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*Note*

# Effect of Royal Jelly Diet on the Testicular Function of Hamsters

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**To investigate the long-term effect of feeding royal jelly (RJ) on the testicular function, 32-week old male golden hamsters were fed diet containing RJ at doses of 0 μg/g diet (control), 50 μg/g diet or 500 μg/g diet for 12 weeks. At the end of the experiment, the hamsters were assessed for testicular function in terms of the amounts of intra-testicular free testosterone (TS) and histopathological changes. RJ diet groups showed higher TS levels and more intensive spermatogenesis than the control group in a dose-dependent manner. The intensity of spermatogenesis and TS levels in the 500 μg of RJ/g diet group showed significant differences of $p < 0.01$ and $p < 0.05$, respectively, when compared with those in the control group. These results indicate that the long-term feeding of RJ inhibits the age-associated decline in the testicular function of male hamsters.**

Keywords: royal jelly, testis, testosterone

The oldest historical reference to the honeybee is considered to be that on a rock painting approximately 7,000 years old, located in Arana Cave in Spain (Rembold, 1965). Although only honey was used as a source of sugar up to the Middle Ages, in recent times, other beehive products have generated interest as therapeutic and nutritive agents, namely royal jelly (RJ), propolis and bee pollen. RJ that is produced by the hypopharyngeal and mandibular glands of the worker honeybees is well known to be a necessary food for the queen honeybee, and the physical properties of RJ are well known. It is a creamy, opalescent, and white liquid, and its major components are carboxylic acids including 10-hydroxy-2-decenoic acid, free amino acids, proteins, sugars, minerals, and vitamins (Rembold, 1965; Lercker *et al.*, 1982). RJ has been used for many years as an anti-aging agent, a hormonal stimulant, an energy enhancer, for cholesterol control, as a wound healing agent, and for general healthcare use (Allen *et al.*, 1958; Elkins, 1996). Various scientific works have shown that RJ has biological functions in mammals. We have also shown that RJ has collagen-inducing activity (Koya-Miyata *et al.*, 2002), anti-allergic activity (Kataoka *et al.*, 2002), anti-atopic activity (Taniguchi *et al.*, 2003), anti-inflammatory activity (Kohno *et al.*, 2004), and anti-aging functions (Inoue *et al.*, 2003).

Regarding hormone-like effects, Townsend *et al.* (1940) reported that the fruit fly (*Drosophila melanogaster*) experienced a remarkable influence on the number of eggs and on the rate of reaching sexual maturity after being fed an ether-soluble fraction of RJ. Kato *et al.* (1988) also demonstrated that the weight of the testes, epididymides, seminal vesicles and prostate glands of male mice increased after the subcutaneous injection of RJ. Furthermore, Takahashi *et al.* (1962) reported that the intramuscular injection of the ether-soluble fraction of RJ increased spermatogenesis in the testes of mice and rats. Thus, various studies concerning the biological effects of RJ on the mammalian genital organs have been carried out parenterally. However, it remains to be resolved whether orally administered RJ promotes the testicular functions. In this study, we therefore fed RJ to old male hamsters and investigated the histopathological changes of the genital organs.

Abbreviations: RJ, royal jelly; TS, testosterone; LPO, Serum lipid hydroperoxide

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## Materials and Methods

*Experimental Animals* This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals, of the National Institutes of Health (1978). Male golden hamsters (31-week-old) maintained as a closed colony at our facility were used. The hamsters were acclimatized by feeding a commercial powdered diet NMF (Oriental Yeast Co., Osaka, Japan) *ad libitum* for 7 days. Two hamsters were housed per polycarbonate cage in an animal facility maintained at 23 ± 2°C with a 12 h light-dark cycle (light, 0700–1900 h). After acclimatization, 32-week-old hamsters weighing 150 g–190 g, were used.

*Experimental diets* Samples of native RJ were collected from the Paraibuna region of Sãn Paulo, Brazil, and were kept frozen at –40°C until use. Pulverized RJ was prepared by adding nine volumes of the disaccharide trehalose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan), which prevents the degeneration of protein and fatty acid (Oku *et al.*, 2002), to one volume of natural RJ. Experimental diets were prepared by adding the pulverized RJ to NMF diet at the amount of 50 μg or 500 μg of RJ per gram of diet. The compositions are summarized in Table 1.

**Table 1.** Composition of experimental diets.

| Diet | Control | 50 μg of RJ/g diet | 500 μg of RJ/g diet |
|---|---|---|---|
| NMF-powdered diet | 99.5% | 99.5% | 99.5% |
| Trehalose | 0.500% | 0.495% | 0.450% |
| Royal jelly | 0.000% | 0.005% | 0.050% |

*Experimental design* After the adaptation period, hamsters were weighed and randomly assigned to each experimental group ($n = 10$). The hamsters were given free access to the respective diet and water for 12 weeks. Daily food intake and weekly body weight changes of individual animals were monitored throughout the experiment.

*Sampling and analytical procedures* After 12 weeks of the experimental period, at the age of 44 weeks, blood was collected from the heart under anesthesia induced by diethyl ether inhalation, and transferred to a blood collection tube (Venoject II, Terumo Co. Ltd., Tokyo, Japan). The serum was separated by centrifugation at 2,500 rpm at room temperature for 20 min, and stored at –40°C until analysis. After blood collection, both testes were removed and weighed. The right testis was fixed in 15% buffered formalin, and embedded in paraffin for histology to examine any morphological changes. Paraffin blocks were cut into 2 μm thick sections and stained with hematoxylin-eosin for microscopic examination. The other testis was stored at –40°C until measurement of free testosterone (TS) levels.

Serum lipid hydroperoxide (LPO) levels, as a marker of oxidative stress that increases during senescence (Yanagawa *et al.*, 1999; Yasui *et al.*, 2003), were measured with commercial kits (Lipid Hydroperoxide Assay Kit, Funakoshi Co. Ltd., Tokyo).

To determine male genital function, the testis was homogenized by using an ultrasonic homogenizer (Sonifier, Branson Ultrasonics Corp., Danbury, CT, USA) for approximately 30 sec, and the supernatant was separated by centrifugation (15,000 rpm × 1 min). TS levels in the supernatants were measured using commercially available RIA kits (DPC Corp., Los Angels, CA, USA).

Histopathological analysis of the testicular tissues was performed under the light microscope according to the method of Dostal *et al.* (1988). In brief, from 5 randomly chosen fields (magnification 40 ×) of the testicular tissues, both normal and abnormal seminiferous tubules showing atrophy, degeneration and loss of germ cells were separately counted (magnification 100 ×). After calculating the sum of the seminiferous tubules in the 5 chosen fields, the intensity of spermatogenesis was presented as the proportion of normal seminiferous tubules.

*Statistical analyses* Data were expressed as the mean ±SD. Statistical analyses were performed by the Bartlett and one-way ANOVA methods, using a suitable computer software package (StatFlex Version 5.0, Artech INC., Osaka, Japan), and the differences between the means were assessed by Dunnett's multiple comparison methods. $p$-values less than 0.05 were considered statistically significant.

## Results

*Food intake and weight gain* Hamsters weighing an initial average of 163 g to 164 g were fed the experimental diets. They consumed 7.9 g to 8.3 g of their diet per day during 12 weeks of feeding period (Table 2). There were no significant differences in food intake and body weights during the experimental period among the 3 groups.

*Testicular free TS levels* Since it is well known that spermatogenesis depends on the action of TS (Sharpe *et al.*, 1988), we measured the intra-testicular levels of TS in the RJ-fed hamsters. As shown in Fig.1, the long-term feeding of RJ resulted in an increase in the intra-testicular TS levels in a dose-dependent manner. In particular, TS levels in the 500 μg of RJ/g diet group increased significantly ($p < 0.05$).

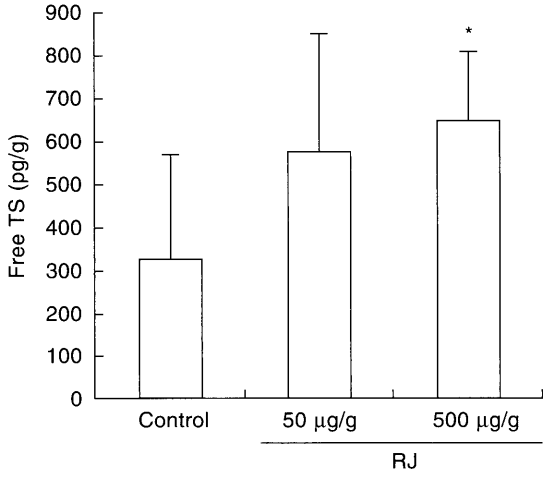


**Fig. 1.** Intra-testicular free TS levels per gram of testis at 44 weeks of age. Each value represents the mean ± S.D. of 10 hamsters for each group. *$p < 0.05$, significantly different from controls by Dunnett's test.

*Histopathological observations* There was no difference in testis weight among the three groups. However, the testicular tissues in the control group (Fig. 2A) showed severe degeneration (atrophy, detachment and diminution of germ cells), which was not observed in younger hamsters at 17 weeks of age (Fig. 2D). In marked contrast, the

**Table 2.** Comparison of daily food consumption.

| Group | No. of hamsters | Daily food intake (g) | Daily RJ intake (mg/kg body weight) | Body weight (g) | |
|---|---|---|---|---|---|
| | | | | Initial | Final |
| Control | 10 | 7.9 ± 0.38 | 0 | 164.0 ± 10.8 | 169.8 ± 8.9 |
| 50 μg of RJ/g diet | 10 | 8.1 ± 0.30 | 2.3 ± 0.2 | 163.4 ± 13.6 | 171.7 ± 14.3 |
| 500 μg of RJ/g diet | 10 | 8.3 ± 0.62 | 24.2 ± 2.6 | 163.9 ± 12.3 | 163.4 ± 7.7 |

Each value represents the mean ±S.D. for each group.

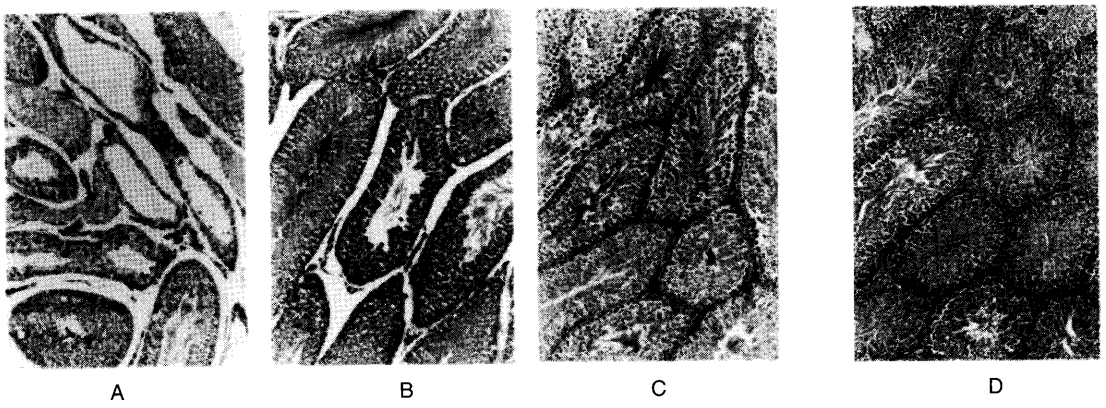


**Fig. 2.** Histological findings in the testicular tissues at 44 weeks of age after feeding a control diet (A) or RJ diets (B, C) for 12 weeks, and those in younger hamsters at 17 weeks of age (D). (A) Control group shows severe loss of germ cells and large lumens within the seminiferous tubules. (B) The 50 μg of RJ/g diet group exhibits a mixture of degenerative changes and vivid spermatogenesis in the testicular tubules. (C) The 500 μg of RJ/g diet group shows remarkably intense spermatogenesis in the same tissues. (D) Severe degeneration was not observed in younger hamsters at 17 weeks of age.

severe degenerative changes were not observed in RJ-fed hamsters (Fig. 2B and C). The proportion of the normal seminiferous tubules, which was calculated by counting the number of normal and abnormal seminiferous tubules in the 5 randomly chosen fields, is shown in Fig. 3. The percentage of testicular tubules showing vivid spermatogenesis was significantly ($p < 0.01$) higher in 500 μg of RJ/g diet-fed hamsters than that in hamsters of the control group.

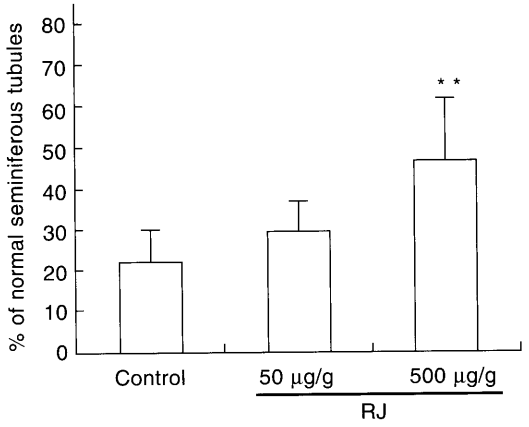


**Fig. 3.** Long-term feeding of RJ inhibits the decline in the number of normal testicular seminiferous tubules showing strong spermatogenesis. Each value represents the mean ±S.D. of 10 hamsters for each group. $^{**}p < 0.01$, significantly different from controls by Dunnett's test.

*Serum LPO levels* It has been shown that LPO, as a marker of oxidative stress, increases with age (Yanagawa *et al.*, 1999; Yasui *et al.*, 2003). We therefore measured serum LPO levels in the RJ-fed hamsters. Interestingly, serum LPO levels in the hamsters of the RJ-fed groups decreased compared with those in hamsters of the control group (Fig. 4). Significant decrease in the serum LPO levels was observed in the 500 μg of RJ/g diet group ($p < 0.05$). These results suggest that the long-term feeding of RJ inhibits the age-related increase of serum LPO levels.

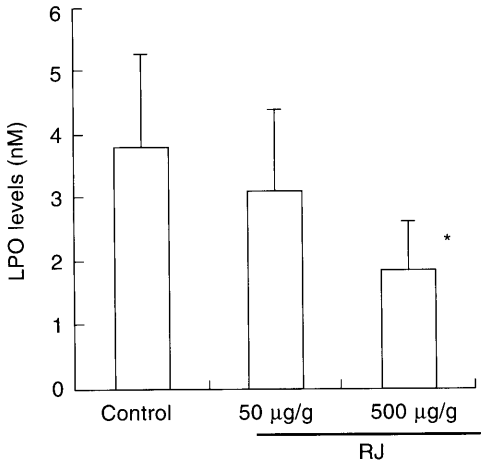


**Fig. 4.** Long-term feeding of RJ decreases the serum LPO. Values of LPO were shown as the means ± S.D. of 10 hamsters for each group. $^{*}p < 0.05$, significantly different from controls by Dunnett's test.

## Discussion

RJ has been traditionally used for years as an anti-aging agent, hormonal stimulant, energy enhancer, natural antidepressant, cholesterol control, and for many other physiological functions. However, the biological effects of RJ have not been fully elucidated. Although early studies reported on the gonadotropic or estrogenic effects in experimental animals by parenteral RJ administration (Townsend *et al.*, 1940; Kato *et al.*, 1988; Takahasi *et al.*, 1962; Heyl, 1939), no experiments to examine the effect of orally administered RJ on the testicular function have been reported. In this study, we confirmed that the feeding of RJ delays the decline in the testicular function of male hamsters. Histological degenerative changes, such as atrophy, diminishment and detachment of germ cells, were not observed in the RJ-fed hamsters. Furthermore, long-term feeding of RJ inhibited the age-associated decline in the intra-testicular TS levels. Considering that TS plays crucial roles in spermatogenesis, these results suggest that orally administered RJ inhibited the loss of TS secreting cells such as Leydig cells in the testis. In this regard, Castro *et al.* (2002) have shown that the number of Leydig cells per gram of testis correlates with both plasma and testicular levels of TS.

The hormone-like effects of RJ extracts in experimental animals were reported in a very early study (Heyl,

1939), and later by Vittek *et al.* (1982) who demonstrated that TS-like compounds are present in RJ as determined by a RIA method. These results further suggest that the compounds like a gonadotropic hormone contained in the RJ may inhibit the decline of testicular function in mammals via activating Leydig cells.

It is well known that the decline of testicular function is attributable to age-associated changes. The mechanism of aging at the biochemical level can be explained by the production of free radicals, which are induced by oxidative stress, resulting in the cellular membrane damage. Free radicals attack proteins, nucleic acids, carbohydrates and lipids of the cells (Günther *et al.*, 1991; Leibovitz *et al.*, 1980; Sohal *et al.*, 2002). It is widely accepted that free radicals induce LPO and play an important role in age-related pathological phenomena including the clustering of degenerative diseases (Günther *et al.*, 1991; Harman, 1981; Tokumaru *et al.*, 1996). Sugawara *et al.* (1990) demonstrated that testicular LPO levels in 28 week-old mice increase significantly compared with the levels in 6 week-old mice. Therefore, we investigated serum LPO levels as a marker of oxidative stress. Our results showed that feeding 500 μg of RJ/g diet significantly suppressed LPO levels compared with those in the hamsters of control group. The finding that the long-term daily intake of RJ inhibited the generation of LPO suggests that RJ could protect organs from free radical-induced cellular damage. These results further prompt us to speculate that RJ may have the oxygen free radical scavenging function in addition to the gonadotropic hormone function, and thereby inhibited the decline of male testicular function. Further studies will help to fully elucidate the mechanisms involved.

In conclusion, our study demonstrated for the first time that the long-term feeding of RJ inhibits the decline of male hamster testicular function.

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## References

Allen, R. and Lust, J. (1958). "The Royal Jelly Miracle." Benedict Lust Publications, New York.

Castro, A.C.S., Berndtson, W.E. and Cardoso, F.M. (2002). Plasma and testicular testosterone levels, volume density and number of Leydig cells and spermatogenic efficiency of rabbits. *Braz. J. Med. Biol. Res.*, **35**, 493–498.

Dostal, L.A., Chapin, R.E., Stefanski, S.A., Harris, M.W. and Schwetz, B.A. (1988). Testicular toxicity and reduced Sertoli cell numbers in neonatal rats by di(2-ethylhexyl) phthalate and the recovery of fertility as adults. *Toxicol. Appl. Pharmacol.*, **95**, 104–121.

Elkins, R. (1996). "Bee Pollen, Royal Jelly, Propolis and Honey. An Extraordinary Energy and Health Promoting Ensemble." Woodland Publishing Inc., Pleasant Grove.

Günther, T. (1991). Magnesium deficiency, oxygen radicals and aging. *Magnesium-Bulletin*, **13**, 78–81.

Harman, D. (1981). The aging process. *Proc. Natl. Acad. Sci. USA*, **78**, 7124–7128.

Inoue, S., Koya-Miyata, S., Ushio, S., Iwaki, K., Ikeda, M. and Kurimoto, M. (2003). Royal jelly prolongs the life span of C3H/HeJ mice: correlation with reduced DNA damage. *Exp. Gerontol.*, **38**, 965–969.

Heyl, H.L. (1939). An observation suggesting the presence of a gonadotropic hormone in royal jelly. *Science*, **89**, 540–541.

Kataoka, M., Arai, N., Taniguchi, Y., Kohno, K., Iwaki, K., Ikeda, M. and Kurimoto, M. (2001). Analysis of anti-allergic function of royal jelly. *Natural Medicines*, **55**, 174–180 (in Japanese with English abstract).

Kato, A., Onodera, M. and Ishijima, Y. (1988). Effect of royal jelly on development of genital organ in male mice. *J. Tokyo Vet. Anim. Sci.*, **35**, 1–4.

Kohno, K., Okamoto, I., Sano, O., Arai, N., Iwaki, K., Ikeda, M. and Kurimoto, M. (2004). Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Biosci. Biotechnol. Biochem.*, **68**, 138–145.

Koya-Miyata, S., Takei, Y., Ushio, S., Iwaki, K., Ikeda, M. and Kurimoto, M. (2002). Royal jelly and ascorbic acid 2-O-alpha-glucoside (AA-2G) increase collagen synthesis in normal hamster skin fibroblast cultures. *Natural Medicines*, **56**, 191–194 (in Japanese with English abstract).

Leibovitz, B.E. and Siegel, B.V. (1980). Aspects of free radical reactions in biological systems: Aging. *J. Gerontol.*, **35**, 45–56.

Lercker, G., Capella, P., Conte, L.S., Ruini, F. and Giordani, G. (1982). Components of royal jelly II. The lipid fraction, hydrocarbons and sterols. *J. Apic. Res.*, **21**, 178–184.

Oku, K., Sawatani, I., Sugimoto, S., Kanbe, M., Takeuchi, K., Murai, S., Kurose, M., Kubota, M. and Fukuda, S. (2002). Functional properties of trehalose. *J. Appl. Glycosci.*, **49**, 351–357 (in Japanese with English abstract).

Rembold, H. (1965). Biologically active substances in royal jelly. *Vitam. Horm.*, **23**, 359–382.

Sharpe, R.M., Donachie, K. and Cooper, I. (1988). Re-evaluation of the intratesticular level of testosterone required for quantitative maintenance of spermatogenesis in the rat. *J. Endocrinol.*, **117**, 19–26.

Sohal, R.S., Mockett, R.J. and Orr, W.C. (2002). Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic. Biol. Med.*, **33**, 575–586.

Sugawara, N. and Chen, B.Q. (1990). Concentration in, and binding of several metals by myelin from mice. *Res. Commun. Chem. Pathol. Pharmacol.*, **67**, 387–394.

Takahashi, K., Kiuchi, K., Endô, M., Furuno, Y., Shinozuka, T. and Hasegawa, S. (1962). Fractions actives de la gelée royale. *Société Franco-Japonaise de Biologie*, **30**, 428–430 (in French).

Taniguchi, Y., Kohno, K., Inoue, S., Koya-Miyata, S., Okamoto, I., Arai, N., Iwaki, K., Ikeda, M. and Kurimoto, M. (2003). Oral administration of royal jelly inhibits the development of atopic dermatitis-like skin lesions in NC/Nga mice. *Int. Immunopharmacol.*, **3**, 1313–1324.

Tokumaru, S., Iguchi, H. and Kojo, S. (1996). Change of the lipid hydroperoxide level in mouse organs on ageing. *Mech. Ageing Dev.*, **86**, 67–74.

Townsend, G.F. and Lucas, C.C. (1940). Chemical examination of the lipid fraction of royal jelly. *Science*, **92**, 43.

US Dept of Health, Education and Welfare, Public Health Service, National Institute of Health. Guide for the care and use of laboratory animals. DHEW Publication, 1978; No.: (NIH) 78–23.

Vittek, J. and Slomiany, B.L. (1982). Testosterone in royal jelly. *Experientia*, **40**, 104–106.

Yanagawa, K., Takeda, H., Egashira, T., Sakai, K., Takasaki, M. and Matsumiya, T. (1999). Age-related changes in alpha-tocopherol dynamics with relation to lipid hydroperoxide content and fluidity of rat erythrocyte membrane. *J. Gerontol. A Bio. Sci. Med.*, **54**, B379–383.

Yasui, F., Ishibashi, M., Matsugo, S., Kojo, S., Oomura, Y. and Sasaki, K. (2003). Brain lipid hydroperoxide level increases in senescence-accelerated mice at an early age. *Neurosci. Lett.* **350**, 66–68.