Effects of treadmill exercise training on cerebellar estrogen and estrogen receptors, serum estrogen, and motor coordination performance of ovariectomized rats

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Objective(s): The present study aims at examining the motor coordination performance, serum and cerebellar estrogen, as well as ERβ levels, of ovariectomized rats (as menopausal model) following regular exercise.

Materials and Methods: Ten female Sprague Dawley rats aged 12 weeks old were randomly divided into two groups; all of which underwent ovariectomy. The first group was treated with regular exercise of moderate intensity, in which the rats were trained to run on a treadmill for 60 min per day for 12 weeks. The second group served as control. Rotarod test was carried out before and after exercise treatment. All rats were euthanized thereafter, and blood and cerebellums of the rats were collected. The serum and cerebellar estrogen as well as cerebellar ERβ levels were measured using ELISA assays.

Results: The number of falls in the rotarod task of the exercise group was significantly lower than that of control group. The cerebellar estrogen level of the exercise group was significantly higher than that of control group. Accordingly, there was a significantly negative correlation between the number of falls and cerebellar estrogen level in the exercise group.

Conclusion: The present study shows that a lengthy period of regular exercise improves the cerebellar estrogen level and motor coordination performance in ovariectomized rats.

Introduction
Menopausal period in women is characterized with a considerable decrease of estrogen hormonal level (1-3). The decrease of estrogen hormone (hypoestrogenicity) interrupts the normal functioning of female organs, including the brain (2, 3). Low level of estrogen in the brain results in the decrease of cognitive, affective, nociceptive, movement, and motor coordination functions (4). These alterations of movement and motor coordination functions, which are pivotal functions of cerebellum (5), indicate that cerebellum appears to be a target organ of estrogen. It seems that the function of cerebellum is strongly influenced by hormonal status (6).

In rodents, menopause-induced reduction of estrogen level can be appropriately mimicked by bilateral ovariectomy procedure. Adult mice underwent ovariectomy have been shown to suffer from sensorimotor (including equilibrium capacity) and exploratory behavior deficits comparable to their old counterparts. These behavioral impairments were apparently associated with the decrease of plasma estradiol levels (7). Previous studies have also shown a decrease in total protein synthesis (8), an increase in estrogen receptor β mRNA containing Purkinje cells (9), but no change in estrogen receptor α (ERα) (10) in the cerebellum of ovariectomized rats.

The physiological effects of estrogens in the brain are accomplished through genomic pathway (1, 11) which are mediated by two estrogen receptors, i.e. ERα and estrogen receptor β (ERβ) (12). Both ERα and ERβ are widely distributed in cerebellum (6, 13, 14). However, the physiological effects of estrogen in cerebellum of adults are mainly mediated by ERβ (13).

Several investigations discovered that physical exercise accelerates motor coordination improvement in both humans and animals (15-17). Physical exercise was also observed to be associated with increased extragonadal aromatization (18) and neurotrophin synthesis (19-21), all of which affect estrogen receptors...
expression (18, 22-24). It remains unclear, however, whether physical exercise affects motor coordination performance, cerebellar and serum estradiol levels, as well as ERβ in ovariec-tomized adult rats; and how these variables correlate to each other. The present study aims at investigating such effects and correlations.

Materials and Methods

Animals

Ten female Sprague Dawley rats aged 12 weeks old which were initially weighing 170-200 g, were used in this study. Sprague Dawley strain was chosen as experimental animals since this strain is considered to be suitable for studies on hormonal changes (25). The rats were obtained from Animal House of Gadjah Mada University. They were housed in cages under 12 hr of naturally light-dark cycle. Food and water were given ad libitum. They were allowed to acclimatize for one week. The experimental protocol and animal handling was approved by the Ethics Committee of Faculty of Medicine, Gadjah Mada University (approval number KE/FK/194/EC).

The rats were randomly assigned into two groups, i.e. exercise group (n=5), and control group (n=5). After one week of acclimatization, both ovaries of all rats of both groups were removed via a 2-3 cm ventral midline incision on the abdomen under anesthesia (ketamine HCl 40 mg/kg body weight; PT Guardian Pharmatama, Jakarta, Indonesia). Seven days after ovariec-tomy, exercise training started.

Exercise training protocol

The exercise protocol referred to that adopted by Hao et al (18) with slight modifications. Briefly, the protocol consisted of two periods, i.e. adaptation period and exercise period. The rats of exercise group were adapted to the exercise protocol and treadmill apparatus (Gama Tread version 2010, Faculty of Medicine, Gadjah Mada University) in a training room for one week. During the adaptation period, the running speed, the treadmill slope, and the duration of exercise were increased gradually. The speed was increased from 10 m/min up to 18 m/min; the slope was increased from 0° up to 5°; while the duration was increased from 15 min up to 60 min. Subsequently, during the exercise period the rats were trained to keep running constantly on the treadmill at a speed of 18 m/min and at a slope of 5° for a total duration of 60 min per day. This regimen of exercise was designed to be of moderate intensity and was calculated to obtain VO2 max of approximately 56%, based on the regimen developed by Bupha-Intr et al (26). The exercise was performed five times per week (every Mondays, Tuesdays, Wednesdays, Fridays, and Saturdays) for 12 weeks with two days of rest period in each week (every Thursdays and Sundays). The control group was only moved to the training room at the same time when the exercise group performed exercise.

Rotarod task

The motor coordination of rats was assessed on a rotarod apparatus (The Ugo Basile model 7700, Veresi, Italy). The protocol of rotarod test was based on those described in previous studies (15, 27, 28) with slight modifications. The tests were carried out in two series, namely seven days after ovariec-tomy and on the last day of exercise. Each series consisted of three trials, which were performed at the intervals of 60 min (15). The duration of each trial was 3 min (27, 28).

In order to habituate to the apparatus, prior to the tests, each rat was left for 1 min on the running surface of the stationary rotarod. The rat was then removed from the rotarod and the rotarod was turned on to rotate at a speed of 16 rounds per min. The rat was returned to the surface of the rotarod. It had to walk forward in order to maintain its position on the running surface of the rotarod during the three minutes trial. The number of falls of the rats was recorded for further statistical analyses. The number of falls was defined as the average of the total number of falls of the rats during the three trials of each series.

Serum and tissue collection

The rats were euthanized approximately 24 hr after the last exercise training. Prior to euthanasia, 2 ml of blood was collected from retro-orbital sinus of each rat under anesthesia (ketamine HCl 40 mg/kg body weight; PT Guardian Pharmatama, Jakarta, Indonesia) and it was allowed to clot for 2 hr at room temperature. The blood was subsequently centrifuged at 1800 ×g for 10 min at a temperature of 4 °C (29). Serum was separated from the blood and stored at -20 °C freezer prior to estrogen level measurements.

Immediately after blood collection, the cerebellums of the rats were removed from their skulls and subdivided into left and right parts. The extracted left cerebellums were homogenized in TEGM (10 mM Tris-HCl, 5 mM EDTA, 10% glycerol, and 2.3 mM MgCl2 pH 6.8). Every 50 mg of the cerebellums was dissolved in 1 ml TEGM. The homogenates were subsequently incubated for 18 hr in 4 °C refrigerator. The homogenates were then centrifuged at 1000 ×g for 20 min, and the supernatants were used for the determination of ERβ concentration. The concentration was determined using rat estrogen receptor 2 (ER beta) ELISA kit (Cusabio Biotech Co., Ltd., PR China) in a Biorad microplate reader (Benchmark, Japan) operated at a wavelength of 450 nm.

The right cerebellums were homogenized in phosphate buffered saline (PBS). Every 100 mg of the cerebellums was dissolveld in 1 ml PBS. The homogenates were incubated for 12 hr in -20 °C freezer.
Exercise, motor coordination, estrogen

The homogenates were then centrifuged at 5000 ×g for 5 min. The supernatants of these homogenates were used for the examination of estradiol concentration. The concentration was determined using rat estradiol ELISA kit (DRG instruments GmbH, Germany) in a Biorad microplate reader (Benchmark, Japan) operated at a wavelength of 450 nm.

**Statistical analysis**

The data of body weights of rats were analyzed using Mann-Whitney test, since these data did not pass the normality test. On the other hand, the data of cerebellar weights were analyzed using unpaired t-test, since these data passed the normality and variance tests. The mean differences of the number of falls before and after treatment (within groups) for both exercise and control groups were analyzed using paired t-test. The differences of the number of falls between groups were analyzed using unpaired t-test. In addition, unpaired t-test was used to measure the mean differences of serum and cerebellar estrogen as well as cerebellar ERβ levels between groups. Spearman correlation test was used to assess the correlation between the number of falls and the levels of cerebellar and serum estrogen as well as ERβ levels.

The statistical analyses were performed using SPSS version 19 software. All data were presented in mean ± standard error of mean (SEM) and significance levels were set at P<0.05.

**Results**

**Body and cerebellar weights**

Table 1 presents data on the body and cerebellar weights of all rats. There was no significant difference of body weights between groups both before and after exercise. There was also no significant difference in cerebellar weights between exercise and control groups.

**Rotarod test**

Table 2 presents data on the number of falls in motor coordination task before and after exercise in both control and exercise groups. There was no significant difference in the number of falls between groups before exercise. On the other hand, the number of falls of exercise groups was significantly lower than that of control group (P<0.05) following exercise. In the exercise group, the number of falls was significantly lower after exercise than that before exercise. In contrast, in the control group, the number of falls after treatment was significantly higher than that before treatment.

**Levels of estrogen and cerebellar ERβ**

Table 3 shows data on the serum and cerebellar estrogen as well as cerebellar ERβ levels in both control and exercise groups. The levels of cerebellar estrogen were significantly higher in the exercise group than in the control group. On the other hand, there were no significant differences in the serum estrogen and cerebellar ERβ levels between exercise and control groups.

**Correlation**

Spearman correlation test showed that there was a significantly negative correlation between the number of falls and cerebellar estrogen levels (r = -0.650; P = 0.042). However, no correlation between the number of falls and cerebellar ERβ levels (r = 0.49; P = 0.893) as well as between the number of falls and serum estrogen level (r = 0.36; P = 0.920) was found.

**Discussion**

The present study found that a regularly and lengthy period of physical exercise prevented ovariectomy-induced motor coordination performance deficits in rats. Exercise also prevented the decline of cerebellar estrogen levels due to ovariectomy. In addition, motor coordination performance correlated significantly with cerebellar estrogen levels.

We cannot compare our study with others since literature search in the biomedical research database to date does not reveal any study of the effects of ovariectomy on the behavior of rats similar to ours.

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**Table 1. The body and cerebellar weights in control and trained ovariectomized rats**

| Weights          | Exercise group (n=5) | Control group (n=5) | P  |
|------------------|----------------------|---------------------|----|
| Body weights (g) | 164 ± 4              | 170 ± 4             | 0.829 |
| Before exercise  | 89.20 ± 11.84        | 99.20 ± 11.84       | 0.04 |
| After exercise   | 10.60 ± 1.47         | 9.82 ± 1.47         | 0.002 |
| Cerebellar weights (mg) | 325.02 ± 9.48b  | 325.02 ± 9.48b      | 0.287 |

*Values are expressed as Medians; a Values are expressed as Means±SEM; b Mann-Whitney test (between groups); c Unpaired t-test test (between groups)*

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**Table 2. Effect of exercise training on the number of falls in motor coordination test before and after treatment in the exercise and control ovariectomized rats**

|                  | Exercise group (n = 5) | Control group (n = 5) | P  |
|------------------|------------------------|-----------------------|----|
| Before exercise  | 23.60 ± 3.74           | 24.20 ± 10.78         | 0.959 |
| After exercise   | 10.60 ± 1.47           | 9.82 ± 1.47           | 0.002 |
| P                | 0.006                  | 0.040                 |    |

*Values are expressed as Means±SEM; a P-values of unpaired t-test (between groups); b P-values of paired t-test (within groups)*
Table 3. Effect of exercise training on the levels of serum and cerebellar estrogen and cerebellar ERβ in the exercise and control ovariectomized rats

| Exercise group (n=5) | Control group (n=5) | P* |
|----------------------|---------------------|----|
| Serum estrogen levels (pg/ml) | 26.06 ± 4.06 | 24.54 ± 8.66 | 0.876 |
| Cerebellar estrogen levels (pg/100 mg tissue) | 31.64 ± 3.54 | 19.66 ± 2.70 | 0.027 |
| Cerebellar ERβ levels (ng/50 mg tissue) | 0.033 ± 0.008 | 0.026 ± 0.004 | 0.418 |

Values are expressed as Mean±SEM; *P=values of unpaired t-test (between groups)

The only study on rodents which may be comparable to ours is that of Baeva and colleagues (7). Our study corroborates this study in which ovariectomy brought about the decrease of sensorimotor abilities such as reflex, balance, muscle strength, and motor coordination in adult mice. Such deficits of motor coordination performance might be reversed by physical exercise as was shown in previous studies using rats model of cerebellar injury and ataxia (15, 30).

Physical exercise, a type of physical activity assumed in a planned and structured movement to maintain or improve physical fitness (31), may modify motor coordination function by affecting neuronal plasticity of cerebellum (32), a key part of brain participating in regulating motor coordination (5). It is plausible that the effects of physical exercise on cerebellum are mediated through estrogen, as the present study demonstrated that cerebellar estrogen levels, but not serum estrogen levels, had a significantly negative correlation with motor coordination performance. Estrogen may influence cerebellum (4) by preventing neuronal deaths, inducing the formation of synapses and increasing information transmission through nerve impulses (33, 34) as well as maintaining the structure (1, 35) and function of cerebellum (34).

At the time of shortage of estrogen in the body, such as following ovariectomy or menopausal period, serum estrogen level may decline. However, estrogen level in tissues, including in the cerebellum, might be maintained through extragonadal production in order to ensure the sufficient availability of estrogen for normal functioning of steroid-dependent organs. It has been demonstrated that cerebellum is an organ that can produce steroid hormones de novo (36). Estrogen biosynthesis in the cerebellum might be enabled by the presence of P450 aromatase enzyme (29). It has been put forth that P450 aromatase enzyme is responsible for converting testosterone into estradiol, and androstenedione into estron (37-39). Exercise possibly leads to an increase to synthesis of this enzyme. Previous studies in our laboratory have indicated that regular exercise might give rise to an increase of CYP19 aromatase expressions in adrenal cortex and adipose tissues of ovariectomized rats (unpublished results). CYP19 aromatase is thought to be implicated in the production of P450 aromatase enzyme (40). The precise mechanism of how exercise increases cerebellar estrogen levels by extragonadal aromatization remains unclear, but it may involve IL-6. Physical exercise increases skeletal muscle’s production of IL-6 that are subsequently released in large quantities into the circulation (41). It has also been reported that IL-6 was released in human brain following a 60 min-physical exercise (42). IL-6 in circulation triggers extragonadal steroidogenesis by increasing aromatase activity in the adrenal cortex, bone, and fat tissue (43). In addition, it is considered to be involved in the regulation of neurosteroid synthesis in the brain (44).

Estrogen normally mediates its functions via estrogen receptors (ER) (12, 34, 37). Direct genomic pathway which involves the interaction between ER and its ligand (estrogen) is the common mechanism of estrogen action on brain tissue (1, 35). In adolescent cerebellum, estrogen acts mainly on ERα rather than ERβ (13, 45). Furthermore, ERαs are more widely distributed in various types of cells of the cerebellar cortex (14, 46), whilst ERβ seems to be more confined to Purkinje cells only (13). Hence, it had been anticipated that, consistent with the increase of cerebellar estrogen levels, the cerebellar ERα levels also increased accordingly. However, this was not the case of our study. The fact that ERα levels remained constant following regular exercise raises questions of whether estrogen might actually have exerted its effects through ERβs instead of ERαs. Alternatively, estrogen might have stimulated other yet unknown pathways.

Previous studies have shown that the expression of ER in neurons at the central nervous system is regulated by insulin-like growth factor-1 (IGF-1) (47) and depends on estrogen concentration (47, 48). In a circumstance when estrogen in tissue is lacking, IGF-1 increases the transcriptional activity of ERα. Otherwise, if the tissue estrogen level is sufficient, IGF-1 suppresses the transcriptional activity of ERα through phosphatidylinositol 3-kinase (PI3K) pathway. Meanwhile, ERβ is a target gene of the transcriptional activity of ERα (49). This implies that the expression of ERα may actually reflect to that of ERβ. In the present study, the higher estrogen level in the exercise group as compared to control group may suppress the transcriptional activity of both ERs.

Conclusion

The present study found that regular physical exercise prevents ovariectomized-induced deterioration of motor coordination and cerebellar estrogen levels of rats. It is likely that the beneficial effects of exercise are exerted through estrogen. Nevertheless, the precise mechanisms by which exercise and estrogen
alter cerebellar functioning require further investigations.

Acknowledgment
The results reported in this article were part of Saidah Rauf’s thesis (Koordinasi motorik tikus Sprague Dawley yang disiovarietIkoni meningkat dengan latihan fisik teratur dan terukur, kajian terhadap kadar estrogen cerebellum dan kadar reseptor estrogen B cerebellum). This research was funded by the grant of The Agency for the Development and Empowerment of Human Resources, Ministry of Health, the Republic of Indonesia (No. HK.03.05/1/II/11984/2010) and SAME project of the DGHE, Ministry of Education and Culture, the Republic of Indonesia. The authors would like to thank Suparno (Department of Physiology, Faculty of Medicine, Gadjah Mada University) and Rumbiwati (Department of Parasitology, Faculty of Medicine, Gadjah Mada University) for their technical assistance.

Conflict of interests
The authors declare that there is no conflict of interest.

References
1. Chakraborty TR, Gore AC. Aging related changes ovarian hormones, their receptors, and neuroendocrine function. Biol Med 2004; 229:777-987.
2. Rao SS, Singh M, Parkar M, Sugumar A. Health maintenance in postmenopausal women. Am Fam Physician 2008; 78:583-591.
3. Wise PM, Dubal DB, Wilson ME, Rau SW, Bottner M. Minireview: Neuroprotective effects of estrogen - new insight into mechanism of action. Endocrinology 2001; 142:969-973.
4. McEwen BS, Alves SE. Estrogen actions in the central nervous system. Endocr Rev 1999; 20:279-307.
5. Pope P, Miall C. How might the cerebellum participate in motor control, if life without one is possible? Adv Clin Neurosci Rehabil ACNR 2011; 10:16-18.
6. Koibuchi N, Kimura-Kuroda J, Ikeda Y, Tsutsui K. Neurosci Rehabil ACNR 2011; 10:16-18.
7. Baeza I, De Castro NM, Giménez-Llort L, De la Fuente M. Ovarioectomy, a model of menopause in rodents, causes a premature aging of the nervous and immune systems. J Neuroimmunol 2010; 219:90–99.
8. Hayase K, Tanaka M, Tujioka K, Hirano E, Habuchi O, Yokogoshi H. 17-b-estradiol affects brain protein synthesis rate in ovariectomized female rats. J Nutr 2001; 131:123–126.
9. Shima N, Yamaguchi Y, Yuri K. Distribution of estrogen receptor β mRNA-containing cells in ovariectomized and estrogen-treated female rat brain. Anat Sci Int 2003; 78:85–97.
10. Mohamed MK, Abdel-Rahman AA. Effect of long-term ovarioectomy and estrogen replacement on the expression of estrogen receptor gene in female rats. Eur J Endocrinol 2000; 142:307-314.
11. Stoffel-Wagner B. Neurosteroid metabolism in human brain. Eur J Endocrinol 2001; 145:669-679.
25. Sokol RZ, Okuda H, Stanczyk FZ, Wolfe GW, Delaney JC, Chapin RE. Normative reproductive indices for male and female adult Sprague Dawley rats. Contraception 1999; 59:203-207.
26. Bupha-Intr T, Laosiripisarn J, Wattanapermpool J. Moderate intensity of regular exercise improves cardiac SR Ca2+ uptake activity in ovariectomized rats. J Appl Physiol 2009; 107:1105-1112.
27. Partadiredja G, Sutarman, Yahya TN, Nuryana CN, Susilowati R. Curcumin alters motor coordination but not total number of Purkinje cells in the cerebellum of adolescent male Wistar rats. J Integr Med 2013; 11:32-38.
28. Partadiredja G, Bedi KS. Mice undernourished before, but not after, weaning perform better in motor coordination and spatial learning tasks than well-fed controls. Nutr Neurosci 2011; 14:129-137.
29. Sakamoto H, Mezaki Y, Shikimi H, Ukena K, Tsutsui K. Dendritic growth and spine formation in response to estrogen in the developing Purkinje cell. Endocrinology 2003; 144:4466-4477.
30. Gorton LM, Vuchovic MG, Vertelkina N, Petzinger GM, Jalousc MW, Wood RI. Exercise effects on motor and affective behavior and catecholamine neurochemistry in the MPTP-lesioned mouse. Behav Brain Res 2010; 213:253-262.
31. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: Definitions and distinctions for health-related research. Pub Health Rep 1985; 100:126-131.
32. Kobuchi N. Hormonal regulation of cerebellar development and plasticity. Cerebellum 2008; 7:1-3.
33. Jung ME, Yang SH, Brun-Zikerbagel A, Simpkin JW. Estradiol protects against cerebellar damage and motor deficit in ethanol-withdrawn rats. Alcohol 2002; 26:89-93.
34. Zeng-Li C, Guang-Li F, Chun-Mei Z, Yi-Dan H. Effects of estrogen on ER, NGF and CHAT expression in cerebellum of aging female Sprague Dawley rat. Agric Sci China 2007; 6:368-374.
35. Smith CL. Cross-talk between peptide growth factor and estrogen receptor signaling pathways. Biol Reprod 1998; 58:627-632.
36. Hedges VL, Elbner TJ, Meisel RL, Mermelstein PG. The cerebellum as a target for estrogen action. Front Neuroendocrinol 2012; 33:403-417.
37. Gruber CJ, Tschugguel W, Schneebberger C, Huber JC. Production and actions of estrogens. N Engl J Med 2002; 346:340-352.
38. Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: From bench to treatment. Pharmacol Rev 2005; 57:359-383.
39. Simpson ER, Davis SR. Minireview: aromatase and the regulation of estrogen biosynthesis—some new perspectives. Endocrinology 2001; 142:4589-4594.
40. Tsutsui K. Neurosteroid biosynthesis and action in the Purkinje cell. J Exp Neurosci 2009; 2:1-12.
41. Pedersen BK, Steensberg A, Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol 2001; 536:329-337.
42. Nybo L, Nielsen B, Pedersen BK, Møller K, Secher NH. Interleukin-6 release from the human brain during prolonged exercise. J Physiol 2002; 542:991-995.
43. Simpson E, Rubin G, Clynge C, Robertson K, O’Donnell L, Davis S, et al. Local estrogen biosynthesis in males and females. Endocr Relat Cancer 1999; 6:131-137.
44. Guzmán C, Hernández-Bello R, Morales-Montor J. Regulation of steroidogenesis in reproductive, adrenal and neural tissues by cytokines. Open Neuroendocrinol J 2010; 3:161-169.
45. Andreescu CE, Milockovic BI, Haasdijk ED, Kramer P, Jong FH, Krust A, et al. Estradiol improves cerebellar memory formation by activating estrogen receptor β. J Neurosci 2007; 40:10832-10839.
46. Qin J, Suh JM, Kim B-J, Yu C-T, Tanaka T, Kodama T, et al. The expression pattern of nuclear receptors during cerebellar development. Dev Dyn 2007; 236:810-820.
47. Mendez P, Wandosell F, Garcia-Segura LM. Cross-talk between estrogen receptors and insulin-like growth factor-I receptor in the brain: Cellular and molecular mechanisms. Front Neuroendocrinol 2006; 27:391-403.
48. Garcia-Segura LM, Arevalo M-A, Aznaita I. Interactions of estradiol and insulin-like growth factor-I signalling in the nervous system: New advances. In: Martini L, Chrousos GP, Labrie F, Pacak K, Pfaff DW, editors. Progress in Brain Research. Neuroendocrinology: The Normal Neuroendocrine System. Amsterdam: Elsevier; 2010. p. 251-272.
49. Wilk A, Hellsten Y, Berthelson P, Lundholm L, Fischer H, Jansson E. Activation of estrogen response elements is mediated both via estrogen and muscle contractions in rat skeletal muscle myotubes. Am J Physiol Cell Physiol 2009; 296:C215-C220.