Original Article

Quercetin Affects Spermatogenesis-Related Genes of Mouse Exposed to High-Cholesterol Diet

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ABSTRACT A high-cholesterol diet can reduce male fertility. However, it is not known whether a high-cholesterol diet can regulate the expression of genes involved in sperm maturation and sperm fertilizing ability. Quercetin, a natural product, is known to have cytoprotective effects by regulating lipid metabolism in various cell types. This study aimed to confirm the expression of genes involved in sperm maturation in the testes of mice fed a high-cholesterol diet and to determine whether quercetin can reverse the genetic regulation of cholesterol. Mice were divided into groups fed a normal chow diet and a high-cholesterol diet. Mice fed the high-cholesterol diet were dose-dependently supplemented with quercetin for 6 weeks. Investigations using quantitative PCR and in situ hybridization revealed that the high-cholesterol diet alters the expression of genes associated with sperm maturation in the testes of mice, and this was reversed with the supplementation of quercetin. In addition, the high-cholesterol diet regulated the expression of genes related to lipid metabolism in the liver of mice. Under a high-cholesterol diet, quercetin can improve male fertility by regulating the expression of genes involved in sperm maturation.

Keywords: cholesterol, lipid metabolism, quercetin, spermatogenesis, testis

INTRODUCTION

Approximately 30% of cases of male infertility have no known cause (Cavallini, 2006). Several studies have shown that obesity-related diseases are associated with a decrease in male reproductive potential, which is evidenced by a decrease in sperm parameters and testosterone levels (Lotti et al., 2013; Leisegang et al., 2014). The testis is the organ responsible for fertility by producing sperm and it functions through the physiological action of various types of cells, including Leydig cells, Sertoli cells, germ cells, and peritubular cells (Majumdar and Bhattacharya, 2013). Cholesterol is a structural component that

maintains the fluidity of cell membranes and plays an important role in regulating the physiological function of mammalian cells. Moreover, cholesterol is a precursor to steroid synthesis, which is essential for the production of normal sperm (Yokoyama, 2000). Cholesterol homeostasis is also disturbed by exposure to environmental toxins, which in turn disrupts spermatogenesis and the development of gonads (Samova et al., 2018). In rabbits, hypercholesterolemia damages Sertoli and Leydig cells in the testes and reduces sperm quality (Saez Lancellotti et al., 2010). Moreover, in male mice fed a high-cholesterol diet, the testes have been shown to have low steroidogenic enzymes and high oxidative stress (Wang et al., 2015). How-

ever, it is not known whether a cholesterol diet can affect the expression of genes related to sperm maturation and sperm fertilizing ability.

Quercetin (3,3',4',5,7-pentahydroxyflavone) is a polyphenolic flavonoid widely found in vegetables and fruits and has been shown to improve various health functions with anti-cancer, antioxidant, and anti-inflammatory effects. Quercetin also alters gene transcription and can affect signal transduction pathways that regulate cell proliferation (Brito et al., 2015). In addition, quercetin has been shown to reduce cholesterol synthesis and regulates lipid metabolism in several cell types, including pancreatic β -cells and glial cells (Carrasco-Pozo et al., 2016; Damiano et al., 2019). Moreover, quercetin is known to mitigate the harmful effects of various chemicals that damage the testes and cause reproductive toxicity in mammals (Izawa et al., 2008; Abdel Aziz et al., 2018). However, it is unclear whether quercetin can control genetic changes in the testes caused by a high-cholesterol diet.

In our previous studies, we found that high-cholesterol diets cause histological alterations in the male reproductive organs in rodents (Lim et al., 2015). In this study, we examined the change in *sperm adhesion molecule 1* (*Spam1*), *ADAM metalloproteinase domain 5* (*Adam5*), *Adam29*, *steroid 5 α -reductase 2* (*Srd5a2*), and *Srd5a3* that play a role in sperm maturation and sperm fertilizing ability in the testes of mice fed a high-cholesterol diet. In addition, quercetin was added to the feed to determine whether the changes in gene expression caused by cholesterol could be controlled. Moreover, we investigated the expression of lipid metabolism-related genes in the liver tissues of mice fed high-cholesterol and quercetin supplements. Taken together, we verified that quercetin could reverse changes caused by high-cholesterol diets in the expression of genes responsible for sperm quality in the testis.

MATERIALS AND METHODS

Experimental animals and diets

The experimental use of mice in this study was approved by the Animal Care and Use Committee of Korea University. Four-week-old male C57BL/6 mice were maintained for an adaptive period of 10 days with ad libitum diet in conditions of 12 h light and 12 h dark, according to the institutional guidelines for animal care. Afterward,

a total of 48 mice were divided into six groups. Group 1 ($n = 8$) was fed a normal diet and Groups 2 through 6 ($n = 8$ per each group) were fed a high-cholesterol diet containing 3% cholesterol and 0.5% cholic acid. Groups 3, 4, and 5 were additionally fed 25, 50, and 100 mg/kg quercetin, respectively. Finasteride (10 mg/kg) was added to the feed of group 6. Each diet was provided for 6 weeks. The composition of normal and high-cholesterol diets is provided in Table 1.

Tissue samples

Mice were euthanized after starving for 12 h. The testis and liver tissues were then stored in liquid nitrogen for RNA extraction and in 4% paraformaldehyde in phosphate buffered saline for tissue fixation.

Quantitative RT-PCR analysis

Trizol (Invitrogen, Carlsbad, CA, USA) was used to extract the total RNA. AccuPower RT premix (Bioneer, Daejeon, Korea) was used to synthesize complementary DNA. Gene expression was measured using a StepOnePlus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Green (Sigma, St. Louis, MO, USA). The C_t value, i.e., the number of cycles in which the fluorescence signal is statistically greater than that of the background, was used to quantify the expression of the target gene based on the $2^{-\Delta\Delta C_t}$ method. The primer sets used are listed in Table 2. Expression of *Gapdh* was used to normalize the expression of target genes.

Table 1. Percentage composition of normal chow and the high-cholesterol diet

Ingredients	Chow diet	High-cholesterol diet
Casein	20	20
Corn starch	40	36.5
Maltodextrin	13.2	13.2
Sucrose	7	7
Tallow	8	8
Soybean oil	2	2
Cellulose	5	5
Mineral mixture	3.5	3.5
Vitamin mixture	1	1
DL-methionine	0.3	0.3
Cholic acid	–	0.5
Cholesterol	–	3

Table 2. Primer sets used in quantitative RT-PCR analyses

Gene symbol	Sense primer (5'→3')	Antisense primer (5'→3')
<i>Gapdh</i>	AACTTTGGCATTGTGGAAGG	ATGCAGGGATGATGTTCTGG
<i>Spam1</i>	GAATGGAGGCCTACCTGGTT	CTTCCTTCCTGCCTCTTCAA
<i>Adam5</i>	AGGAGAATCTGTGGCAATGG	CCGTGCAATCTTGACTACAGC
<i>Adam29</i>	CCATGAATGTCCAGATGATGC	TTGCCTACAGTGCTCATTGC
<i>Srd5a2</i>	AGAGGCAGGCCTTTATCAGC	GAAGACACCGACGCTAAACC
<i>Srd5a3</i>	CCGCCATCAGTATAAATGC	CTCGAACCAGTCTCCAAAGG
<i>Mxlipl</i>	CTTCAAAGGCCTCAAGTTGC	GTCACGAAACCACACACTGG
<i>Pparα</i>	GCAGATGACCTGGAAAGTCC	TCTGCAAAACCAAGCTTCC
<i>Cd36</i>	AAAATCGTGGAGTGGAATGG	CTGACGGGAAGAGGTACAGG
<i>Fabp1</i>	GAGCCAGGAGAACTTTGAGC	CATGCACGATTCTGACACC
<i>Fatp5</i>	TCGGATCTGGGAATTCTACG	CAAGCTCAAAGGGAGTCAGC
<i>Cpt1</i>	GACAGACACCATCCAACACG	AGGTGTCATCCAGGATCTGC

In situ hybridization analysis

PCR products generated from cDNA were cloned using TOPO vector (Invitrogen) to synthesize hybridization probes. Plasmids with the correct gene sequence verified were amplified using T7- and SP6-specific primers. The DIG RNA labeling kit (Roche, Indianapolis, IN, USA) was then used to transcribe DIG-labeled RNA probes. Testis tissues fixed in 4% paraformaldehyde were embedded in paraffin blocks and sectioned (5 µm thick) on slide glass. The sections were deparaffinized with xylene and rehydrated in DEPC-treated water containing a gradual alcohol series. Then, the sections were treated with 1% Triton X-100 for 20 min and washed twice with DEPC-treated phosphate buffered saline. The sections were then digested at 37°C with 5 µg/mL of proteinase K (Sigma) diluted in TE buffer. Next, the sections were exposed twice for 5 min in a buffer containing 0.25% (v/v) acetic anhydride and 0.1 M triethanolamine. The sections were exposed for 10 min at room temperature in a prehybridization mixture containing 50% formamide and 4X standard saline citrate (SSC). They were then incubated in a hybridization mixture and DIG-labeled cRNA probe for 16 h at 42°C. The sections were washed for 15 min in 2X SSC, 30 min in 1X SSC, 30 min in NTE buffer, and 30 min in 0.1X SSC. Normal sheep serum (2%) was used for blocking and sections were exposed for 16 h to sheep anti-DIG antibody conjugated with alkaline phosphatase. Signals for visualizing the expression of target genes were detected using solutions containing 5-bromo-4-chloro-3-indolyl phosphate, nitroblue tetrazolium, and levamisole.

Statistical analyses

The statistical significance of quantitative PCR cells was confirmed based on the analysis of variance using SAS (SAS Institute). Differences with a probability value of $p < 0.05$ were considered statistically significant. Data are presented as mean $\pm$ SEM unless otherwise stated.

RESULTS

Effect of quercetin supplementation on high-cholesterol diet-altered expression of *Spam1*

We first confirmed the mRNA level of the *Spam1* gene in the testes of mice fed the high-cholesterol diet using quantitative PCR (Fig. 1A). *Spam1* expression was reduced by 56.0% ($p < 0.001$) in the testes of mice fed a high-cholesterol diet compared to *Spam1* expression in mice fed a normal chow diet. We added quercetin to the feed in a dose-dependent manner (0, 25, 50, and 100 mg/kg) to determine if the effect of reduced expression of *Spam1* owing to the high-cholesterol diet could be reversed by quercetin. We confirmed that 25 mg/kg of quercetin restored the mRNA level of *Spam1* to that of the control and found that even higher doses of quercetin increased the expression of SPAM1 in a dose-dependent manner. Finasteride, a competitive inhibitor of 5 α -reductase, responsible for the conversion of testosterone to DHT, the most active androgen, restored the expression of *Spam1*, reduced by a high-cholesterol diet, similar to that by quercetin. Using in situ hybridization assays, we histologically identified that quercetin can restore the expression of the *Spam1* gene reduced owing to a high-cholesterol diet (Fig. 1B). In the testes of mice supplemented with high-cholesterol and 100 mg/kg of quercetin, *Spam1* was highly expressed in the spermatogonia (Spg), spermatocytes (Spc), spermatids (Spt), and spermatozoa (Spz). Spg showed the highest expression of *Spam1* during sperm maturation in the group fed quercetin supplementation. *Spam1*, in contrast, was weakly detected in the testes from mice fed a high-cholesterol diet without quercetin supplementation.

Effect of quercetin supplementation on high-cholesterol diet-altered expression of *Adam5* and *Adam29*

Next, we identified the effects of high-cholesterol diets and quercetin supplementation on the expression of the *Adam5* and *Adam29* genes. The high-cholesterol diet increased the mRNA levels of the *Adam5* gene 1.5-fold

Fig. 1. Effects of a high-cholesterol diet and quercetin supplementation on the expression of *Spam1* in the testes of male mice. (A) Using quantitative RT-PCR, *Spam1* mRNA levels were estimated in the testes of mice fed normal chow and a high-cholesterol diet. Quercetin (0, 25, 50, and 100 mg/kg) and finasteride (10 mg/kg) were added to a high-cholesterol diet and *Spam1* mRNA levels were investigated in the testes. The relative quantities of mRNA were normalized to the quantity of *Gapdh* mRNA. The asterisks denote significant differences ($***p < 0.001$). The letter "a" means that there is a significant difference in the expression of *Spam1* in the group with quercetin or finasteride supplementation as compared to the expression of *Spam1* in the high-cholesterol diet group. (B) Localization of *Spam1* mRNA in the testes was detected through in situ hybridization analyses. Cross and longitudinal sections of the testes were hybridized with antisense or sense *Spam1* cRNA probes. Scale bars represent 10 μ m (the first horizontal panels and sense) and 5 μ m (the second horizontal panel). Spc, spermatocyte; Spg, spermatogonium; Spt, spermatid; Spz, spermatozoa.

($p < 0.01$) in the testes of male mice (Fig. 2A). Meanwhile, feeding quercetin or finasteride reduced *Adam5* expression to levels similar to that in the testes of mice fed normal chow diet. Histologic analysis of the testes also showed increased expression of *Adam5* by high-cholesterol diet compared to normal chow diet but decreased with the addition of quercetin (Fig. 2B). The expression of

Adam5 in Spg, Spc, Spt, and Spz in the testes of mice fed a high-cholesterol diet was stronger than in mice fed a normal chow diet. Spg and Spc showed the highest levels of *Adam5* expression in the testes of mice fed a high-cholesterol diet. In contrast, weak expression of *Adam5* was observed in Spg, Spc, Spt, and Spz in the testes of mice fed diets supplemented with quercetin or finasteride.

Fig. 2. Effects of a high-cholesterol diet and quercetin supplementation on the expression of *Adam5* in the testes of male mice. (A) Using quantitative RT-PCR, *Adam5* mRNA levels were estimated in the testes of mice fed normal chow and a high-cholesterol diet. Quercetin (0, 25, 50, and 100 mg/kg) and finasteride (10 mg/kg) were added to a high-cholesterol diet and *Adam5* mRNA levels were investigated in the testes. The relative quantities of mRNA were normalized to the quantity of *Gapdh* mRNA. The asterisks denote significant differences (** $p < 0.01$). The letter 'a' means that there is a significant difference in the expression of *Adam5* in the group with quercetin or finasteride supplementation as compared to that in the high-cholesterol diet group. (B) Localization of *Adam5* mRNA in the testes was detected through in situ hybridization analyses. Cross and longitudinal sections of the testes were hybridized with antisense or sense *Adam5* cRNA probes. Scale bars represent 10 μ m (the first horizontal panels and sense) and 5 μ m (the second horizontal panel). Spc, spermatocyte; Spg, spermatogonium; Spt, spermatid; Spz, spermatozoa.

The expression of *Adam29* was significantly reduced in the testes by the high-cholesterol diet (Fig. 3A). Quantitative PCR analysis revealed that a high-cholesterol diet reduced the expression of *Adam29* by approximately 77.3% ($p < 0.001$). The addition of 25 mg/kg quercetin to the high-cholesterol diet restored the expression level of *Adam29* in the testes to that of the control. In addition, feed

supplementation with 100 mg/kg quercetin or 10 mg/kg finasteride increased the expression of *Adam29* 7.9-fold and 10.9-fold, respectively, compared to the expression of *Adam29* by the high-cholesterol diet. Histological analysis through in situ hybridization also revealed that quercetin could restore the expression of mRNA levels of *Adam29* reduced by a high-cholesterol diet (Fig. 3B). In the testes

Fig. 3. Effects of a high-cholesterol diet and quercetin supplementation on the expression of *Adam29* in the testes of male mice. (A) Using quantitative RT-PCR, *Adam29* mRNA levels were estimated in the testes of mice fed normal chow and a high-cholesterol diet. Quercetin (0, 25, 50, and 100 mg/kg) and finasteride (10 mg/kg) were added to a high-cholesterol diet and *Adam29* mRNA levels were investigated in the testes. The relative quantities of mRNA were normalized to the quantity of *Gapdh* mRNA. The asterisks denote significant differences ($***p < 0.001$). The letter 'a' means that there is a significant difference in the expression of *Adam29* in the group with quercetin or finasteride supplementation as compared to that in the high-cholesterol diet group. (B) Localization of *Adam29* mRNA in the testes was detected through in situ hybridization analyses. Cross and longitudinal sections of the testes were hybridized with antisense or sense *Adam29* cRNA probes. Scale bars represent 10 μm (the first horizontal panels and sense) and 5 μm (the second horizontal panel). Spc, spermatocyte; Spg, spermatogonium; Spt, spermatid; Spz, spermatozoa.

of mice with a high-cholesterol diet, *Adam29* was rarely expressed in Spg, Spc, Spt, and Spz. However, with the addition of quercetin, high levels of *Adam29* were observed during sperm maturation, especially in Spg.

Effect of quercetin supplementation on high-cholesterol diet-altered expression of *Srd5a2* and *Srd5a3*

In the testes of mice with a high-cholesterol diet, *Srd5a2* expression increased 6.8-fold ($p < 0.001$) (Fig. 4A). The addition of 25, 50, and 100 mg/kg of quercetin in a high-cholesterol diet reduced *Srd5a2* expression by 57.7%, 65.0%, and 80.9%, respectively, compared to the *Srd5a2*

Fig. 4. Effects of a high-cholesterol diet and quercetin supplementation on the expression of *Srd5a2* in the testes of male mice. (A) Using quantitative RT-PCR, *Srd5a2* mRNA levels were estimated in the testes of mice fed normal chow and a high-cholesterol diet. Quercetin (0, 25, 50, and 100 mg/kg) and finasteride (10 mg/kg) were added to a high-cholesterol diet and *Srd5a2* mRNA levels were investigated in the testes. The relative quantities of mRNA were normalized to the quantity of *Gapdh* mRNA. The asterisks denote significant differences (*** $p < 0.001$). The letter 'a' means that there is a significant difference in the expression of *Srd5a2* in the group with quercetin or finasteride supplementation as compared to that in the high-cholesterol diet group. (B) Localization of *Srd5a2* mRNA in the testes was detected through in situ hybridization analyses. Cross and longitudinal sections of the testes were hybridized with antisense or sense *Srd5a2* cRNA probes. Scale bars represent 10 μ m (the first horizontal panels and sense) and 5 μ m (the second horizontal panel). Spc, spermatocyte; Spg, spermatogonium; Spt, spermatid; Spz, spermatozoa.

expression for the high-cholesterol diet. The addition of finasteride also reduced *Srd5a2* expression by approximately 61.9% compared to that for the high-cholesterol diet. In situ hybridization analysis also revealed that the increased expression of *Srd5a2* in the testes of mice owing to the high-cholesterol diet decreased with quercetin addition (Fig. 4B). *Srd5a2* was strongly expressed in Spg,

Spc, Spt, and Spz of mice fed the high-cholesterol diet.

Similar to *Srd5a2*, the expression of *Srd5a3* in the testes was also increased 1.7-fold (Fig. 5A) owing to the high-cholesterol diet. In addition, quercetin supplementation lowered the expression of *Srd5a3* that was increased by the high-cholesterol diet compared with the expression of *Srd5a3* in the testes of mice fed a normal chow diet. His-

Fig. 5. Effects of a high-cholesterol diet and quercetin supplementation on the expression of *Srd5a3* in the testes of male mice. (A) Using quantitative RT-PCR, *Srd5a3* mRNA levels were estimated in the testes of mice fed normal chow and a high-cholesterol diet. Quercetin (0, 25, 50, and 100 mg/kg) and finasteride (10 mg/kg) were added to a high-cholesterol diet and *Srd5a3* mRNA levels were investigated in the testes. The relative quantities of mRNA were normalized to the quantity of *Gapdh* mRNA. The asterisks denote significant differences (*** $p < 0.001$). The letter 'a' means that there is a significant difference in the expression of *Srd5a3* in the group with quercetin or finasteride supplementation as compared to that in the high-cholesterol diet group. (B) Localization of *Srd5a3* mRNA in the testes was detected through in situ hybridization analyses. Cross and longitudinal sections of the testes were hybridized with antisense or sense *Srd5a3* cRNA probes. Scale bars represent 10 μ m (the first horizontal panels and sense) and 5 μ m (the second horizontal panel). Spc, spermatocyte; Spg, spermatogonium; Spt, spermatid; Spz, spermatozoa.

tological analysis also revealed that quercetin may lower the increased expression of *Srd5a3* in the testes owing to a high-cholesterol diet (Fig. 5B). *Srd5a3* expression was especially high in Spg, Spt, and Spc owing to the high-cholesterol diet.

Expression of genes involved in lipid metabolism with the high-cholesterol diet and quercetin supplementation

Next, we examined the expression of genes involved in the uptake, transport, and synthesis of lipid content in the livers of mice fed diets containing cholesterol and quercetin. The expressions of *carbohydrate-responsive element-binding protein* (also known as *MLX interacting protein-*

Fig. 6. Relative expression of genes involved in lipid metabolism in the liver of male mice fed a diet with high-cholesterol and quercetin (100 mg/kg). Quantitation of *Mlxip1* (A), *Ppara* (B), *Cd36* (C), *Fabp1* (D), *Fatp5* (E), and *Cpt1* (F) was validated using quantitative RT-PCR. The asterisks denote significant differences (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$). The letter 'a' means that there is a significant difference in the expression of genes in the group with quercetin supplementation as compared to that in the high-cholesterol diet group.

like (*Mlxip1*)), *peroxisome proliferator-activated receptor alpha* (*Ppara*), *Cd36*, *fatty acid binding protein 1* (*Fabp1*), *fatty acid transport protein 5* (*Fatp5*), and *carnitine palmitoyltransferase I* (*Cpt1*) were all significantly reduced by the high-cholesterol diet (Fig. 6). Among these genes, the expression of *Mlxip1*, *Ppara*, and *Fabp1* was significantly recovered by quercetin supplementation. In contrast, the expression of *Cd36* was further decreased in the liver by supplementation of quercetin, whereas the expression of *Fatp5* and *Cpt1* was not significantly affected by supplementation of quercetin.

DISCUSSION

In this study, we identified changes in the expression of *Spam1*, *Adam5*, *Adam29*, *Sre5a2*, and *Sre5a3* involved in

sperm maturation and sperm fertilizing ability in the testis tissues of mice fed normal chow and high-cholesterol diets. We also found that the addition of quercetin to a high-cholesterol diet mitigates changes in gene expression caused by cholesterol. In addition, a high-cholesterol diet with quercetin supplementation was shown to regulate the expression of genes related to lipid metabolism in the liver tissues of mice. These findings suggest that a disruption of cholesterol homeostasis poses a risk to normal sperm production and that quercetin has preventive effects on these abnormalities in male mice.

Several studies have reported that the expression of proteins involved in lipid metabolism can affect testicular function in mice (Wang et al., 2017; Sharma et al., 2019). Lipids and cholesterol are essential for Sertoli cells to remodel the membranes of germ cells. Therefore, cho-

lesterol is needed to produce large amounts of germ cells during spermatogenesis. The absence of the 24-dehydrocholesterol reductase gene, the enzyme responsible for synthesizing cholesterol, causes infertility in male mice (Wechsler et al., 2003). Cholesterol is not only a major component of cell membranes but is also involved in intracellular signaling. In addition, cholesterol can be converted into bioactive substances such as steroid hormones, vitamin D, and bile acids. The production of immature sperm is one of the main causes of male infertility. In a previous study, we identified changes in the expression of apolipoprotein D, a cholesterol-binding lipoprotein in male reproductive organs of rats fed a high-cholesterol diet (Lim et al., 2016). However, no studies, to the best of our knowledge, have identified the expression of genes involved in sperm maturation and fertilization in the testes of mammals fed cholesterol.

SPAM1 is the most widely conserved mammalian sperm antigen and is expressed in both the testes and sperm of humans and rodents (Martin-Deleon, 2011). SPAM1 plays an important role in the penetration of sperm into the cumulus during fertilization. The ADAM family of proteins are distributed throughout the male reproductive track, including in the testes and epididymis (Edwards et al., 2008). Adam1 and Adam2 are involved in membrane interactions of the sperm and oocytes. Sperm in male mice deficient in Adam1 and Adam2 lack mobility in the oviduct and ability to bind to the membrane of oocytes (Cho et al., 1998; Kim et al., 2006a). Adam5 is located on the surface of mature sperm in mice (Kim et al., 2006b). Adam29 is also involved in sperm-oocyte interaction, and reduced expression of Adam29 is considered a potential indicator of male infertility (Schiza et al., 2019). Therefore, SPAM1, ADAM5, and ADAM29 are considered molecular markers of sperm maturation and sperm fertilizing ability (Jaiswal et al., 2015; Liu et al., 2019). SRD5A1 and SRD5A2 belong to the steroid 5 α -reductase family and are responsible for producing dihydrotestosterone, a derivative of testosterone (Saartok et al., 1984). In this study, a high-cholesterol diet reduced the expression of *Spam1* and *Adam29*, whereas increased the expression of *Adam5*, *Sre5a2*, and *Sre5a3* in the testes of mice. A high-cholesterol diet is suggested to affect male fertility by regulating the expression of genes involved in sperm maturation and sperm fertilizing ability. The association between these genes and the level of cholesterol in the body requires

further study.

Quercetin has cytoprotective effects by regulating lipid metabolism in many types of cells. In glial cells, expression of sterol regulatory element-binding protein (Srebp)-1, Srebp-2, and Mlx1pl at the mRNA and protein levels was reduced by quercetin (Damiano et al., 2019). Quercetin-3-glucoside, a glucoside form of quercetin, can alleviate hyperlipidemia and hyperinsulinemia in mice fed a high-cholesterol diet (Mbikay et al., 2018). Moreover, quercetin contributes to alleviating male reproductive toxicity caused by environmental factors. Quercetin restores a reduced number of Sertoli cells in mice treated with diesel exhaust particles (Izawa et al., 2008). Quercetin also modulates proteins in apoptotic pathways such as Bax, Bcl-xl, and caspase-3 in germ cells of male mice and mitigates the testicular toxicity of 4-nitrophenol (Mi et al., 2013). Quercetin also mitigates increased cholesterol levels and decreased 3 β -hydroxysteroid dehydrogenase (HSD) and 17 β -HSD activity in the testes of rats exposed to cadmium (Ujah et al., 2018). High-cholesterol diets cause oxidative stress, inflammatory responses, and mitochondrial dysfunction in the pancreas of rats; however, a diet supplemented with quercetin mitigates these detrimental effects (Carrasco-Pozo et al., 2016). In addition, our study found that quercetin can mitigate changes caused by a high-cholesterol diet in gene expression in the testes of mice. Quercetin also has a protective effect against the negative effects of the herbicide atrazine on serum testosterone levels and the expression of CYP17A1, a major protein responsible for steroidogenesis in the testes of rats (Abdel Aziz et al., 2018). In humans and rodents, testosterone is synthesized mainly in Leydig cells, and maintaining proper concentrations of testosterone is essential for spermatogenesis (Woolveridge et al., 1998). This study also revealed that finasteride, which inhibits the conversion of testosterone to its most active form, regulates changes in genes involved in spermatogenesis, similar to the effects of quercetin.

The liver is the main organ that regulates the homeostasis of cholesterol in the body. The liver regulates absorption and synthesis to prevent cholesterol from accumulating in the plasma or tissues (Goldstein et al., 2006). Mlx1pl is a glucose-sensing transcription factor that has been reported to contribute to hepatoprotection by preventing excessive cholesterol synthesis (Zhang et al., 2017). Ppara is responsible for lipid metabolism in the liver and

its transcriptional regulation has been shown to prevent hepatotoxicity from cholesterol overload (Lu et al., 2019). CD36 is involved in the intracellular uptake of cholesterol and fatty acids (Nassir et al., 2007). FABP1 combines with various hydrophobic ligands, including fatty acids in the liver, to help prevent cytotoxicity. A high-cholesterol diet is known to inhibit the expression of FABP1 (Newberry et al., 2003; Martin et al., 2006). FATP5 is also involved in the transport of fatty acids in the liver, and the deletion of FATP5 has been shown to lower serum cholesterol levels (Doege et al., 2006). CPT1 is responsible for fatty acid transport to the mitochondria, regulating fatty acid oxidation (Irvin et al., 2014). In this study, we found that high-cholesterol diets reduce the expression of lipid-related genes in the liver. The effects of quercetin in restoring the expression of genes that decreased under high-cholesterol conditions could be observed in *Mlxipl*, *Ppara*, and *Fabp1*. Further research is needed to determine whether quercetin can counterbalance the lipid metabolism in the liver caused by a high-cholesterol diet.

CONCLUSION

Taken together, we uncovered new genes related to sperm maturation and fertilization in male mice whose expression in the testes is altered with a high-cholesterol diet. In addition, confirmation that quercetin supplementation could reverse genetic changes caused by a high-cholesterol diet suggests that quercetin is a potential biologically active substance that can prevent male infertility caused by a high-cholesterol diet.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

C.Y., G.S., and W.L. conceived and designed all experiments; C.Y., H.B. collected experimental samples and conducted all experiments; G.S., and W.L. analyzed, interpreted the data, and contributed to the development of the manuscript. All authors contributed to its critical review and agreed on the final version. All authors have read and agree to the published version of the manuscript.

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