviors are highly correlated to tissue-level changes (e.g. collagen fiber orientation).

## 5. Effects on tissue-level (material) properties

In our previous studies, we used mathematical methods to estimate tissue-level biomaterial properties. Through calculating long bone's cross-sectional moment of inertia, we normalized load-displacement data to stress and strain. Under such conditions, ERT rats' worse structural material properties disappeared. Additionally, exercise and control groups showed no differences in yield stress, yield toughness or elastic modulus (Young's modulus),<sup>55</sup> suggesting that endurance training is not harmful for bone material properties. ERT's benefits on the post-yield biomaterial behaviors seemed to be more size-independent and associated with tissue-level (e.g. bone matrix, collagen) changes. Because measuring post-yield mechanical properties using beam bending theory is only valid in the pre-yield regime,<sup>75</sup> reporting post-yield stress, strain or toughness is inappropriate. Therefore, we discussed this tissue-level adaptation base on the post-yield parameters measured from load-displacement data. As shown in our two recent studies, either moderate ERT or endurance swimming training benefits bone tissue more in terms of energy absorption capacity,<sup>55,77</sup> especially in post-yield energy. Similar results of enhanced post-yield behavior were provided by another ERT animal study, which showed a short-term treadmill running (21 days) enhanced tibia post-yield deformation in mice.<sup>78</sup> Moreover, such effects on post-yield behavior changes seem to apply not only to endurance training. After a short-term (5 days) freefall landing exercise, Wistar rats revealed an increased post-yield energy absorption in ulnae.<sup>79</sup> Such an enhanced absorption capability is more likely due to tissue-level (e.g. bone matrix, collagen orientation etc) changes rather than structural adaptation. As mentioned in previous studies, tissue-level properties can be divided into the inorganic mineral phase (e.g., hydroxyapatite), which determines tissue stiffness and strength,<sup>80, 81</sup> and the organic bone matrix, which plays a key role in energy absorption,<sup>82, 83</sup> It has been suggested that the networks of collagen, one of the major components of bone matrix, could affect the energy dissipation between the yield point and fracture point in bone tissue.<sup>84-86</sup>

Collagen fiber orientation (CFO) has been measured by circularly polarized light microscopes as one parameter to represent the collagen network and to predict post-yield energy of bone tissue.<sup>84</sup> Hence, the post-yield behavior revealed by ERT rats' bone tissue could partially stem from a highly organized collagen fiber network. Though the information regarding collagen orientation in the present study is not available in our previous rodent studies,<sup>55,77</sup> it has been reported that dogs after one-year of intensive endurance running (40km/day) revealed a higher organization of collagen fibers in bone tissues.<sup>87</sup> Such highly organized collagen fibers seemed to be able to compensate for the 10% BMD decrease in running dogs. Thus, a highly organized CFO would be expected in rodent studies. As mentioned above, rats subjected to short-term freefall landing exercise (5 days, 10 or 30 times per day) from a height of 40cm also showed enhanced post-yield energy of ulnae.<sup>79</sup> In that study, authors tried to measure the CFO of cross-sectional ulnae. Unfortunately, no difference in CFO between exercise and control groups was found. One major reason for this lack of significant results could be species difference. That is, CFO analysis might not be sensitive enough to detect biomaterial differences in rodents. To date, CFO-related analysis in bone tissue specimens have all been obtained from big mammals,<sup>88, 89</sup> which have more mature Haversian's systems and visible osteons. However, in smaller mammals (e.g. young adult rodents), it is not possible to find Haversian's systems or complete osteon.<sup>90, 91</sup> Per our observation, the organization of collagen fiber tends to be relatively irregular in rats and, thus, CFO analysis might not be sensitive enough to predict post-yield material properties. On the other hand, cross-links within collagen networks might be another contributor to changes in tissue post-yield behaviors.<sup>92</sup> In an exercise-related study, Kohn and colleagues verified that cortical toughness enhanced by a 21-day ERT could be correlated with the overall maturity of collagen cross-links.<sup>93</sup>

In addition to individually measure CFO or cross-links, the biomaterial properties (e.g. tissue strength or tissue post-yield behaviors) might benefit from better integration between collagen and its crosslinks. Related measurement methods are awaited and are worthy of further study.

Finally, microdamage is another factor influencing tissue's post-yield behavior. Accumulation of microdamages (or microcracks) would lead to a fragile bone with lower capability in post-yield energy dissipation.<sup>84, 94</sup> However, such accumulated microdamages seemed to be more related to aging. Also, as in CFO-related studies, microdamage studies have been more frequently done in big mammals. Whether microdamage measurement can be performed on exercise-related rodent study needs further verification.

## 6. Summary

Endurance running is a popular aerobic activity and typical training type. However, related human studies reveal no significant benefits to bone health based on densitometric measurement of bone mineral. On the other hand, animal studies apparently indicate that ERT enhances biomaterial of bone tissue in a size-independent way. The effects of endurance running on the organic bone matrix or other parameters, as well as their relationship to mechanical properties of bone tissues, are worthy of further study.

## 7. Acknowledgments

This study was supported by a grant from the National Science Council: NSC 99-2410-H-006 -114 -MY2. Miss Jae Cody is appreciated for her editorial assistance.

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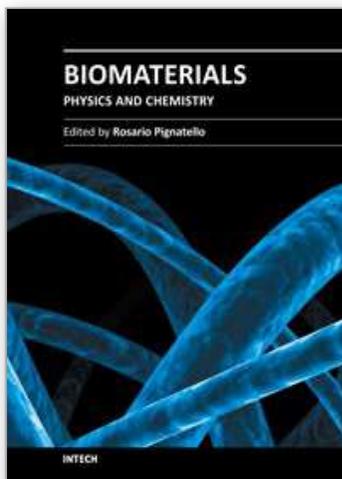
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Edited by Prof. Rosario Pignatello

ISBN 978-953-307-418-4

Hard cover, 490 pages

**Publisher** InTech

**Published online** 14, November, 2011

**Published in print edition** November, 2011

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