Dietary rapeseed (canola) oil suppresses testosterone production and increases plasma aldosterone level in stroke-prone spontaneously hypertensive rats (SHRSP)

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ABSTRACT — The present study was conducted to survey the influence of canola oil (CAN) ingestion on the steroid hormone production in stroke-prone spontaneously hypertensive rats (SHRSP). Male SHR-SP were fed a diet containing 10 wt/wt% soybean oil (SOY, the control) or CAN as the sole dietary fat for 8 weeks. Plasma concentration of luteinizing hormone (LH) was similar in the 2 dietary groups. However, the plasma testosterone level in the CAN group, 1.36 ± 0.271 ng/mL, was lower than in the SOY group, 2.79 ± 0.514 ng/mL (p < 0.05, unpaired t-test; n = 10), and plasma concentration of aldosterone in the CAN group, 345 ± 79.6 pg/mL, was higher than in the SOY group, 159 ± 33.7 pg/mL (p < 0.05, unpaired t-test; n = 10). In the testis, the expressions of mRNA for StAR, CYP11A1, CYP17, 3βHSD and 17βHSD and the amounts of the corresponding proteins were significantly decreased. However, in the adrenal gland, the expressions of mRNA for StAR, CYP11A1, 3βHSD and CYP11B1 in the CAN group were not different from those in the SOY group, but the expression of mRNA and the amount of the corresponding protein for CYP11B2 were increased significantly in the CAN group. These findings are indicative of a peripheral, testicular toxicity of CAN. The decreased testosterone and the concomitantly increased aldosterone may play a role in the aggravation by CAN of the genetic diseases (i.e., metabolic syndrome-like complications) in male SHRSP.

Key words: Rapeseed (canola) oil, Testosterone, Aldosterone, Testicular toxicity, Stroke-prone spontaneously hypertensive rat (SHRSP)

INTRODUCTION

Rapeseed oil (canola oil, CAN) is known to shorten the survival of male stroke-prone spontaneously hypertensive rats (SHRSP) compared to animals given soybean oil (SOY, the control) (Huang et al., 1996, 1997; Miyazaki...
The life-shortening by CAN is attributable to aggravation of the inherent diseases of SHRSP (i.e., metabolic syndrome-like conditions), including hypertension (Naito et al., 2003), increased plasma lipids (Ohara et al., 2006; Papazzo et al., 2011a), decreased antioxidant enzymes (Papazzo et al., 2011b); vascular injuries (Miyazaki et al., 1998) and thrombopenia (Ohara et al., 2006). On the other hand, CAN is reported to suppress testicular testosterone production in SHRSP compared to control animals given a diet containing SOY (Okuyama et al., 2010). Testosterone deficiency has been known to cause hypertension and cardiovascular disease, which are included in the major symptoms of metabolic syndrome in both, men (Saad and Gooren, 2009; Kelly and Jones, 2013) and in rodents (Hermoso et al., 2020; Baik et al., 2020; Kelly et al., 2016). Since SHRSP, which exhibit a predisposition to the development of complications similar to metabolic syndrome, have been used as a model animal for metabolic syndrome (Hiraoka-Yamamoto et al., 2004; de Artiñano and Castro, 2009; Tanaka et al., 2013), the suppressed testosterone production by CAN may give rise to an early onset of hypertension and leads to life-shortening. It is unknown if CAN also decreases the testosterone level in men. However, considering those effects are induced by one of the most widely consumed oils in the dietary life of humans, the effects of CAN in SHRSP are worth investigating in more detail.

In addition to the suppression of testosterone production in male SHRSP (Okuyama et al., 2010), CAN elevates plasma aldosterone in male Wistar rats (Ohara et al., 2008). These findings make us suspect the influence of CAN on the metabolic pathway of steroids. However, so far, there are no reports referring to the influence of CAN on steroid hormone production. Thus, the present study was conducted to survey the influence of CAN in SHRSP are worth investigating in more detail.

Table 1. Fatty acid compositions (%) of in the diets.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>SOY</th>
<th>CAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>14: 0 Myristic acid</td>
<td>0.14</td>
<td>0.22</td>
</tr>
<tr>
<td>16: 0 Palmitic acid</td>
<td>9.01</td>
<td>4.37</td>
</tr>
<tr>
<td>16: 1 Palmitoleic acid</td>
<td>0.07</td>
<td>0.21</td>
</tr>
<tr>
<td>18: 0 Stearic acid</td>
<td>4.38</td>
<td>1.93</td>
</tr>
<tr>
<td>18: 1 Oleic acid</td>
<td>23.64</td>
<td>63.67</td>
</tr>
<tr>
<td>18: 2 n-6 Linoleic acid</td>
<td>54.50</td>
<td>19.56</td>
</tr>
<tr>
<td>18: 3 n-3 Linolenic acid</td>
<td>7.23</td>
<td>8.00</td>
</tr>
<tr>
<td>20: 0 Arachidic acid</td>
<td>0.39</td>
<td>0.59</td>
</tr>
<tr>
<td>20: 1 Eicosenoic acid</td>
<td>0.20</td>
<td>1.15</td>
</tr>
<tr>
<td>22: 0 Behenic acid</td>
<td>0.45</td>
<td>0.32</td>
</tr>
<tr>
<td>Total fatty acids (g/100 g diet)</td>
<td>9.88</td>
<td>9.22</td>
</tr>
</tbody>
</table>

SOY, 10 w/w% soybean oil diet (control); CAN, 10 wt/wt% canola oil diet.

MATERIALS AND METHODS

Diets

SOY and CAN were gifts from the Japan Oilseed Processors Association (Tokyo, Japan). A fat-free AIN-93G powder diet supplemented with 10 wt/wt% (24.8 energy percentage) SOY and the same diet containing, instead, 10 wt/wt% CAN were prepared in CLEA Japan (Tokyo, Japan). The fatty acid compositions of the diets are shown in Table 1.

Animal husbandry

Twenty male SHRSP (Izm), at 4 weeks of age were purchased from Japan SLC (Shizuoka, Japan). Two to three animals were housed per plastic cage, with a bedding of wood chips, and acclimatized for 7 days in a room with a conditioned temperature of 23 ± 1°C and a relative humidity of 50 ± 5% and with lighting from 8:00–20:00. After acclimation, the animals were divided into 2 groups of 10 animals each. The animals in each group were given, ad libitum, the SOY diet or the CAN diet and tap water for 8 weeks. The diets were replaced with fresh ones every 3 days. The SOY diet was used as the control, since general rat chows contain SOY as the major fat ingredient, and the life-span of SHRSP fed one of several different diets, each of which contained a single oil or fat as the sole dietary fat (Huang et al., 1996).

All the animals were used following the requirements set by the Committee for Ethical Usage of Experimental Animals in Kinjo Gakuin University.

Blood and tissue samples

At the end of the feeding period, the animals were anes-
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Determination of plasma FSH and LH

Plasma concentrations of FSH and LH were measured using commercially available ELISA kits, E-EL-R0391 with a detection range of 3.31–200 ng/mL (Elabscience Biotechnology, Houston, TX, USA) and ENZ-KIT107 with a detection range of 0.14–32.9 ng/mL (ENZO Life Sciences, Lausen, Switzerland), respectively, following the instructions provided in the kits, and the results were obtained by subjecting 96-well plates to colorimetric detection at 450 nm.

Determination of steroids in plasma and testis

The concentrations of steroids were determined using a method described previously (Takagi et al., 2010). Briefly, 2H- or 13C-labelled steroids were added to the plasma as internal standards, and steroids were extracted in diethyl ether. The standards used were as follows: testosterone-13C3 and aldosterone-d7 (Isosciences, King of Prussia, PA, USA). The extracts were then purified using an InertSep pharma column (GL Sciences, Tokyo, Japan) and then derivatized with picolinyl ester. The resulting derivatives were placed in an InertSep SI column (GL Sciences), and the eluted fractions were injected into liquid chromatography-mass spectrometry/mass spectrometry (LC/MS/MS) equipment, comprising a Nexera UHPLC system (Shimadzu, Kyoto, Japan) and an API4000 mass spectrometer (SCIEX, Framingham, MA, USA).

Quantitative analysis of mRNA expression

The total RNA was extracted from the frozen testicular and adrenal tissues using Trizol™ reagent (Thermo Fisher Scientific, Tokyo, Japan) and chloroform-isopropanol with phase-separation by centrifugation. The concentrations of total RNA obtained was determined by absorbance at 260 nm using an Ultra-micro spectrophotometer NanoDrop™ 1000 (Thermo Fisher Scientific). Two μg of total RNA was reverse-transcribed to cDNA using a High Capacity RNA-to cDNA™ kit (Thermo Fisher Scientific). The cDNA obtained was diluted (1:2) and used with Eagle’Taq universal MMX™ (Roche Diagnostics, Tokyo, Japan) and a probe/primer kit, TaqMan Assay™ (Thermo Fisher Scientific). The target genes and TaqMan Assays™ used were as follows: glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Rn01777563_g1; steroidogenic acute regulatory protein (StAR), Rn00580695_m1; CYP11A1, Rn00568733_m1; CYP11B1, Rn02607234_g1; CYP11B2, Rn01767818_g1; CYP17, Rn00562601_m1; 3βHSD, Rn01789220_m1; 17βHSD, Rn00562601_m1.

Real-time PCR was performed using a 7300 Real-Time PCR System (Applied Biosystems-Thermo Fisher Scientific, Tokyo, Japan) programmed as follows: a 10-min holding period at 95°C, followed by 40-cycle amplification for 15 sec at 95°C, and 1-min at 60°C. Expression values were calculated according to the ΔΔCT method. The expression of each mRNA relative to that for glyceraldehyde-3-phosphate dehydrogenase was compared between the 2 diets.

Western blotting

The expression of protein corresponding to every mRNA whose expression was significantly different between the 2 dietary groups was investigated by Western blotting. Aliquots of the tissues of the testes and adrenal glands were homogenized in 4 volumes of 10 mmole/L Tris-HCl buffer (pH 7.4) containing 137 mmole/L NaCl, 0.1 mmole/L EDTA, 20% glycerol and protease inhibitors cocktail (Thermo Fisher Scientific) using Ultra-Turrax™ (Ika, Staufen, Austria). The homogenates were centrifuged at 1,000 x g for 15 min at 4°C and the supernatants were obtained. The protein concentrations of the supernatants were determined by BCA protein assay reagents (Nacalai Tesque) and each sample was diluted to a protein concentration of 1 mg/mL. The sample was mixed with an equivalent volume of Laemmlı buffer (pH 6.8), consisting of 62.5 M Tris, 2% SDS, 12% 2-mercaptoethanol, 25% glycerol and 0.01% bromphenol blue and denatured at 95°C for 5 min. A 15 μL aliquot of each sample was separated by 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane...
using a Trans-Blot™ semi-dry electrophoretic transfer cell (Bio-Rad Laboratories, Tokyo, Japan). The membrane was soaked in Bullet Blocking One™ (Nacalai Tesque) for 10 min at room temperature and washed in 20 mmole/L Tris-HCl buffer containing 137 mmole/L NaCl, 0.1% Tween 20 (TBS-T buffer, pH = 7.6). Then, the specimens were incubated in a primary antibody to β-actin, diluted to 1: 5000–20000 (8457, Cell Signaling Technology, Danvers, MA, USA), GAPDH, diluted to 1: 2000 (sc-32233, Santa Curz Biotechnology, Dallas, TX, USA), StAR, diluted to 1: 100–500 (8449, Cell Signaling Technology), CYP11A1, diluted to 1: 1000 (AB1244, Sigma-Aldrich Merck, Tokyo, Japan), CYP17, diluted to 1:200–1000 (sc-66850, Santa Curz Biotechnology), 3βHSD, diluted to 1:1000 (sc-515120, Santa Curz Biotechnology), 17βHSD diluted to 1:100–200 (sc-32872, Santa Curz Biotechnology) or to CYP11b2 diluted to 1:1000 (PA-5-67694, Invitrogen, Thermo Fisher) overnight at 4°C. The membrane was rinsed in TBS-T buffer and incubated with a suitable secondary IgG conjugate to horseradish peroxidase (Goat Anti-Mouse IgG HRP Conjugate 71045 or Goat Anti-Rabbit IgG Antibody or HRP-conjugate 12-348, Sigma-Aldrich Merck) for 1 hr at room temperature or Simple Stain MAX-PO MULTITM (Nichirei Biosciences) and incubated for 30 min at room temperature. After washing with PBS containing 0.005% Tween20 the specimens were covered with 1 drop of 3,3’-diaminobenzidine, tetrahydrochloride, DAB solution (peroxidase stain DAB kit, Nacalai Tesque) and incubated strictly for 10–12 seconds. The specimens were washed with distilled water followed by PBS containing 0.005% Tween20, and nuclear staining was done using hematoxylin. The DAB-stained preparations were placed under a BZ X-800 microscope (Keyence, Osaka, Japan) and the total number of Leydig cells within 10 microscopic fields at a magnification of 200 were counted for each specimen.

**Statistical methods**

The results are presented as means ± S.E. The differences of the variables between the dietary groups were compared by unpaired t-test when the data sampled from two populations followed a Gaussian distribution or by Mann-Whitney test when the distribution was non-Gaussian. In all cases significance was set at p < 0.05. Prism v.9.10 (GraphPad Software, San Diego, CA, USA) was used for the statistical analyses.

**RESULTS**

**Plasma FSH and LH**

Plasma concentrations of FSH and LH are shown in Table 2. Neither of the gonadotropins was different between the SOY diet and the CAN group.

**Steroids in plasma and testis**

Plasma concentrations of pregnenolone, progesterone, corticosterone, 11-dehydrocorticosterone, deoxycorticosterone and dehydroepiandrosterone in the CAN group tended to be increased but were not significantly different from those in the SOY group. In contrast, the plasma concentrations of both, aldosterone and testosterone, were significantly different between the dietary groups (i.e., the aldosterone concentration was significantly higher and the testosterone concentration significantly lower, in the CAN group than in the SOY group). In the testis, the tissue concentrations of both, pregnenolone and testosterone, were significantly decreased in the CAN group compared to the SOY group. Plasma androstenedione and
Canola oil suppresses testosterone production

Table 2. Plasma FSH and LH levels in male SHRSP given SOY diet or CAN diet for 8 weeks.

<table>
<thead>
<tr>
<th>Gonadotropins</th>
<th>SOY</th>
<th>CAN</th>
<th>p value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (pg/mL)</td>
<td>351 ± 19.2</td>
<td>347 ± 50.0</td>
<td>0.9366 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>LH (ng/mL)</td>
<td>18.7 ± 1.88</td>
<td>19.5 ± 0.634</td>
<td>0.6831 n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

SOY, 10 wt/wt% soybean oil diet (control); CAN, 10 wt/wt% canola oil diet.
Values are means ± S.E. of 6 animals.
n.s., Group mean values are not significantly different (unpaired t-test).

Table 3. Plasma and testicular tissue concentrations of steroids in male SHRSP given SOY diet or CAN diet for 8 weeks.

<table>
<thead>
<tr>
<th>Steroids</th>
<th>SOY</th>
<th>CAN</th>
<th>p value</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnenolone (ng/mL plasma)</td>
<td>0.373 ± 0.0731</td>
<td>0.554 ± 0.175</td>
<td>0.3128 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Progesterone (ng/mL plasma)</td>
<td>965 ± 299</td>
<td>1600 ± 816</td>
<td>0.4755 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Corticosterone (ng/mL plasma)</td>
<td>305 ± 57.2</td>
<td>332 ± 52.0</td>
<td>0.7315 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>11-Dehydrocorticosterone (ng/mL plasma)</td>
<td>29.4 ± 5.25</td>
<td>33.4 ± 3.67</td>
<td>0.5402 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Deoxy corticosterone (ng/mL plasma)</td>
<td>2.66 ± 0.840</td>
<td>3.67 ± 1.53</td>
<td>0.5700 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (ng/mL plasma)</td>
<td>3.77 ± 1.79</td>
<td>2.61 ± 0.22</td>
<td>0.2241 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Androstenedione (ng/mL plasma)</td>
<td>512 ± 94.3</td>
<td>301 ± 58.0</td>
<td>0.0729 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Aldosterone (ng/mL plasma)</td>
<td>159 ± 33.7</td>
<td>345 ± 79.6</td>
<td>0.0433 s.d.</td>
<td>Mann-Whitney test</td>
</tr>
<tr>
<td>Testosterone (ng/mL plasma)</td>
<td>2.79 ± 0.514</td>
<td>1.36 ± 0.271</td>
<td>0.0247 s.d.</td>
<td>Unpaired t-test</td>
</tr>
<tr>
<td>Pregnenolone (ng/g testis)</td>
<td>36.4 ± 1.98</td>
<td>27.2 ± 3.20</td>
<td>0.0251 s.d.</td>
<td>Unpaired t-test</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (ng/g testis)</td>
<td>1.63 ± 0.386</td>
<td>0.784 ± 0.172</td>
<td>0.0630 n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Testosterone (ng/g testis)</td>
<td>167 ± 28.1</td>
<td>92.2 ± 21.4</td>
<td>0.0492 s.d.</td>
<td>Unpaired t-test</td>
</tr>
</tbody>
</table>

SOY, 10 wt/wt% soybean oil diet (control); CAN, 10 wt/wt% canola oil diet.
Values are means ± S.E. of 10 animals.
s.d., Group mean values are significantly different (p < 0.05 by Man-Whitney test or unpaired t-test).
n.s., Group mean values are not significantly different.

Testicular dehydroepiandrosterone concentrations in the CAN group tended to be lower than in the SOY group.

**mRNA expression for proteins involved in steroid metabolism in the testis and the adrenal gland**

In the testis, the expressions of mRNA for StAR, CYP11A1, CYP17, 3βHSD and 17βHSD in the CAN group were significantly depressed without exception compared to the SOY group (Fig. 1A). In the adrenal gland, the expressions of mRNA for StAR, CYP11A1 and 3βHSD in the CAN group tended to be increased but were not significantly different compared to those in the SOY group. Interestingly the expression of mRNA for CYP11B2 in the CAN group was significantly increased and 3 times as high as that in the SOY group. The expressions of mRNA for CYP11B1 in the 2 dietary groups were similar (Fig. 1B).

**Expressions of proteins involved in steroid metabolism**

The expression of every protein corresponding to mRNA whose expression was significantly different
between the 2 dietary groups was investigated by Western blotting. The protein expressions of StAR, CYP11A1, CYP17, 3βHSD and 17βHSD in the testis in the CAN group were significantly depressed compared to those in the SOY group. In the adrenal gland, the expression of CYP11B2 in the CAN group was significantly increased compared to that in the SOY group (Fig. 2).

**Leydig cell number**

Leydig cell numbers were comparable in the 2 die-
Fig. 2. Amounts of protein of StAR and enzymes involved in the steroid metabolism in the testis and the adrenal gland (CYP11B2) of male SHRSP given SOY diet or CAN diet for 8 weeks. SOY, 10 wt/wt% soybean oil (control) group; CAN, 10 wt/wt% canola oil group. *P < 0.05, ***P < 0.001, significantly different from the values in the soybean oil group (unpaired t-test).

DISCUSSION

Male SHRSP given the CAN diet showed a significant overall suppression of the gene expression and protein production in the testis for StAR, CYP11A1, CYP17, 3βHSD and 17βHSD, all of which are responsible for testosterone production. In these animals, the concentrations of pregnenolone and testosterone in the testicular tissue were significantly decreased compared to those in the animals given the SOY diet. The decreased production of testosterone resulted in a significantly lower plasma testosterone level in the animals given the CAN diet. In contrast to the testis, the CAN diet did not affect the gene expression for StAR, CYP11A1or 3βHSD in the adrenal gland. Therefore, CAN appears to affect the 2 organs differently, although they both possess similar steroid metabolism pathways. The CAN effect may not involve central but only peripheral action (i.e., suppression of the steroid metabolism in the testis), because the decrease in testosterone level was not accompanied by changes in plasma LH concentration. Since Leydig cell numbers were comparable in the animals given the 2 different diets, the CAN-induced suppression might not be due to organic or pathological changes but to a functional change in the Leydig cells.

Since StAR is the rate-determining protein in the steroid metabolism and responsible for the transport of cholesterol into mitochondria (Clark et al., 1994; Stocco and Clark, 1996; Christenson and Strauss, 2000), a shortage or a malfunction of StAR by CAN may result in the suppressed expressions of the enzymes (i.e., CYP11A1, CYP17, 3βHSD and 17βHSD), and in the suppressed production of the intermediate products which are regulated by these enzymes in the steroid metabolism pathway. Thus, StAR and transcription factors for StAR (Rust et al., 1998; Tremblay et al., 2002; Manna et al., 2003) cannot be ruled out as possible targets in CAN toxicity. However, even though cholesterol is the immediate precursor of pregnenolone in the steroid metabolism pathway in both, the testis and the adrenal gland, and the role of StAR is common to the pathways in both organs, CAN-induced suppression of the gene expressions of the molecules were found only in the testis. If StAR or a tran-
scription factor for StAR is a target in CAN toxicity, the depressed gene expression for StAR should have been seen not only in the testis but also in the adrenal gland. Although concentrations of steroids in the adrenal gland were not determined in the present study, plasma concentrations of pregnenolone, progesterone, corticosterone, deoxycorticosterone and 11-deoxycorticosterone, all of which might be associated with the amounts of the intermediate products in the pathway for glucocorticoid and mineralocorticoid production in the adrenal glands, and the expressions of mRNAs for StAR, CYP11A1, CYP17 and 3βHSD in the adrenal gland in the CAN group were not decreased but, rather, tended to be increased compared to those in the SOY group. On the basis of these findings, the adrenal gland is not, but the testis is, likely to be a target organ of CAN toxicity.

In the CAN group, plasma aldosterone concentration was significantly greater than in the SOY group, and the expressions of mRNA and the protein for CYP11B2 in the adrenal gland in the CAN group were also increased significantly compared to the SOY group. Since there were no significant increases in precursors of aldosterone nor in the expressions of mRNA for the enzymes involved in producing the precursors upstream in the metabolic pathway, the increased plasma aldosterone along with the increased expressions of mRNA and the protein for CYP11B2 should have been induced by some stimulus from outside of the adrenal gland. One of the possible causes is an increased plasma renin level owing to the CAN-induced vascular injuries in the kidney. In fact, it was reported that an increased incidence of nephropathy with thickening in the arterioles was found in male SHRSP given a 10 wt/wt% CAN diet compared to those given a 10 wt/wt% SOY diet (Ohara et al., 2006). Moreover, severer renal injury accompanied by a significantly increased expression of mRNA for renin was found in male SHRSP after a 9-week ingestion of a diet containing 10% CAN as compared to animals given a 10% SOY diet (Miyazaki et al., 2000). Thus, increased renin release due to renal vascular injuries is a possible cause of the increased plasma aldosterone by CAN in male SHRSP.

The existence of another possible cause for the CAN-induced increase in plasma aldosterone concentration may be indicated by the fact that the CAN-induced decrease in testosterone was accompanied by an increase in aldosterone. It is possible that the decreased plasma testosterone directly inhibited aldosterone production in the adrenal gland. According to the report by Kau et al. (1999), plasma aldosterone level was elevated in orchiectomized rats, and the aldosterone level was restored by testosterone replacement. They also reported that in primary cultured rat zona glomerulosa cells, testosterone in the range of 10⁻⁹–10⁻⁷ mol/L concentration-dependently inhibited aldosterone production. In the present study, the plasma testosterone concentration in the SOY group was approximately 0.97 x 10⁻⁹ mol/L, but that in the CAN group was 0.47 x 10⁻⁹ mol/L. These findings are suggestive of an inhibition by the CAN diet of the regulation via the suppression of testosterone production in the testis. In other words, plasma testosterone in male SHRSP may physiologically negatively regulate the production of aldosterone at the adrenal gland, and the CAN-induced decrease in testosterone would inhibit the negative regulation of aldosterone production. The testosterone deficiency is associated with metabolic syndrome in men and
induces metabolic syndrome-like symptoms in rodents (Hermoso et al., 2020; Baik et al., 2020; Kelly et al., 2016). In addition, SHRSP exhibits a predisposition to the development of complications similar to metabolic syndrome and is therefore used as a model animal of metabolic syndrome in men (Hiraoka-Yamamoto et al., 2004; de Artiñano and Castro, 2009; Tanaka et al., 2013). Thus, the suppressed testosterone production is a possible trigger for the early onset of metabolic syndrome-like symptoms and life-shortening in male SHRSP given the CAN diet.

In conclusion, it is demonstrated that the CAN diet decreases plasma testosterone concentration in male SHRSP, and the lowered testosterone level is accompanied by an increased plasma aldosterone level. The CAN diet suppresses the expressions of mRNA for StAR, CYP11A1, CYP17, 3βHSD and 17βHSD, the amounts of corresponding proteins and the intermediate products, which are regulated by those molecules in the steroid metabolism pathway. Since plasma luteinizing LH level was not affected, the decrease in testosterone with the suppression of the expressions of the molecules involved in the steroid metabolism may be due to the testicular toxicity of CAN. Although the relevancy between the 2 steroid hormones and the role of each one in the CAN-induced adverse events still remain to be elucidated, both steroids are presumed to be involved in the aggravation of metabolic syndrome-like conditions in male SHRSP.

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

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