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Physical activity in the androgen receptor knockout mouse: Evidence for reversal of androgen deficiency on cancellous bone

Jill Ophoff [a], Filip Callewaert [a], Katrien Venken [a], Karel De Gendt [a], Claes Ohlsson [b], Ghislaine Gayan-Ramirez [c], Marc Decramer [c], Steven Boonen [a], Roger Bouillon [a], Guido Verhoeven [a], Dirk Vanderschueren [a,*]

[a] Laboratory for Experimental Medicine and Endocrinology, Department of Experimental Medicine, Katholieke Universiteit Leuven, Herestraat 49, B-3000 Leuven, Belgium
[b] Division of Endocrinology, Department of Internal Medicine, Sahlgrenska University Hospital, Göteborg, Sweden
[c] Respiratory Muscle Research Unit, Laboratory of Pneumology, Department of Pathophysiology, Katholieke Universiteit Leuven, Leuven, Belgium

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ABSTRACT

Disruption of the androgen receptor (AR) in male mice reduces cortical bone expansion and muscle mass during puberty and results in high bone turnover-related cancellous osteopenia. We hypothesized that voluntary wheel running during growth is able to rescue the effects of AR disruption on bone. To this end, 5-week-old AR knockout (ARKO) mice were randomized to a running group (cage with running wheel) and a sedentary group (cage without wheel) and followed-up until 16 weeks of age. Voluntary wheel running in ARKO mice did not influence body weight, muscle mass or periosteal bone expansion. Interestingly, voluntary running significantly reduced bone turnover in ARKO mice and prevented cancellous bone loss due to a preservation of trabecular number. Thus, voluntary running in ARKO mice was able to reduce cancellous bone resorption, suggesting that sustained exercise may potentially compensate the effects of androgen disruption on cancellous bone.

Men have a 13% lifetime risk for osteoporotic fractures [1,2]. Since at least 90% of the bone mass is acquired by age 18, an important potential strategy to increase bone strength in late adulthood would be to maximize peak bone mass during growth [3]. Androgen action is an important determinant of bone mass acquisition in males. Hypogonadal men have severely reduced bone mineral density and increased risk for fractures [4]. Similarly, castration reduces cortical and trabecular bone growth in rodents [4]. Androgen therapy rescues bone loss following orchidectomy in normal mice, but not in a mouse model with androgen receptor disruption (ARKO) [5]. These and other findings provide compelling evidence that optimal bone mass acquisition in males requires a functional AR [5–7].

Physical exercise affects bone mass accrual and may be important as well. According to the mechanostat theory, physical activity creates mechanical stress on the bone and induces a bone strength adaptation to keep bone strains below a threshold [8]. Several studies have demonstrated that both in humans and rodents physical exercise (even moderate exercise) increases bone mass, at least during growth [9–12]. In addition, exercise may protect against bone loss induced by gonadectomy [13–15]. Exercise might therefore potentially be a low-cost bone forming therapy with lifelong benefits to bone strength [16,17].

However, the potential interaction of physical exercise with hormonal stimuli, such as androgen action, is unknown and needs further study. The ARKO mouse model provides an opportunity to investigate this interaction. The aim of this study was to explore the bone forming potential of voluntary exercise in the absence and presence of androgen activity.

Materials and methods

Animals. ARKO mice were generated using Cre/*loxP* technology, as previously described [18] and were backcrossed to the C57Bl/6N background for at least 12 generations. Genotyping was performed using PCR amplification [18]. Mice were housed in conventional conditions: 12-h light/dark cycle, standard diet (0.87% calcium, 0.65% phosphate), and water *ad libitum*.

Experimental design. At weaning (3 weeks of age), male WT and ARKO littermates were put in a cage with a running wheel for a training period of 2 weeks. At 5 weeks of age, mice were randomly divided in 3 groups (8–10 animals/group). ARKO mice were randomly assigned to either a cage with (running group) or without (sedentary control group) a running wheel (1 animal/cage), while WT mice were assigned to a cage without a running wheel. The running group was able to run voluntarily with free access to the running wheel 24 h/day from 5 to 16 weeks of age. Food consumption was monitored weekly throughout the experiment. *In vivo*

* Corresponding author. Fax: +32 16345934.
E-mail address: Dirk.Vanderschueren@uz.kuleuven.be (D. Vanderschueren).

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peripheral quantitative computed tomography (pQCT) of the tibia was performed and body weight was measured at 5, 8, 12, and 16 weeks of age. At 16 weeks of age, whole body *in vivo* dual-energy X-ray absorptiometry (DEXA) was performed and mice were put in metabolic cages to collect urine for measurement of collagen cross-links. At sacrifice, serum was collected, stored at −20 °C, and used for osteocalcin measurement. Left musculus quadriceps and seminal vesicles were dissected and weighed. Left femur was dissected and used to perform microCT.

In a separate experiment, voluntary running capacity and treadmill endurance capacity of ARKO and WT mice was compared. Similar as with ARKO mice, WT mice were put in a running cage from week 5 to 16 (after a two-week training period) and running activity was monitored daily. At 16 weeks of age, sedentary male WT, female WT and male ARKO littermates were tested by an *in vivo* treadmill running experiment.

All experimental procedures were conducted after obtaining formal approval from the ethical committee of the K.U. Leuven.

Voluntary wheel running exercise. Running cages contained a hamster-sized metal cage wheel with a diameter of 12 cm (Nobby, Bocholt, Germany), which was fitted with a digital magnetic counter (M9 Race, Mafac Ertedis, Achères, France). The digital counter measured the total distance, total time, and average speed. Every morning, distance, time, and speed of running were monitored and counter was reset. Running data were collected every day from week 5 to 16.

Peripheral quantitative computed tomography (pQCT). Trabecular bone mineral density (BMD) and cortical bone parameters (cortical perimeters, cortical thickness, cortical area, and strength strain index [SSI]) were assessed using the Stratec XCT Research M^+ densitometer (Norland Medical Systems, Fort Atkinson, WI, USA), as described earlier [5].

Muscle cross-sectional area was measured by analyzing the *in vivo* pQCT scan of the tibia with its surrounding tissue of the lower leg, using contmode 1, peelmode 2 and separation mode 1. Total soft tissue was measured mid-diaphyseal, at 7 mm from the proximal end of the tibia, and using a density threshold between 35 and 710 mg/cm^3.

Whole body dual-energy X-ray absorptiometry (DEXA). Body composition was analyzed in vivo by DEXA (PIXImus densitometer; Lunar Corp., Madison, WI, USA), using ultra-high resolution (0.18 × 0.18 pixels, resolution of 1.6 line pairs/mm) and software version 1.45. DEXA was performed at the end of the experimental period.

MicroCT. MicroCT analysis was performed on the distal femur by using a Skyscan 1072 scanner (Skyscan N.V., Kontich, Belgium). Trabecular bone volume, number and thickness were determined as previously reported [19].

Assays. Serum osteocalcin was measured by an in-house radioimmunoassay (RIA) [20]. Urinary collagen cross-links (deoxypyridinoline [DPD]) were measured by high-performance liquid chromatography (HPLC) with fluorescence detection after acid hydrolysis [21]. The concentration of DPD was corrected for creatinine excretion, which was measured colorimetrically.

Endurance treadmill experiment. A four-lane treadmill with a speed and inclination adaptable belt was used (Columbus Instruments, Columbus, OH, USA). The first day, a familiarization trial was performed in which the mice had to run three times for 15 min with a tread speed of 5 m/min, 10 m/min, and 15 m/min respectively, (10° inclination) and a rest period of 5 min after each run. The experiment was performed the day after the training test. Endurance was tested at a speed of 20 m/min (10° inclination) until mice stopped running from exhaustion. A mild electric shock was presented to promote running and mice were removed from the experiment after being on the shock grid for 15 consecutive seconds. The time (in seconds) of running until exhaustion was recorded for each mouse. ARKO and WT littermates were tested on the same day.

Statistical analysis. Statistical analysis of the data was performed using NCSS software (Kaysville, UT, USA). The data were compared between WT and ARKO and between control group and running group by Student's *t*-test to assess significant differences. A multiple linear regression model was used to test the effect of the ARKO genotype the correlation of SSI with body weight gain. Data are represented as means ± SE and $p < 0.05$ was accepted as significant.

Results

Effect of androgen receptor disruption on bone and body characteristics

ARKO mice gained less body weight from 5 to 16 weeks of age compared to WT (Fig. 1A), due to a lower lean body mass (−17%) but no change in fat mass (Table 1). Accordingly, ARKO mice had reduced muscle cross-sectional area and quadriceps weight (Fig. 1B, Table 1). Food intake was 12% lower in ARKO compared to WT mice (Table 1).

In addition, ARKO mice achieved a smaller bone size with, compared to WT, significantly lower cortical area, cortical thickness and periosteal perimeter from 8 weeks of age on (Fig. 1C, E, and F). Linear regression analysis was performed to investigate the correlation between muscle cross-sectional area and bone strength (estimated by SSI), as well as the effect of AR disruption on this correlation. Linear regression analysis showed that the SSI was positively related with the calf muscle cross-sectional area in 16-week-old WT mice (Fig. 2). AR disruption significantly influenced the relationship since no correlation between SSI and calf muscle cross-sectional area could be detected in ARKO mice (Fig. 2, dashed line).

Trabecular bone mass was significantly lower in ARKO mice at all time points (Fig. 1G) and associated with elevated levels of osteocalcin (a marker for bone formation) and DPD (a marker for bone resorption) (Table 2).

Effect of androgen receptor disruption on running activity

AR disruption had a significant impact on voluntary wheel running (Fig. 3). At all time points, distance, running time as well as average speed were much lower in ARKO mice compared to WT. In contrast, in a forced treadmill experiment, the endurance capacity was significantly (+109%) higher in ARKO mice compared to male WT mice (Fig. 4). No difference in endurance capacity between ARKO and female WT mice could be found (Fig. 4).

Effect of voluntary wheel running on bone

Although food consumption increased (Table 1), voluntary running did not influence body weight (22.9 ± 0.3 g). Total fat mass (Table 1), lean mass (Table 1), quadriceps weight (Table 1) and calf muscle cross-sectional area (25.95 ± 0.34 mm^2) are also unchanged in ARKO mice after voluntary running.

Similarly, voluntary running did not affect cortical bone gain in ARKO mice. Periosteal perimeter (4.258 ± 0.026 mm), endosteal perimeter (3.125 ± 0.043 mm), cortical thickness (180 ± 4 mm^2), cortical area (0.67 ± 0.01 mm^2) and SSI (0.19 ± 0.01 mm^3) were not significantly different between ARKO controls and ARKO runners.

Voluntary running changed trabecular bone mass in ARKO mice. Trabecular BMD of the tibia was 14% higher after voluntary running (Table 3). Accordingly, μCT analysis showed a 31% increase in trabecular bone volume in the femur (Table 3). The enhanced trabecular bone volume resulted from an increase in trabecular number, without difference in trabecular thickness (Table 3). Fur-

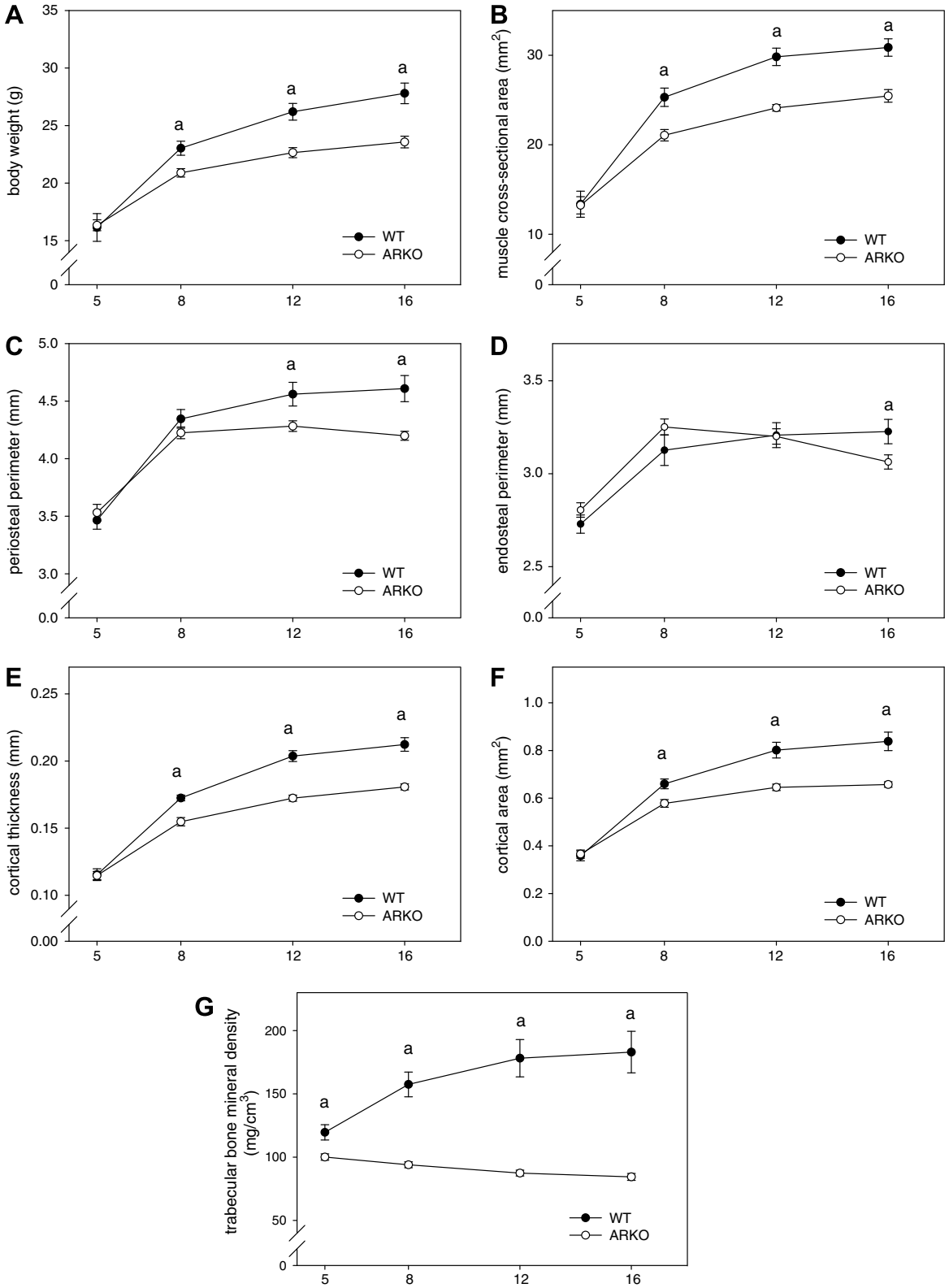

Fig. 1. Longitudinal follow-up of (A) body weight (g), (B) muscle cross-sectional area (mm^2), and (C) periosteal perimeter (mm), (D) endosteal perimeter, (E) cortical thickness (mm), (F) cortical area (mm^2), and (G) trabecular BMD (mg/cm^3) of the tibia from 5 till 16 weeks of age, as measured by *in vivo* pQCT in male WT ($n = 7$) and ARKO mice ($n = 8$). Values are expressed as means ± SE. $^a p < 0.05$ WT vs. ARKO at respective time points.

ther evidence for an anti-resorptive effect of voluntary running on trabecular bone in ARKO mice was provided by reduced levels of osteocalcin and DPD compared to WT controls (Table 2).

Discussion

Physical exercise might potentially be a convenient strategy to optimize bone strength during growth. In this longitudinal study

Table 1
Body composition and food consumption in WT and ARKO at 16 weeks of age.

	WT	ARKO	
		Con	Run
Lean body mass (g)	21.20 ± 0.55	17.6 ± 0.43 [a]	18.03 ± 0.13
Quadriceps (mg)	114.7 ± 19.9	66.0 ± 2.2 [a]	83.0 ± 12.0
Quadriceps/weight (mg/g)	4.2 ± 0.7	2.8 ± 0.1 [a]	3.6 ± 0.6
Total body fat mass (g)	6.20 ± 0.55	5.82 ± 0.40	5.15 ± 0.25
Food consumption (g/week)	30.22 ± 0.66	26.45 ± 0.32 [a]	33.14 ± 0.71 [b]

5-week-old male ARKO mice were randomly divided into a sedentary (con) and voluntary wheel running (run) group. Body composition and food consumption were measured at 16 weeks of age. Values are expressed as mean ± SE.
[a] $p < 0.05$ ARKO con vs. WT.
[b] $p < 0.05$ run vs. con. ($n = 8$–10 mice/group).

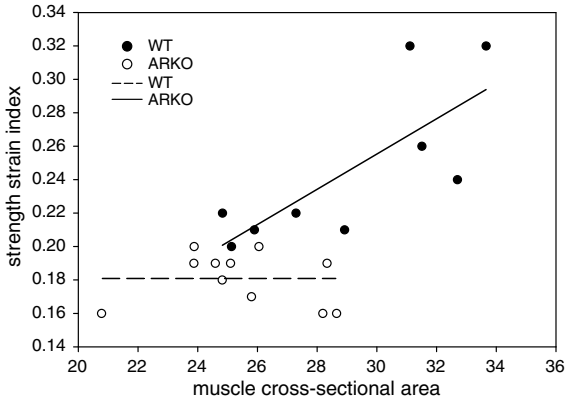

Fig. 2. Linear regression analysis of the *dependent* variable strength strain index (mm^3) of 16-week-old male WT and ARKO mice with muscle cross-sectional area and genotype (WT or ARKO) as *independent* variables. In WT mice, SSI was positively correlated with the muscle cross-sectional area. A statistically significant interaction could be found between genotype and muscle-cross-sectional area, and no correlation could be found between SSI and muscle cross-sectional area in ARKO mice. The model ($R^2 = 0.76$) was highly significant: $p < 0.01$.

Table 2
Biochemical markers of bone turnover in WT and ARKO at 16 weeks of age.

	WT	ARKO	
		Con	Run
Osteocalcin (ng/ml)	35.7 ± 3.1	57.0 ± 3.4 [a]	43.5 ± 4.0 [b]
Deoxypyridinoline (nM/mM creatinine)	20.8 ± 1.1	28.5 ± 1.6 [a]	22.6 ± 0.9 [b]

5-week-old male ARKO mice were randomly divided into a sedentary (con) and voluntary wheel running (run) group. Values are expressed as means ± SE.
[a] $p < 0.05$ ARKO con vs. WT.
[b] $p < 0.05$ run vs. con. ($n = 8$–10 mice/group).

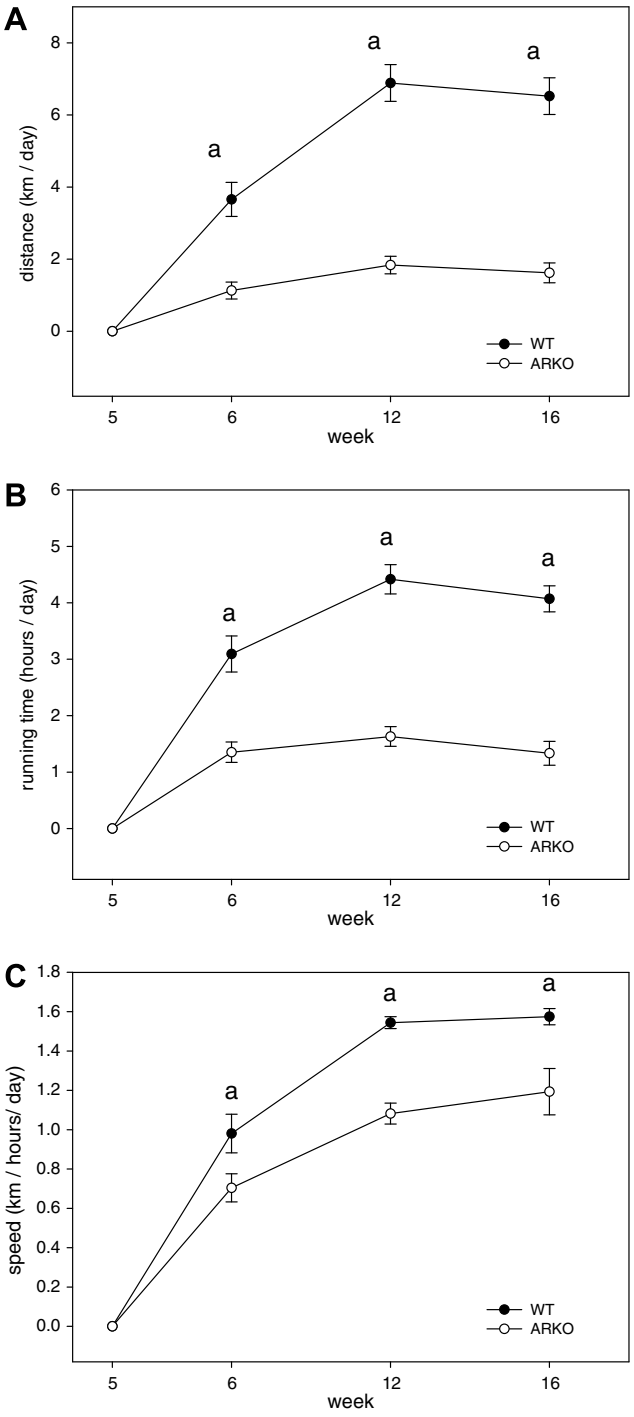

Fig. 3. Effect of androgen receptor knockout on voluntary running activity. (A) Distance (km/day), (B) running time (hours/day), and (C) speed (km/hours/day) of ARKO ($n = 9$) and WT ($n = 8$) mice from 5 till 16 weeks of age. Values are expressed as means ± SE. [a]$p < 0.05$ WT vs. ARKO at respective time points.

in growing pubertal mice, we evaluated the effect of voluntary wheel running on mineral acquisition in the absence and presence of androgen action.

Disruption or even a delay of androgen action during puberty represents one of the most serious threats for male skeletal bone mineral accumulation, both in humans and in rodents [5,22]. In this study, not only bone mass acquisition was reduced, but also body weight and muscle gain were severely impaired in ARKO compared to WT controls. Moreover, the SSI – a measure of biomechanical competence – was found to be positively related with calf muscle cross-sectional area in WT mice. This association is most likely due to the muscle-associated increase in skeletal loading, which needs to be fully matched by an appropriate adaptation of bone strength (as reflected by the SSI). Absence of androgen action reduced the gain in muscle and impaired skeletal development. Compared to WT, no correlation between SSI and calf muscle cross-sectional area could be found in ARKO mice. These findings suggest that androgens may have a direct anabolic effect on bone strength, independently from their well-documented effects on muscle growth.

Both cortical and trabecular bone mass gain and maintenance were severely reduced in ARKO compared to WT mice. In line with earlier findings [5,6], bone turnover parameters were increased in sedentary ARKO mice, reflecting higher cancellous bone remodeling

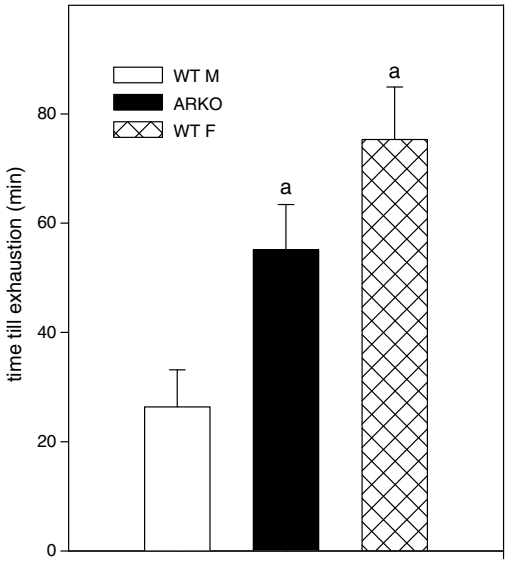

Fig. 4. Effect of androgen receptor knockout on endurance. Time till exhaustion (in minutes) of male ARKO ($n = 10$) and their corresponding male (M WT) ($n = 11$) and female wild-types (F WT) ($n = 6$) mice was measured in a treadmill experiment. Values are expressed as means ± SE. [a] $p < 0.05$ compared with corresponding M WT. No significant difference between ARKO and F WT.

Table 3
Trabecular bone characteristics in ARKO mice at 16 weeks of age.

	ARKO	
	Con	Run
Trabecular bone mineral density (mg/cm^3)	84.4 ± 2.8	97.1 ± 7.0 [a]
Percentage bone volume (%)	3.56 ± 0.29	4.65 ± 0.29 [a]
Trabecular thickness (μm)	37.52 ± 1.36	39.66 ± 0.79
Trabecular number (1/mm)	0.944 ± 0.062	1.167 ± 0.021 [a]

5-week-old male ARKO mice were randomly divided into a sedentary (con) and voluntary wheel running (run) group. Trabecular bone characteristics were measured at 16 weeks of age. Values are expressed as means ± SE.

[a] $p < 0.05$ con vs. run. ($n = 7$–9 mice/group).

activity. Taken together, these data support the concept that androgens are not only essential to optimize cortical bone mineral acquisition, but even more so for the development and integrity of the cancellous bone network during growth.

An unexpected observation in our study was the severely reduced voluntary wheel running intensity in ARKO compared to WT mice. This observation is in line with earlier findings in ARKO mice [23]. Intriguingly however, this lower voluntary running performance was not reflected by a reduced endurance capacity as measured by a forced treadmill running experiment. These data suggest that the lower voluntary running intensity, in line with the reduced food consumption in ARKO mice, is due to androgen-related behavioral changes, rather than to differences in running capacity.

In our study voluntary running did not increase puberty-associated cortical bone gain in ARKO mice. This is in agreement with data in humans where high impact physical exercise was more effective than running [24]. Less intense voluntary running in ARKO mice did not impair body weight gain or muscle mass in androgen disrupted mice and, consequently, did not affect cortical perimeters, thickness or SSI.

Of potential interest from a clinical perspective, moderate physical activity was found to partly prevent trabecular bone loss in ARKO mice. Markers of bone remodeling were reduced in running compared to sedentary ARKO mice, suggesting that moderate physical activity may reduce bone resorption and prevent trabecular bone loss in the context of androgen deficiency. In accordance, running was shown to reduce trabecular bone loss and resorption following orchidectomy in rodents in some [14,15] but not all studies [25,26]. Overall, our data suggest that ARKO mice remain responsive to the beneficial effects of running therapy. It is tempting to speculate that, in menopausal women and hypogonadal men, moderate (rather than intensive) running might potentially reduce bone turnover and treat osteoporosis.

In conclusion, puberty represents a critical and vulnerable period during which androgen action, muscle mass gain, and physical exercise have both independent and interactive effects on trabecular and cortical bone compartments. We showed that androgens are the most important determinants of cortical and trabecular bone mass. In addition, voluntary physical exercise may reduce bone resorption and prevent trabecular bone loss in the context of androgen deficiency-induced excessive bone turnover.

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