Influence of coenzyme Q10 supplementation on skin follicle characteristics in gonadectomized mice

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Abstract. Several factors including steroid hormones and oxidative stress are associated with cyclic growth of hair follicle and fibre diameter. This study aimed to investigate the effect of gonadectomy and dietary supplementation of CoQ10 on skin follicle characteristics and hair diameter in mice. Twenty four 8-weeks-old male NMRI mice were randomly selected. Eighteen mice were bilaterally gonadectomized (Gdx) and the remaining six mice received a sham operation and randomly assigned into four experimental groups of six animals per group (control, Gdx, Gdx125 and Gdx250 received 0.0, 0.0, 125 and 250 mg CoQ10/kg/d, respectively). At the end of experiment, blood, skin and fibre samples were collected. The mice in Gdx and CoQ10 groups had significantly lower plasma testosterone concentration than those in control group. Similar skin TAC concentrations were recorded in mice of control, Gdx and CoQ10 supplemented groups, whereas plasma TAC concentration was greatest in Gdx250 and lowest in control and Gdx groups (P < 0.05). The value of over-hair (O) follicle per mm² skin and its percentage were greater in Gdx group in compared with those in control and CoQ10 supplemented Gdx, while the density of under-hair (U) follicle per mm² skin and its percentage were lowest in Gdx group. The lowest values for percentage of active U follicle, U/O ratio and greater value for fibre diameter were observed in Gdx group (p < 0.05). It is concluded that the gonadectomy and dietary supplementation of CoQ10 influence both skin follicle characteristics and hair diameter in mice.

Key words: gonadectomy, over-hair, under-hair, follicle activity, testosterone, total antioxidant capacity.

Introduction

The hair follicle is an epidermal derivative capable of periodic remodeling and regeneration. The hair follicle cycle involves a period of follicle growth and cell proliferation (anagen), followed by a brief transitional phase (catagen), where the follicle shortens to one-third of its anagen length and finally by a rest period (telogen), during which the follicles are quiescent and there is no hair growth (Alonso & Fuchs 2006). In mice, a large collection of hair follicles in any particular body site cycle together, where synchronous follicle growth occurs in large waves (Stenn & Paus 2001).

Several factors including hormones and growth factors affect the cyclic growth of hair follicle. In this regard, studies revealed that the sex steroids influence the cyclic activity of hair follicle. For example, it has been demonstrated that androgens perform a crucial role in regulation of the hair follicle growth cycle (Randall 1994). In male mice, testosterone inhibits hair follicle growth and blocks the transition from telogen to anagen, while gonadectomy in male mice stimulates hair follicle growth to anagen phase (Azzi et al. 2004). Moreover, the incidence of low testosterone concentration represents a condition of oxidative stress (Mancini et al. 2008). Oxidative stress is defined as an imbalance between production and destruction of reactive oxygen species. It has been indicated that the excess in reactive oxygen species plays a vital role in creating an abnormal hair follicle cycling (Liu et al. 2013, Naito et al. 2008). On the other hand, a positive association between plasma total antioxidant capacity (TAC) and testosterone has been observed by (Demirbag & Erel 2005). However, the role of sex steroids on skin TAC is not yet completely understood.

Coenzyme Q10 (CoQ10) is a fat-soluble and vitamin-like substance that is an essential component of the mitochondrial respiratory chain (Santos-Ocaña & Clarke 2002). As a potent antioxidant agent, it stimulates cell growth and inhibits its cell death and lipid peroxidation by scavenging free radicals and regenerating α-tocopherol (Miles 2007). Giesen et al. (2009) suggested the beneficial effects of CoQ10 as an anti-aging bioactive on the human hair follicle. However, the possible association between gonadal steroids, CoQ10 and skin follicle characteristics has not yet been investigated. The current study was therefore undertaken to use male mice as a model to investigate whether low blood testosterone concentrations are associated with oxidative stress-affecting hair follicle growth and if these effects can be antagonized by anti-oxidative treatment with CoQ10.

Materials and methods

Animals and experimental conditions

Twenty four male NMRI mice with average 8 weeks of age and 28 g body weight were obtained from the Laboratory of Animal Facility, School of Veterinary Medicine, Razi University, Kermanshah, Iran. The animals were allocated in groups (four) of six individuals per cage and maintained at standard housing facilities (23 °C ± 1 and 12 h light/dark cycle) with free access to food and drinking water. They were fed with a standard laboratory mouse diet (Dane-e-pars Co., Kermanshah, Iran) and water ad-libitum and left to acclimatize for 2 weeks before the onset of experiment. This experiment was approved by the Laboratory Animal Care Committee of the Razi University. The experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

Eighteen mice were bilaterally gonadectomized (Gdx) and the remaining six mice received a sham operation under a Ketamine (50 mg/kg; 80 mg/kg; Alfasan Co., Netherlands)/ diazepam (0.5 mg/kg; Chemi Darou Co., Iran) cocktail, as previously performed by (Cernetich et al. 2006) and (Hannah et al. 2008). Animals were allowed for two weeks to recover from the operation before they were introduced to dietary supplementation period. They were weighed weekly for two weeks prior to initiation of the CoQ10 supplementation. After recovering from the gonadectomy or sham operation, mice were randomly assigned into four experimental groups of six animals per group as follows: 1) Intact (control); 2) Gdx, received 0.0
mg CoQ10/kg/d; 3) Gdx125, received 125 mg CoQ10/kg/d; and 4) Gdx250, received 250 mg CoQ10/kg/d. Starting from the third week after operation and during 14 weeks the animals were received experimental diets.

Preparation of diet: Standard laboratory mouse diet was wet by adding drinking water in equal proportions to 10% (v/w) of the diet. CoQ10 (Nutralife Co., Ontario, Canada) was mixed into the wet diet, to achieve the final concentrations of CoQ10 (0.075% or 0.15% w/w). The mixture was processed into pellet type chow and dried at room temperature overnight. The control diet was processed the same, without addition of CoQ10. The diet was prepared weekly. Based on average body weight through the supplementation period (30 g), a food intake of 5 g/d and the final concentrations of CoQ10 in the diet, the mice in Gdx125 and Gdx250 groups were received 125 and 250 mg/kg/d of CoQ10, respectively. Animals of control and Gdx groups were fed unsupplemented standard diet during the same period.

Plasma testosterone measurement
Blood samples were collected at day 96 by heart puncture, centrifuged and plasma collected. Plasma samples were frozen immediately at -80 °C within 30 minutes and stored until hormone analysis. Testosterone concentration (ng/ml) was measured in thawed plasma samples using ELISA kits (Diometra, Italy), according to the instruction of manufacturer.

Skin Sampling: Mice were sacrificed at day 96 by cardiac puncture under anesthesia with Ketamine and diapason. After shaving the hair, two skin samples (1 cm²) were collected from the right mid-side (flank) of each animal. One Sample was frozen in dry ice and stored at -80 °C until homogenized for measuring total antioxidant capacity (TAC). The other sample was immediately fixed and stored in 10% buffered formalin (w/v) for histological processing.

Estimation of skin and plasma total antioxidant capacity (TAC): Frozen skin samples were thawed and homogenized at 10% w/v in PBS 50 mM, pH 7.4. Homogenates were centrifuged at 40 °C for 15 minutes and supernatants were stored at -80 °C until used for measuring TAC. Quantitative determination of TAC was measured in skin supernatants and plasma samples using a stable free radical a,a-diphenyl-b-picyril hydradyl (DPPH), at the concentration of 0.2 mM in methanol. Aliquots of the frozen skin supernatant and plasma samples were thawed at room temperature, and 0.1 ml of the sample was deproteinized by the addition of 1 ml of methanol, vortexed for 30 s and centrifuged at 3000 rpm for 30 min to separate the proteins. Methanol (1.5 ml) and DPPH solution (0.5 ml) were added to the clear supernatant, mixed thoroughly and absorbance was read at 517 nm against blank. Ascorbic acid was used as a reference standard. The standard graph was plotted using different concentrations of ascorbic acid and the antioxidant status values for skin and plasma were expressed in terms of mM of ascorbic acid.

Follicle density, under-hair/over-hair follicle ratio (U/O ratio) and follicle activity
Fixed skin samples were dehydrated through a series of graded ethanol, cleared in histoclear using a Citadel tissue processor (Histokinette 200, Cambridge Instruments Company) and embedded in paraffin using Leukhardt blocks. Transverse sections of 6 mm thickness were cut using a base sledge microtome (Model Leica rm 213s, Nussloch, Germany). The sections were placed on slides and stained by using the Sapcit staining procedure (Reis & Sahlu 1994). The level immediately under sebaceous gland was used for carrying out microscopically observations.

The over-hair (O) and under-hair (U) follicles were identified according to their size, shape and associated glands and counted per 1 mm² of 12 clusters in each histological section. The counted follicles classified as inactive or active according to the absence or presence of fibre and a distinct bright red-stained inner root sheath, respectively. The recorded data were used to calculate the follicle density (FD) per 1 mm² field, the ratio of follicles (U/O) and the percentage of active follicles. A correction factor (area of mounted skin section/area of the trephine) was used to adjust follicle densities for shrinkage in the diameter of the transverse sections during excision, fixation, and processing (Ryder & Stephenson 1968).

Hair Sampling and Measurements: At the end of experiment, the hair patch samples (1x1 cm) were taken from the left mid-side area (flank) of the mice. The hair samples were then washed in a 10 µm nylon filter folded in a funnel. Six 200-mL aliquots of 0.3% Tween 80 detergent at 60 °C were poured through the hair, followed by 6 rinses with deionized water at 40-50 °C and two 200-mL ethanol (100%) rinses. The washed hair was then air-dried at room temperature. One hundred clean fibres from each sample were randomly selected to measure the mean hair diameter of the samples, using an inverted microscope fitted with an eyepiece measuring graticule. The minimum detectable difference in diameter that could be measured was 1µm.

Statistical analyses
All values were expressed as least square means ± SE. Differences between means analyzed by Tukey’s test. Test values with a P < 0.05 were considered significantly different. The data for both O and U follicle activity were modified by application of a log10 transformation for homogeneity of variance. The statistical analysis was performed using Minitab Statistical software (vers. 17.1, 2013).

Results
Skin follicle measurements
The value of O follicle per mm² skin and their percentage were greatest in Gdx group, while the density of U follicle per mm² skin and their percentage were lowest in Gdx group (Table 1). The lowest values for percentage of active U follicle, U/O ratio and greatest fibre diameter were observed in Gdx group (P < 0.05). There was no significant difference between the mean values for percentage of active O follicles observed in four experimental groups (Table 2, Fig. 1).

Table 1. Effect of CoQ10 supplementation on skin follicle characteristics of intact and Gonadectomized (Gdx) mice.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Control</th>
<th>Gdx</th>
<th>Gdx125</th>
<th>Gdx250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicle Density</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (no/mm²)</td>
<td>75.00±5.1</td>
<td>77.50±4.8</td>
<td>78.55±4.5</td>
<td>72.87±4.8</td>
</tr>
<tr>
<td>Over-hair (no/mm²)</td>
<td>39.85±2.9</td>
<td>46.48±1.1</td>
<td>43.81±2.1</td>
<td>38.50±2.7</td>
</tr>
<tr>
<td>Over-hair (%)</td>
<td>52.49±2.7</td>
<td>59.98±2.2</td>
<td>54.14±1.9</td>
<td>54.55±2.1</td>
</tr>
<tr>
<td>Under-hair (no/mm²)</td>
<td>35.14±1.7</td>
<td>30.50±2.0</td>
<td>36.16±2.9</td>
<td>34.33±1.2</td>
</tr>
<tr>
<td>Under-hair (%)</td>
<td>47.50±2.7</td>
<td>40.01±2.1</td>
<td>45.85±1.8</td>
<td>45.44±2.2</td>
</tr>
<tr>
<td>Active follicle (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over-hair</td>
<td>91.64±1.7</td>
<td>93.24±1.6</td>
<td>90.91±1.2</td>
<td>91.81±1.6</td>
</tr>
<tr>
<td>Under-hair</td>
<td>93.67±1.6</td>
<td>88.53±1.3</td>
<td>91.02±1.1</td>
<td>90.68±1.2</td>
</tr>
<tr>
<td>U/O ratio</td>
<td>0.88±0.04</td>
<td>0.66±0.05</td>
<td>0.85±0.04</td>
<td>0.89±0.03</td>
</tr>
<tr>
<td>Active</td>
<td>0.90±0.06</td>
<td>0.62±0.04</td>
<td>0.85±0.04</td>
<td>0.88±0.05</td>
</tr>
</tbody>
</table>

* Values in the rows with different superscripts are significantly different (P < 0.05).

Body weight and hair diameter
The average body weight and hair diameter values are presented in Table 2. There was no significant difference in the average body weight of the mice in four experimental groups. The mean hair diameter of mice in Gdx group was significantly greater than that of mice in control and CoQ10 treated groups, however no significant difference of hair diameter was observed among the mice in control, Gdx125 and
Table 2. Effect of CoQ10 supplementation on average body weight (g), plasma TAC (nM of ascorbic acid) and hair diameter (µm) of intact and Gonadectomized (Gdx) mice.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Control</th>
<th>Gdx</th>
<th>Gdx125</th>
<th>Gdx250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>31.90±1.2</td>
<td>33.50±1.35</td>
<td>32.38±2.25</td>
<td>34.51±2.23</td>
</tr>
<tr>
<td>Plasma TAC</td>
<td>286.3±63.5c</td>
<td>279.6±40.9c</td>
<td>878.0±15.0b</td>
<td>1213.0±80.0a</td>
</tr>
<tr>
<td>Hair diameter</td>
<td>27.4±0.5b</td>
<td>31.8±0.5a</td>
<td>26.8±0.7b</td>
<td>27.07±0.8b</td>
</tr>
</tbody>
</table>

abc Values in the rows with different superscripts are significantly different (P < 0.05).

Figure 1. Transverse section through the skin sample (at the sebaceous gland level) from adult mice, presenting follicle groups consisting of both over-hair and under-hair follicles.

Gdx250 groups.

Testosterone and TAC
The plasma testosterone concentration in control group varied from 3.0 to 11.2 ng/ml (Fig. 1). The mice in Gdx and CoQ10 treated groups had significantly lower level of plasma testosterone concentration than those in control group, while no significant difference was observed between the values of mice in Gdx, Gdx125 and Gdx250 groups. Similar values for skin TAC were found in mice of control, Gdx as well as CoQ10 treated groups (P > 0.05; Fig. 2), whereas plasma TAC concentration was greatest in Gdx250 and lowest in control and Gdx groups (P < 0.05; Table 2).

Discussion
To the best of our knowledge, this is the first report of Gdx mice being supplemented with the CoQ10, and the recording of the associated skin follicle changes as well as hair diameter and their relationship with blood testosterone and skin and plasma TAC concentrations. An understanding of the factors controlling and affecting the skin follicle activity and hair growth is desirable, because such knowledge lead to exciting new ways to treat hair disorders.

In mouse skin, there are several distinct hair follicle types that differ in the time of initiation, size, shape and presence or absence of kinks in the hair shaft (Schlake 2007, Slee 1962). The earliest formed follicles become largest and produce the over-hair consisting of coarse hairs named monotrichs, awl, and auchenes, and those initiated after birth produce the under-hair of zigzags (Dry 1926).

In the present investigation, skin follicle characteristics of mice have been described using follicle histology. Histological observation on hair follicle pattern is in agreement with the findings of (Duverger & Morasso 2009) who demonstrated that in normal mouse, hair follicles are found in specific arrays, with large follicles being interspersed by smaller follicles throughout the skin (Fig. 1). This pattern of follicle arrangement was uniform in all experimental groups. However, the U/O ratio measured in our control group was less than the range of 1.86 to 2.33 reported by (Duverger & Morasso 2009) in mouse.

It is well known that the hair follicle is one of the androgen-sensitive appendages in the skin (Azziz et al. 2004, Deplewski & Rosenfield 2000). It has been recognized that androgens have paradoxically different effects on human hair follicle. For instance, during and after puberty androgens stimulate tiny vellus follicles to transform into larger and deeper follicles in the face, upper pubic diamond and chest (Randall 1994). In contrast, androgen may cause the reverse transformation of large and deep follicles to miniaturised vellus follicles (Randall 1994). Testosterone is a potent androgenic steroid which is produced primarily by the Leydig cells of the testis and, to a much lesser extent, at peripheral tissue including the skin and its appendages (Chen & Zirkin 1999). Our results demonstrated that the male intact mice had normal levels of plasma testosterone concentration within the physiological range observed in normal mice (Nelson et al. 1975), which was reduced in Gdx animal to a low level as reported for Gdx male BALB/c mice (Frisancho-Kiss et al. 2009). Previous studies have shown that sex hormones including testosterone delay anagen in rats (Chase 1954), while gonadectomy in rats and mice (Azzi et al. 2004, Chase 1954) advance it and triggers a rapid hair growth with a thicker hair shaft diameter in mice. Moreover, it has been indicated that the underhair follicles are affected most in the adult mice (Falconer et al. 1951).

In the present study we observed that the absence of androgens or other mediator released from the testes in Gdx mice did not influence the overall number of follicle per skin surface. In contrast, gonadectomy considerably increased the...
number and percentage of O follicles and reduced the num-
ber and percentage of U follicles per skin surface. These find-
ings indicated that the absence of testosterone in mice stimu-
lates tiny U follicles to transform into thicker O follicles. Such
change in the proportion of O follicles could be the main
reason for the greatest hair diameter (31.8±0.5 µm) ob-
gained in Gdx group of mice. Therefore, an interesting find-
ing of this experiment was that the gonadectomy increased
the hair diameter, but it did not occur in CoQ10 supple-
mented groups (Gdx125 and Gdx250). This could be attrib-
uted to the possible effects of CoQ10 supplementation on
inhibiting the transformation of U into O follicle under a free
testosterone environment.

It has been found that the incidence of low testosterone
concentration in hypogonadal men was associated with a
condition of oxidative stress (Mancini et al. 2008). Oxidative
stress is an imbalance between reactive oxygen species, gen-
erated in all living organism and antioxidant defense (Rizzo
et al. 2010), which represent a potential threat to all organ-
isms. Therefore, the measurement of TAC as the functional
sum of antioxidants represent a key factor for evaluating the
overall antioxidants status resulting from antioxidant intake
or production and their consumption by the increasing lev-
eals of oxidative stress (Butnariu & Samfira 2012). Moreover,
the role of sex hormones on TAC has previously been de-
scribed. In this regard, Demirbag et al (2005) found a strong
association between plasma concentrations of TAC and tes-
stosterone, showing lower TAC in men with decreased testos-
terone than normal men. In the current study, although no
changes were found in the skin TAC concentration among
experimental groups, however, the plasma TAC concentra-
tion was linearly increased with dietary supplementation of
CoQ10. This greater antioxidant capacity can explain lowest
U/O ratio and greatest hair diameter that observed in Gdx
rather than those in control as well as CoQ10 supplemented
groups.

In conclusion, results of this study showed a consid-
erable effect of gonadectomy and dietary supplementation of
CoQ10 on skin follicle characteristics and hair diameter in
mice. The greatest O follicle proportion was observed in Gdx
mice, while the values for CoQ10 supplemented mice were
similar with that of control group. Moreover, hair diameter
was greatest in Gdx mice, whereas similar hair diameter was
observed in CoQ10 supplemented and control groups. These
indicated that the absence of testosterone in mice stimulates
tiny U follicles to transform into the thicker O follicles. The
greater antioxidant capacity attained by dietary CoQ10 sup-
plementation can be a possible reason for inhibiting the trans-
formation of U into O follicle under low testosterone
environment. Further investigation is required to under-
stand the accurate underlying mechanism and association
between hair follicle characteristics, gonadectomy and other
factors such as antioxidant capacity.

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