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Maternal resveratrol intake during lactation attenuates hepatic triglyceride and fatty acid synthesis in adult male rat offspring

Masato Tanaka[a], Tomomi Kita[b], Shojiro Yamasaki[a], Tae Kawahara[a], Yukako Ueno[a], Mai Yamada[a], Yuuka Mukai[b], Shin Sato[c], Masaaki Kurasaki[d], Takeshi Saito[a,e,*]

[a] Graduate School of Health Sciences, Hokkaido University, Sapporo, Japan
[b] School of Nutrition and Dietetics, Faculty of Health and Social Work, Kanagawa University of Human Services, Kanagawa, Japan
[c] Department of Nutrition, Aomori University of Health and Welfare, Aomori, Japan
[d] Environmental Adaptation Science, Faculty of Environmental Earth Science, Hokkaido University, Sapporo, Japan
[e] Laboratory of Environmental Health Sciences, Faculty of Health Sciences, Hokkaido University, Sapporo, Japan

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ABSTRACT

Resveratrol (3,5,4-trihydroxystilbene) is a natural polyphenolic compound found in grapes and red wine and has been shown to exert protective effects on the liver preventing lipid accumulation induced by a high-fat diet. However, no studies have shown that the nutritional resveratrol intake by the parental generation has modified lipogenesis in an adult offspring. The aim of this study was to investigate whether maternal resveratrol intake during lactation affects lipogenesis in adult male rat offspring, and if it does, what is the molecular mechanistic basis. Six male pups born from mothers given a control diets during lactation (CC group) and six male pups born from mothers given a control diet as well as resveratrol during lactation (CR group) were fed a standard diet until sacrifice at 36 weeks. Adult male offspring from mothers given resveratrol during lactation (CR group) had lower body weight from the fourth week of lactation until adulthood, but no significant change was observed in the relative food intake. Low levels of plasma triacylglycerol were found in the CR group compared to the CC group. Histopathological analysis of the livers of adult male rat offspring revealed lipid accumulation in hepatocytes in the CC group, whereas lipid droplets were rare in the CR group. Hepatic protein levels of AMPK-phosphorylated at ser403, Sirt1, and Nampt in the CR group were upregulated significantly compared to the CC group. These results indicated the maternal resveratrol intake during lactation-induced activation of AMPK through Sirt1 upregulation. In this study, significant upregulation of the levels of precursor of sterol regulatory element binding protein-1c (SREBP-1c) and downregulation of the ratio of active-SREBP-1c/precusor-SREBP-1c were observed in the CR group compared to the CC group. These results suggested that proteolytic processing of SREBP-1c was suppressed by AMPK in the livers of the CR group. It is well known that SREBP-1c regulates the lipogenic pathway by activating genes involved in triglyceride and fatty acid synthesis. The present study showed significant downregulation of hepatic fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) levels in the CR group. These results indicated that maternal resveratrol intake during lactation suppressed SREBP-1c cleavage and nuclear translocation and repressed SREBP-1c target gene expression such as FAS and ACC in the livers of adult male offspring. These changes attenuate hepatic triacylglycerol and fatty acid synthesis in adult male offspring.

1. Introduction

Resveratrol (3,5,4-trihydroxystilbene) is a natural polyphenolic compound found in grapes and red wine that has been shown to extend the lifespan of many organisms [1,2]. Resveratrol is well known for its biological effects, including anticancer, anti-inflammatory, and antioxidant properties [1]. Resveratrol exerts protective effects on the liver by preventing lipid accumulation induced by a high-fat diet [1–3]. Despite resveratrol's well-known health benefits, its precise mechanism of action remains controversial [4].

The biological effects of resveratrol depend on activation of Sirtuin 1 (Sirt1), a mammalian ortholog of Sir2 [5,6]. Sirt1 regulates AMP-

Abbreviations: Sirt1, Sirtuin 1; SREBPs, Sterol regulatory element binding proteins; AMPK, AMP-activated protein kinase; ACC, Acetyl-CoA carboxylase; Fas, Fatty acid synthase; Nampt, Nicotinamide phosphoribosyltransferase; LKB1, Liver kinase B1; ER, Endoplasmic reticulum

* Corresponding author at: Laboratory of Environmental Health Sciences, Faculty of Health Sciences, Hokkaido University, Sapporo 060-0812, Japan.
E-mail address: taksaito@med.hokudai.ac.jp (T. Saito).

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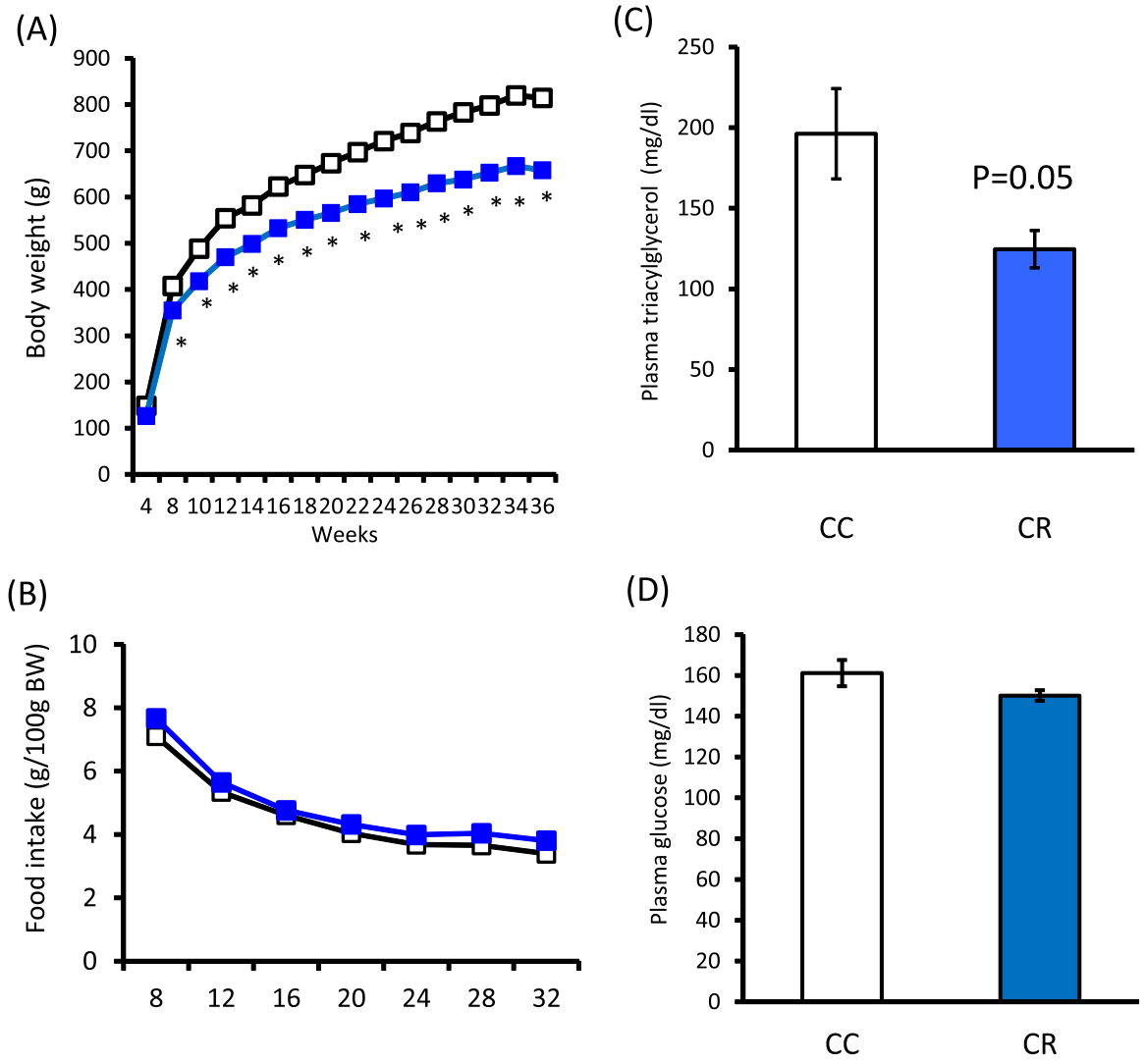

Fig. 1. Body weight and relative food intake. (A) Body weight of rats whose mothers were fed with a control diet (open square) (CC group) or control diet+administration of resveratrol diet (blue square) (CR group) during lactation. (B) The relative food intake (g/100 g body weight) from 8 weeks to 32 weeks. (C) Plasma triacylglycerol concentration (mg/dL) of rats in each group. (D) Plasma glucose concentration (mg/dL) of rats in each group. n=6 in each group. *$p < 0.05$.

activated protein kinase (AMPK) through deacetylation and activation of LKB1 [7]. A previous report has shown that polyphenols, including resveratrol, increased phosphorylation of AMPK and its downstream target, acetyl-CoA carboxylase (ACC) in HepG2 hepatocytes [8]. AMPK activation by polyphenols can explain their beneficial effects on hepatic lipid accumulation, hyperlipidemia, and atherogenesis in type 1 diabetic LDL receptor deficient mice [8].

Sterol regulatory element binding proteins (SREBPs) are key lipid synthesis transcription factors [9,10]. SREBP-1c regulates the lipogenic pathway by activating genes involved in fatty acid and triglyceride synthesis. SREBP-1c is synthesized as a precursor protein that is inserted into the endoplasmic reticulum (ER) membrane. The precursor of SREBP migrates from the ER to the Golgi and undergoes sequential proteolytic processing to release the transcriptionally active N-terminal domain. The active nuclear form of SREBP-1c is translocated into the nucleus; it binds to the sterol regulatory element and activates the transcription of SREBP-responsive genes. The dysregulation of SREBP-1c has been implicated in the pathogenesis of hepatic steatosis, dyslipidemia, and type 2 diabetes [11,12]. AMPK and AMPK activators such as polyphenols phosphorylate inhibit SREBP-1c proteolytic process, nuclear translocation, and gene expression of target lipogenic enzymes activity and it ultimately suppresses hepatocyte lipogenesis [13]. SREBP-1c regulates the lipogenic pathway by activating genes involved in fatty acid and triglyceride synthesis. Fatty acid synthase (FAS) play an essential role in *de novo* lipogenesis by converting the acetyl-CoA into palmitate that subsequently is esterified into triglycerides in the liver. ACC, the rate-limiting enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, is the pivotal enzyme in the biosynthesis of long-chain fatty acids [14].

Fetal and neonatal environmental and nutritional influences may change some physiological parameters in adulthood, a phenomenon known as programing [15–18]. Epidemiological and experimental studies indicate a relationship between the periconceptional, fetal and early infant phases of life and the subsequent development of diseases as an adult [19]. For instance, maternal low-protein diets are early-life inducers of glucose intolerance, hypertension, renal disease and obesity [20–22]. Our previous report showed that green tea extract intake during lactation modulates AMPK expression in the kidneys of adult male offspring of dams fed a protein-restricted diet and may induce long-term alterations in the expression of that protein in the kidneys [23]. Another investigation showed the potential protective effects of vinifera grape skin extract on programming-induced renal endowment in mice offspring of dams submitted to protein restriction during pregnancy, and its possible effects on oxidative stress associated with malnutrition [24]. These results indicated that maternal resveratrol intake during lactation modulates programing-induced changes of

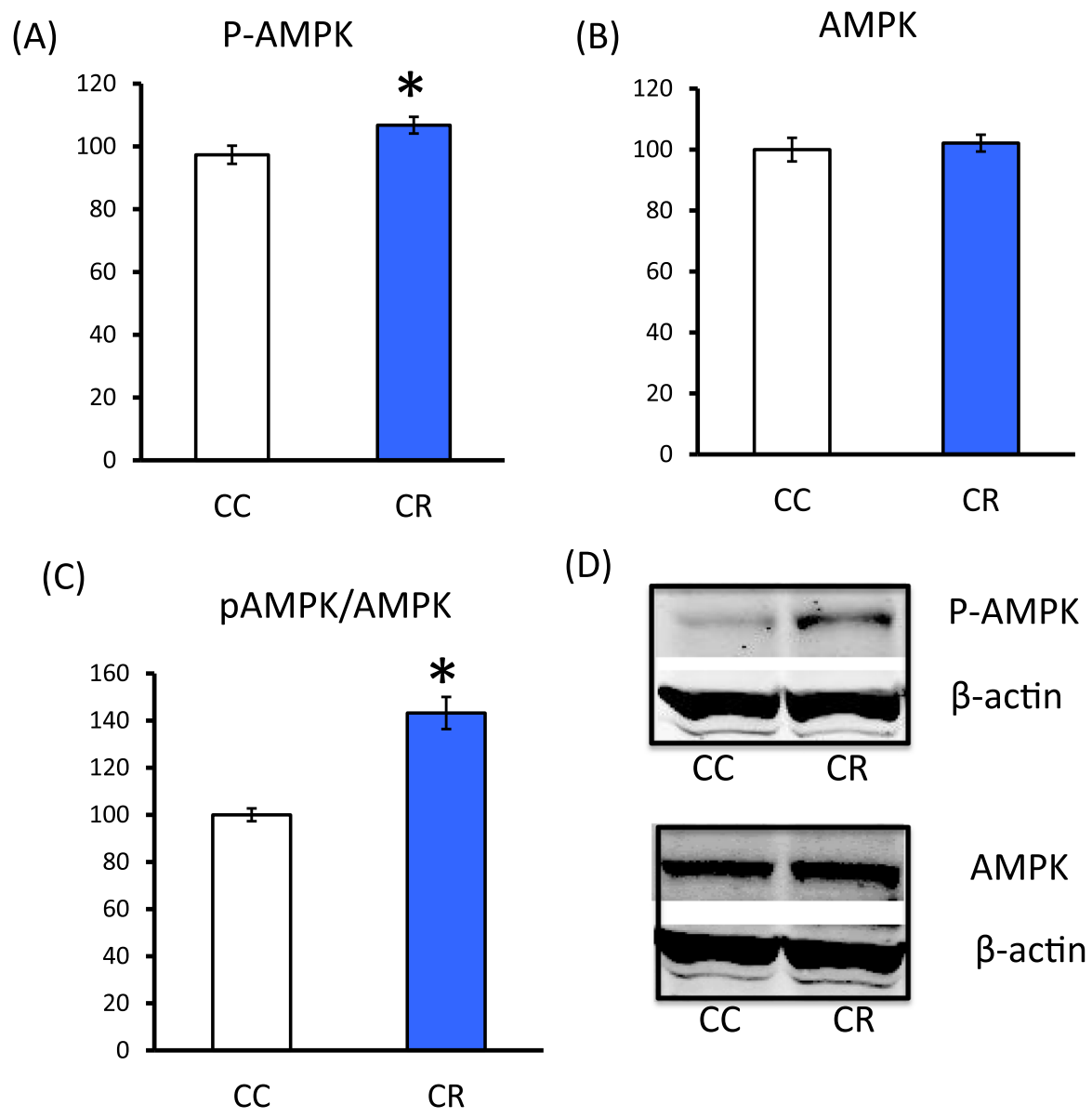

Fig. 2. Hepatic protein expressions of AMPK-phosphorylated at ser403 (*p*-AMPK) (A) and AMPK (B), and the ratio of p-AMPK/AMPK (C) in the CC and CR groups. n=6 in each group. *p < 0.05.

the metabolism in offspring exposed to fetal malnutrition, although the effects of resveratrol intake during lactation on lipogenesis in the adult offspring is unknown.

The aim of this study was to investigate whether maternal resveratrol intake during lactation affects lipogenesis in adult male rat offspring, and if it does, what is the molecular mechanistic basis.

2. Materials and methods

All procedures were performed according to the regulations of the Guidelines for Animal Experimentation, Aomori University of Health and Welfare. Pregnant Wistar rats were divided into two groups: one group was administered a control diet during lactation, and another group was fed a control diet and resveratrol (Sigma-Aldrich Japan) during lactation. Dams in the CR group received orally a 20 mg/kg weight of resveratrol solution once a day during lactation by gavage, and dams in the CC group received orally a vehicle (0.05% carboxymethyl cellulose). After the lactation period (3 weeks), six male pups born from mothers fed a control diets (CC group) and six male pups born from mothers fed a control diet and resveratrol (CR group) were fed a standard diet (MF diet; Oriental Yeast, Tokyo, Japan) and were weighed. Before sacrifice at week 36, the animals were fasted overnight and weighed, and blood samples were collected. Under ether anesthesia, the livers were immediately removed. The livers of all male offspring were stored at −80 °C before evaluation. A portion of liver tissue was fixed in 4% paraformaldehyde phosphate buffer solution (pH 7.4) for histopathology.

3. Blood chemistry

Plasma samples were obtained after centrifugation (800×g for 15 min at 4 °C) and tested for glucose using an autoanalyzer for blood chemistry (Fuji Dry-Chem 3500 V; Fuji Film, Tokyo, Japan) and a commercially available kit (Wako Pure Chemical Industries Ltd), respectively.

4. Western blot analysis

For western blots, the treated livers were homogenized, as shown by Palmiter, Cole, Quaife, and Findley (1996). The liver sample was added

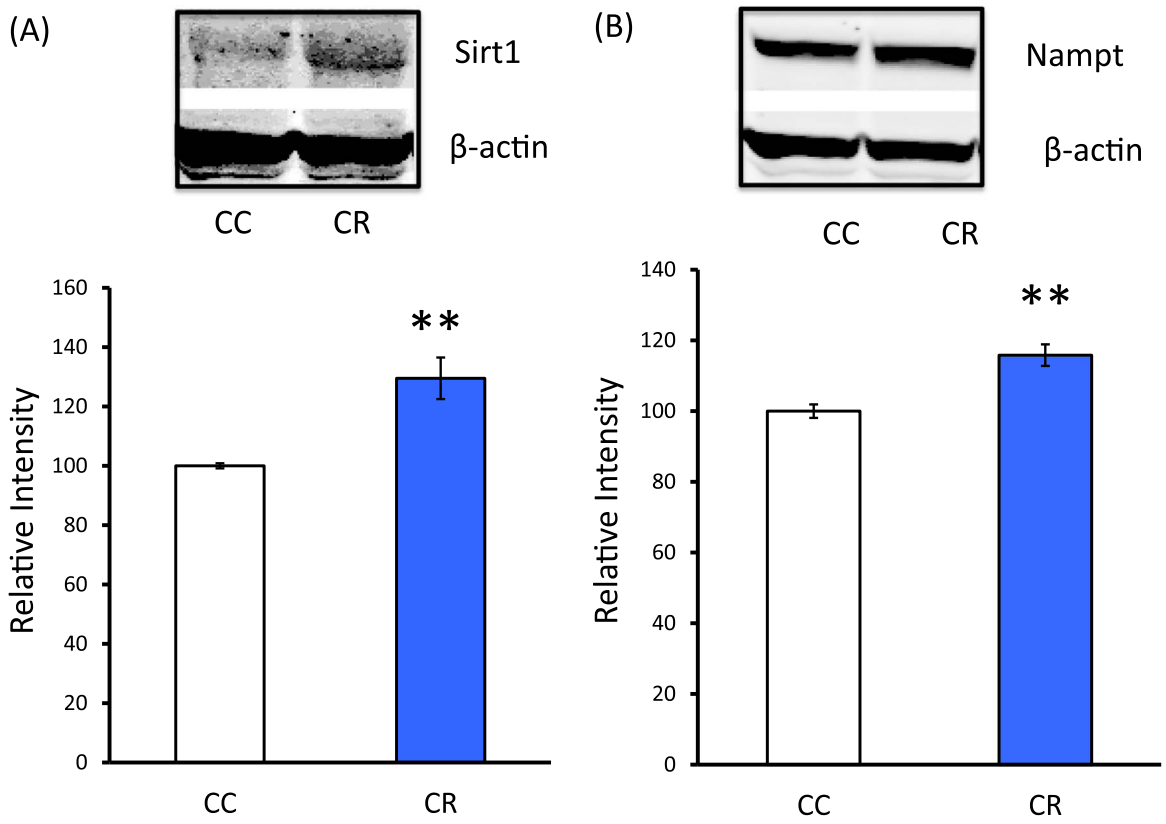

Fig. 3. Hepatic protein expressions of Sirt1 (A) and Nampt (B) in CC and CR groups. n=6 in each group. **p < 0.01.

to 1 mL of homogenate buffer, containing 55 mM Tris (pH 7.4), 2.2% sodium dodecyl sulfate (SDS), 5.5% bmercaptoethanol (b-ME), 11% glycerol, and 55 mM PMSF, in an Eppendorf tube and homogenized with a Polytron (PCU Drehzahlregler, Kinematica, Switzerland) twice for 30 s on ice. The homogenate was then centrifuged at 150×100 rpm, for 20 min at 4 °C, and the supernatant was transferred to a fresh tube. Tubes were heated for 5 min at 100 °C, and 0.1% BPB-glycerol was added. Proteins in the tissue supernatants (20–30 mg) were separated by SDS-PAGE (12.5% e-PAGEL, ATTO, Japan). Biotinylated protein molecular weight markers (M & S TechnoSystems, Japan) were used as protein standards. Proteins were then electrophoretically transferred onto a nitrocellulose membrane (Bio-rad, USA) with blotting buffer that contained 48 mM Tris buffer, 39 mM or by using the iBlot transfer system (Invitrogen, USA). The nitrocellulose membrane was overnight at 4 °C in a 3% blocking solution, containing 40 mM Tris–HCl buffer (pH 7.4), 0.9% NaCl, 0.3% Tween 20%, and 3% blocking reagent, or by using ODYSSEY blocking buffer (M & S TechnoSystems, Japan). The membrane was washed twice with 40 mM Tris–HCl buffer (pH 7.4), 0.9% NaCl, and 0.3% Tween 20, and then exposed to the diluted primary antibody. Antibodies of AMPK, phospho-AMPK and acetyl-coenzyme carboxylase (ACC) (CST, Japan), and Sirt1, nicotinamide phosphoribosyltransferase (Nampt) and fatty acid synthase (FAS) (abcam, Japan), and sterol regulatory element-binding protein-1c (SREBP-1c) and beta-actin (Santa Cruz Biotech, Japan) were incubated with the blot in a 1% blocking solution that contained 40 mM Tris–HCl buffer (pH 7.4), 0.9% NaCl, 0.3% Tween 20%, and 1% blocking reagent or ODYSSEY blocking buffer. Again, the membrane was washed 3 times for 3 min in 40 mM Tris–HCl buffer (pH 7.4), 0.9% NaCl, 0.3% Tween20, and then exposed to the secondary antibody: Anti-rabbit IgG IRDye 680 or Anti-mouse IgG IRDye 800 (M & S Techno Systems, Japan), diluted 1500 times in 1% blocking solution. Finally, the membrane was washed 5 times for 3 min in 40 mM Tris-HCl buffer (pH 7.4), 0.9% NaCl, and 0.3% Tween 20. Protein bands were quantitated with Odyssey infrared imaging system (M & S Techno Systems, Japan). Protein levels were normalized to those of beta-actin from the same sample.

The protein concentration of the obtained homogenate was measured by Bradford assay (Protein Assay, BIO-RAD, USA) [25].

5. Histopathology

For histological examination, paraformaldehyde-fixed liver was embedded in paraffin and sections (4 μm) were stained with hematoxylin and eosin.

6. Statistical analysis

Each value was expressed as mean ± SEM. Statistical analyses were performed by student's *t*-test. In all cases, $p < 0.05$ was considered statistically significant.

7. Results

Body weight and relative food intake from 8 weeks to 32 weeks are shown in Fig. 1. Male offspring from mothers given resveratrol during lactation had lower body weight from week 4 of lactation until adulthood (Fig. 1A), but no significant change was observed in the relative food intake (Fig. 1B). Low level of plasma triacylglycerol was found in the CR group compared to the CC group (p=0.05) (Fig. 1C). No significant change was observed in plasma glucose level between the CC and CR groups (Fig. 1D). Levels of AMPK-phosphorylated at Thr172 (p-AMPK) and the ratio of p-AMPK/AMPK in the CR group were significantly higher than those in the CC group (Figs. 2A and 2B). No significant difference was observed in AMPK level between the CC and CR groups.

The levels of Sirt1 and nicotinamide phosphoribosyltransferase (Nampt), the rate-limiting enzyme for NAD^+ biosynthesis from nicotinamide, were significantly upregulated in the livers of the CR group

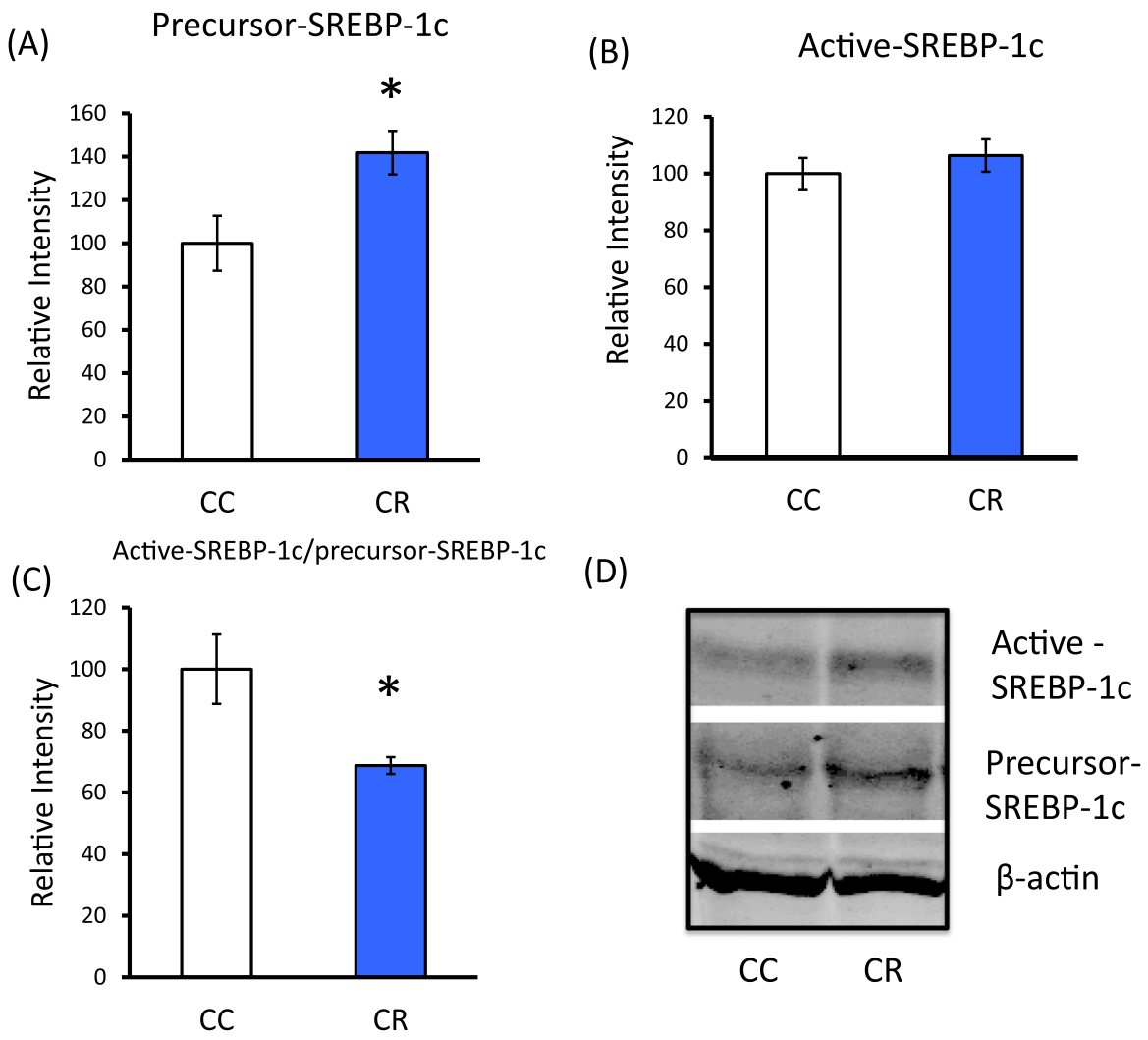

Fig. 4. Hepatic protein expressions of precursor-SREBP-1c (A) and active-SREBP-1c (B), and the ratio of active-SREBP-1c / precursor-SREBP-1c (C) in the CC and CR groups. n=6 in each group. *p < 0.05.

compared to those of the CC group (Figs. 3A and 3B).

Hepatic precursor-SREBP-1c in the CR group was significantly higher than that in the CC group (Fig. 4A). Furthermore, the ratio of active-SREBP-1c/precursor-SREBP-1c in the livers of the CR group was significantly lower than that in the CC group (Fig. 4B).

No significant difference was observed in active-SREBP-1c between the CC and CR groups (Fig. 4C).

Hepatic FAS protein, the rate-limiting enzyme of fatty acid synthesis, was significantly downregulated in the CR group compared to the CC group (Fig. 5A). Hepatic ACC protein, the rate-limiting enzyme in the biosynthesis of long-chain fatty acids, was significantly downregulated in the CR group compared to the CC group (Fig. 5B).

Histopathological analysis of the livers revealed lipid accumulation in hepatocytes around central vein in the CC group, whereas lipid droplets were rare in the CR group (Fig. 6).

8. Discussion

In this study, male rat offspring from mothers administered resveratrol during lactation had lower body weight from week 4 of lactation until adulthood, but no significant change was observed in the relative food intake. Low level of plasma triacylglycerols were found in the CR group compared to the CC group. However, no significant change was observed in plasma glucose level between the CC and CR groups (Fig. 1D). These results indicated that the maternal resveratrol intake during lactation modulated lipid metabolism in adult male rat offspring. The hepatic release of triacylglycerol into the bloodstream, fatty acid oxidation, and *de novo* lipogenesis are three key metabolic pathways that control hepatic triacylglycerol metabolism [26]. Thus we investigated hepatic lipogenesis in adult male rat offspring.

Histopathological analysis of the livers of adult male rat offspring revealed lipid accumulation in hepatocytes in the CC group, whereas lipid droplets were rare in the CR group. The present study showed that maternal resveratrol intake during lactation upregulates protein levels in AMPK-phosphorylated at Thr172, Sirt1, and Nampt in adult male rat offspring. It is well known that resveratrol upregulates Sirt1 [5,6] and Sirt1 induces AMPK activation through deacetylation and activation of LKB1 [7]. In addition, AMPK regulates Sirt1 activation through upregulation of Nampt protein. Thus, these results indicated that maternal resveratrol intake during lactation activates AMPK through Sirt1 upregulation in adult male rat offspring. Our results suggested that the nutritional resveratrol intake of the parental generation has modified lipogenesis in adult offspring and an epigenetic mechanism may be responsible for this effect.

Previous reports have demonstrated that AMPK interacts with and directly phosphorylates SREBP-1c, and phosphorylation of SREBP-1c by AMPK is necessary for inhibition of proteolytic processing and transcriptional activity of SREBP-1c in response to polyphenols [13]. In this study, maternal resveratrol intake during lactation upregulated the level of precursor-SREBP-1c and downregulated the ratio of active-

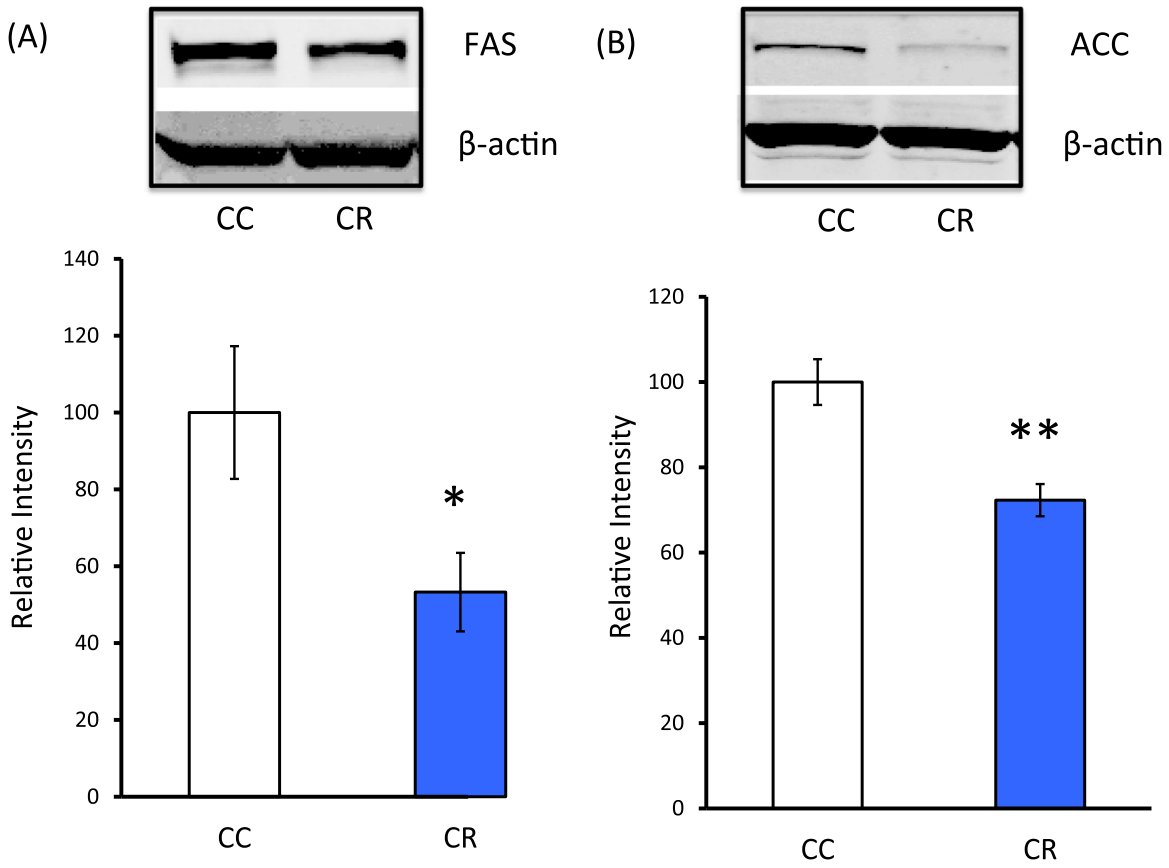

Fig. 5. Hepatic protein expressions of FAS (A) and ACC (B) in CC and CR groups. n=6 in each group. *p < 0.05, **p < 0.01.

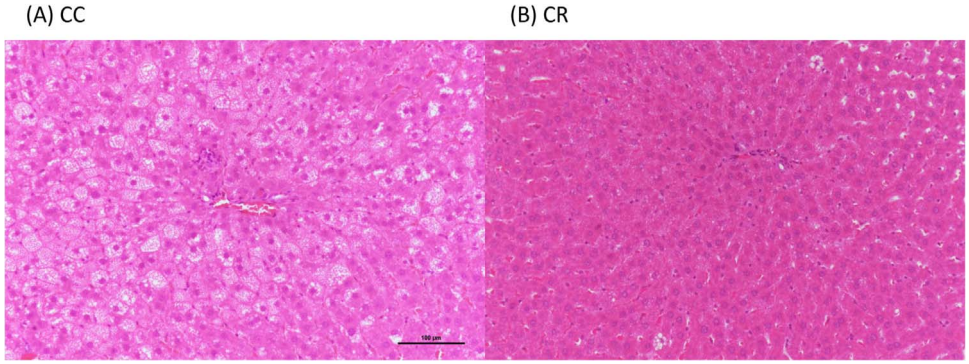

Fig. 6. Light micrographs of livers in CC and CR groups. Sections of liver tissue samples were stained with hematoxylin and eosin (scale bar: 100 μm for all images).

SREBP-1c/precursor -SREBP-1c in adult male rat offspring. These results indicated that proteolytic processing of SREBP-1c was suppressed by AMPK in the livers of the CR group.

It is well known that SREBP-1c regulates the lipogenic pathway by activating genes involved in triglyceride and fatty acid synthesis. The present study showed that maternal resveratrol intake during lactation downregulated the levels of FAS and ACC in adult male rat offspring. FAS plays an essential role in *de novo* lipogenesis by converting acetyl-CoA into palmitate that is subsequently esterified into triglycerides in the liver. ACC, the rate-limiting enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, is a pivotal enzyme in the biosynthesis of long-chain fatty acids [14]. The present results indicated that maternal resveratrol intake during lactation suppresses SREBP-1c cleavage and nuclear translocation and represses SREBP-1c target gene expression, such as FAS and ACC, in the livers of adult male offspring. These changes lead to decreased hepatic triacylglycerol and fatty acid synthesis in adult male rat offspring.

In conclusion, maternal resveratrol intake during lactation attenuated body weight and plasma triacylglycerol in adult male offspring and induced the activation of AMPK through Sirt1 upregulation in the livers of adult male rat offspring. The proteolytic processing of SREBP-1c and SREBP-1c target gene expression, such as FAS and ACC, were suppressed in the livers of adult male offspring. These changes attenuate hepatic lipogenesis in adult male rat offspring. The present study demonstrated that the nutritional resveratrol intake by the parental generation has modified lipogenesis in an adult offspring. However, the mechanism is unknown. These long-lasting downregulation of lipogenesis in adult male rat offspring may be due to epigenetic mechanism that has occurred during the lactation period due to maternal resveratrol intake, although further investigation is needed to clarify the precise mechanism.

Conflict of interest

The authors report no conflict of interest with regard to this paper.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2016.12.011.

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