Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 24 (2013) 118–123

Journal of Nutritional Biochemistry

Quercetin intake during lactation modulates the AMP-activated protein kinase pathway in the livers of adult male rat offspring programmed by maternal protein restriction☆

Shin Sato[a,*], Yuuka Mukai[a], Takeshi Saito[b]

[a]Department of Nutrition, Faculty of Health Sciences, Aomori University of Health and Welfare, Aomori 030-8505, Japan
[b]Faculty of Health Sciences, Hokkaido University, Sapporo 060-0812, Japan

Received 13 July 2011; received in revised form 10 February 2012; accepted 7 March 2012

Abstract

Quercetin, a naturally occurring flavonoid, has been reported to possess numerous biological activities including activation of adenosine-5'-monophosphate-activated protein kinase (AMPK). We investigated the effects of quercetin intake during lactation on the AMPK activation in the livers of adult offspring programmed by maternal protein restriction during gestation. Pregnant Wistar rats were fed control and low-protein diets during gestation. Following delivery, each dam received a control or 0.2% quercetin-containing control diet during lactation as follows: control on control (CC), control on restricted (LPC) and 0.2% quercetin-containing control on restricted (LPQ). At weaning (week 3), some of the pups from each dam were killed, and the remaining pups (CC, $n=8$; LPC, $n=10$; LPQ, $n=13$) continued to receive a standard laboratory diet and were killed at week 23. Blood chemistry and phosphorylation levels of AMPKα, acetyl-CoA carboxylase (ACC), endothelial nitric oxide synthase (eNOS) and mammalian target of rapamycin (mTOR) in the livers of male offspring were examined. At week 3, the level of phosphorylated AMPK protein in LPQ increased about 1.5- and 2.1-fold compared with LPC and CC, respectively, and the level in LPQ at week 23 increased about 1.9- and 2.9-fold, respectively. A significant increase in phosphorylated ACC and eNOS levels was found in LPQ. There was no significant difference among the three groups in the level of phosphorylated mTOR protein. In conclusion, quercetin intake during lactation up-regulates AMPK activation in the adult offspring of protein-restricted dams and modulates the AMPK pathway in the liver.

Keywords: Quercetin; AMP-activated protein kinase pathway; Endothelial nitric oxide synthase; Maternal protein restriction; Liver

1. Introduction

Fetal and neonatal environments are important determinants of disease risk in adult life. 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 [1]. For instance, maternal low-protein diets are early-life inducers of glucose intolerance, hypertension, renal disease and obesity [2–4].

Adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) is a serine/threonine protein kinase that plays a central role in regulating cellular metabolism and energy balance. It is activated under conditions of nutrient deprivation and exercise. For instance, AMPK inhibits the activity of acetyl-CoA carboxylase (ACC) by direct phosphorylation and leads to a blockage of fatty acid synthesis [5,6]. In addition, AMPK phosphorylates and activates endothelial nitric oxide synthase (eNOS) in endothelial cells [7,8]. Nitric oxide (NO) is an important molecule that functions as an endogenous vasodilator and plays a role in liver regeneration [9–11]. Thus, the AMPK pathway is the focus in studies on diabetes, obesity and heart diseases.

Quercetin is one of the most ubiquitous flavonoids and possesses various physiological functions. Several studies have reported that quercetin activated AMPK in cultured cells and animals [12–14]. In addition, quercetin increased the phosphorylation of AMPK and down-regulation of ACC in HeLa cells [15]. Moreover, quercetin increased the phosphorylation of eNOS and NO production in cultured endothelial cells [16] and enhanced eNOS activity in the aorta in a model rat of experimental hypertension [17]. However, little is known about the effects of quercetin intake during lactation on the AMPK pathway in the livers of adult offspring programmed by maternal protein restriction.

The present study was designed to evaluate whether quercetin intake during lactation affects the expression and activity of AMPK in the livers of adult offspring of dams exposed to protein restriction during gestation and whether quercetin intake modulates the AMPK pathway in hepatic metabolic responses in the adult offspring.

☆ Grant: This study was supported by a Grant-in-Aid (no. 20500710) for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.
* Corresponding author. Tel.: +81 17 765 4184; fax: +81 17 765 4184.
E-mail address: s_sato3@auhw.ac.jp (S. Sato).

0955-2863/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2012.03.007

2. Methods and materials

2.1. Animals

All procedures were performed in accordance with the Guidelines for Animal Experimentation of the Aomori University of Health and Welfare. Virgin female Wistar rats aged 5–7 weeks and weighing 158–230 g were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan). They were maintained at a temperature of 23°C±1°C under a 12-h light–dark cycle and had access to food and tap water *ad libitum* until 11 weeks of age. A vaginal impedance reader (Model MK-10C; Muromachi Kikai Co. Ltd., Osaka, Japan) was used to determine whether the female rats were in the appropriate stage of the estrus cycle for mating, as described previously [18]. This was routinely performed in the afternoons, and a reading of >3 kΩ indicated that the female was in proestrus and presumably in estrus. One appropriate female was mated with one male overnight. The next morning, the presence of a vaginal plug indicated successful mating; this day was taken as day 0 of gestation. As shown in Fig. 1, pregnant rats were randomly allocated to feed *ad libitum* on a diet containing either 20% (control group: C, $n=3$) or 8% (low-protein group: LP, $n=9$) casein during gestation. Following delivery, each dam received a control or 0.2% quercetin-containing control diet during lactation as follows: control on control (CC, $n=3$), control on restricted (LPC, $n=4$) or 0.2% quercetin-containing control on restricted (LPQ, $n=5$). The diets were isocaloric, as described in Table 1. The mean human intake of quercetin in the habitual diet typically varies from <5 mg to 40 mg, but daily levels as high as 200–500 mg may be attained by heavy consumers of fruits and vegetables [19]. In addition, male rats fed diet containing 0.2% quercetin for 64 weeks showed no tissue lesions [20]. From these reports, we decided to administer a diet containing 0.2% quercetin, which was equivalent to 80–110 mg/day, at postnatal days 10–22 in our preliminary reproductive experiment. The pups were weighed at postnatal day 4 (PD 4) and kept with six male pups to ensure adequate nutrition during lactation. At weaning, some of the pups were separated from the CC ($n=8$), LPC ($n=10$) and LPQ ($n=13$) groups and killed. The animals were weighed, and blood samples were collected under anesthesia. The liver, kidney and heart were immediately removed and weighed. The remaining pups (CC, $n=8$; LPC, $n=10$; LPQ, $n=13$) continued to receive a standard commercial laboratory diet (MF diet; Oriental Yeast, Tokyo, Japan) and were weighed. At postnatal week 22, 24-h urine samples were collected using metabolic cages. Before sacrificing at week 23, the animals were fasted overnight and then weighed, after which blood samples were collected. Under ether anesthesia, the liver, kidney and heart were immediately removed and weighed. The livers of all offspring were stored at −80°C for the evaluation of mRNA and protein expression.

2.2. Blood chemistry

Plasma samples were separated by centrifugation at 800*g* for 10 min at 4°C and tested for glucose (Glc), triglyceride (Tg), total cholesterol (T-Cho), blood urea nitrogen (BUN) and albumin (Alb) using an autoanalyzer for blood chemistry (Fuji Dry-Chem 3500V; Fuji Film, Tokyo, Japan). Insulin was measured using a rat insulin enzyme-linked immunosorbent assay (ELISA) Kit (TMB; AKRIN-010T, Shibayagi, Gunma, Japan). Adiponectin was determined using a mouse/rat high-molecular-weight adiponectin ELISA kit (Shibayagi, Gunma, Japan).

Table 1
Composition of the diets

Ingredient	Control diet	Low-protein diet	Quercetin-containing control diet
	g/100 g of diet		
Casein	20.000	8.000	20.000
L-Cystine	0.300	0.123	0.300
Cornstarch	39.749	48.826	39.749
α-Corn starch	13.200	16.300	13.200
Sucrose	10.000	10.000	10.000
Soybean oil	7.000	7.000	7.000
Cellulose	5.000	5.000	4.800
Mineral mixture[a]	3.500	3.500	3.500
Vitamin mixture[b]	1.000	1.000	1.000
Choline chlorhydrate	0.250	0.250	0.250
tert-Butylhydroquinone	0.001	0.001	0.001
Quercetin	–	–	0.200

[a] AIN-93G mineral mixture (Oriental Yeast, Tokyo, Japan).
[b] AIN-93G vitamin mixture (Oriental Yeast.)

2.3. Nitrate/nitrite (NOx) content in 24-h urine samples

The NOx content in the 24-h urine samples was measured using the Griess method with the NO_2/NO_3 Assay Kit-C II (Dojindo Laboratories, Kumamoto, Japan). Samples were read at 540 nm with a microplate reader (Model 680; Bio-Rad Laboratories Inc., Hercules, CA, USA).

2.4. Tg analysis in the liver

A total of 100 mg of frozen liver tissue per animal was weighed and homogenized in a 4-ml chloroform–methanol mix (2:1, vol/vol) using a tissue homogenizer. Then, 1 ml of 50 mM NaCl was added to the homogenate and mixed for 1 min, and the ternary phase was separated by centrifugation (1500*g*, 5 min). The organic solvent (100 μl) was resuspended in 20 μl isopropanol containing 1% Triton X-100 and dried. Tg was measured spectrophotometrically using enzymatic assay kits from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.5. Western blot analysis

The livers were homogenized in homogenizing buffer [50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid, 150 mM NaCl, 1 mM dithiothreitol and 0.5% (v/v) Tween-20; pH 7.4] containing protease inhibitor cocktail tablets (Roche Applied Science, Indianapolis, IN, USA). The homogenates were centrifuged at 5000*g* for 45 min at 4°C. Supernatants were collected, and the protein concentration was determined using the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). For Western blot analysis, the proteins were electrophoresed on 10% sodium dodecyl sulfate–polyacrylamide gel and then electrotransferred onto polyvinylidene difluoride membranes (GE Healthcare UK

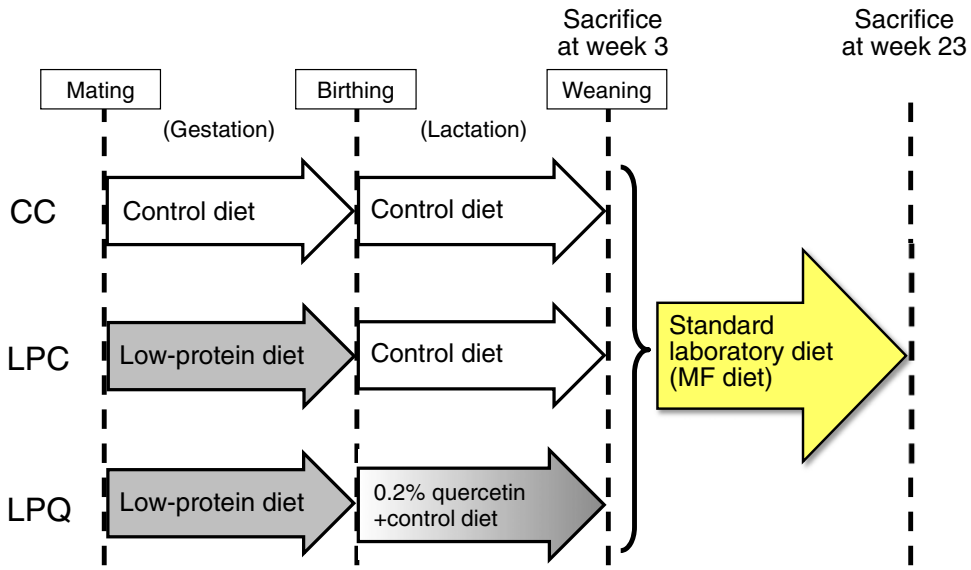

Fig. 1. Experimental design. Pregnant Wistar rats were fed control (20% casein) and low-protein (8% casein) diets during gestation. During lactation, each dam received a control or 0.2% quercetin-containing control diet.

Table 2
Morphological characteristics of the offspring

Group	Week 3			Week 23		
	CC	LPC	LPQ	CC	LPC	LPQ
BW‡ (g)	65.4±2.8	65.2±1.6	62.9±1.2	675±12	626±11[a]	690±15[b]
Liver (g)	2.72±0.13	2.66±0.08	2.47±0.07	16.4±0.8	15.8±0.6	16.8±0.5
Kidney (g)	0.69±0.03	0.63±0.02	0.59±0.01[a]	3.43±0.11	3.21±0.12	3.36±0.09
Heart (g)	0.29±0.01	0.29±0.01	0.28±0.01	1.46±0.03	1.36±0.05	1.42±0.03
L/BW (g/kg)	41.6±0.7	40.8±0.6	39.2±0.6[a]	24.4±1.1	25.2±0.8	24.3±0.6
K/BW (g/kg)	10.54±0.33	9.73±0.22[a]	9.45±0.12[a]	5.10±0.18	5.12±0.16	4.88±0.15
H/BW (g/kg)	4.49±0.09	4.38±0.08	4.38±0.06	2.17±0.06	2.17±0.07	2.07±0.04

‡ At sacrifice. Values are means±S.E. (n=8–13). BW, body weight; L, liver; K, kidney; H, heart.
[a] P<.05 compared with CC.
[b] P<.05 compared with LPC.

Ltd., Buckinghamshire, UK). The membranes were then blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% v/v Tween 20 (TBS-T) for 8 h, followed by incubation overnight at 4°C with rabbit AMPKα, phospho-AMPKα-Thr^{172}, phospho-eNOS-Ser^{1177} and phospho-mammalian target of rapamycin (mTOR)-Ser^{2448} polyclonal antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA); rabbit phospho-ACC-Ser^{79} (1:500; Millipore Corp., Billerica, MA, USA); and mouse eNOS monoclonal antibody (1:500; BD Biosciences, San Jose, CA, USA). The membranes were washed with TBS-T three times for 15 min each. The membranes were then incubated with the appropriate secondary horseradish-peroxidase-conjugated antibodies for 1 h at room temperature. After the membranes were washed, protein bands were visualized with ECL Western Blotting Detection Reagents (GE Healthcare UK Ltd.) on Hyperfilm (GE Healthcare UK Ltd.). Quantitative analysis of the specific band density was performed using ATTO densitometry software (ATTO Corp., Tokyo, Japan). Protein levels were normalized to β-actin expression from the same sample.

2.7. Statistical analysis

Statistical analyses were performed using one-way analysis of variance followed by the Tukey test. Each value was expressed as mean±S.E.

3. Results

3.1. Body weight

Body weight between the C and LP groups during pregnancy did not differ significantly (C, 442±6 g, n=3 vs. LP, 426±10 g, n=9 at day 20 of gestation). The levels of food intake per day during lactation at PD 0–2 were 21±6 g, 16±1 g and 17±1 g in the CC, LPC and LPQ groups, respectively; at PD 10–12, they were 41±4 g, 40 ±3 g and 43±1 g, respectively; and at PD 20–22, they were 56±3 g, 56±3 g and 56±2 g, respectively. As shown in Table 2, body weight differences among CC, LPC and LPQ at week 3 were not significantly different. However, at week 23, the body weights of the LPQ group were higher than those of the LPC group (P<.05) and comparable to those of the CC group.

3.2. Tissue weight, plasma and hepatic parameters of the offspring

Although the LPQ group showed a significant decrease in relative liver weight compared with the CC group at postnatal week 3, there was no significant difference among the three groups at week 23 (Table 2). The relative weights of the kidneys in LPC and LPQ were significantly lower than in CC at week 3.

There was no significant difference among the three groups at weeks 3 and 23 in plasma levels of Glc, T-cho, BUN and Alb. The plasma insulin and adiponectin levels in LPQ at week 23 were significantly higher than in CC and LPC (Table 3). In addition, the insulin level in LPQ at week 3 tended to be higher than in LPC. The Tg level in LPQ at week 3 significantly decreased compared with CC and LPC. However, the level in LPQ at week 23 tended to be higher than in CC and LPC, but a significant increase in the Tg level was not found. With regard to Tg levels in the liver at week 23, although there was no significant difference among the 3 groups (CC, 20.7±2.1 mg/g of liver tissue, n=7; LPC, 31.0±3.5 mg/g, n=8; LPQ, 25.9±2.3 mg/g, n=12), Tg levels in LPQ offspring tended to be lower than in LPC offspring.

3.3. Effect of the quercetin diet on the phosphorylation of AMPK protein

The level of total AMPK protein did not change at postnatal week 3 in any of the groups. However, the abundance of phosphorylated AMPK protein in LPQ increased significantly compared with that in CC and LPC offspring (Fig. 2A). Likewise, the protein abundance of phosphorylated AMPK in LPQ offspring at week 23 was higher than in CC and LPC offspring (Fig. 2B), indicating that quercetin intake during lactation activated AMPK in the livers of adult offspring of dams fed a protein-restricted diet.

3.4. Effect of the quercetin diet on the phosphorylation of ACC protein

In order to investigate the effect of ACC, an important enzyme for the synthesis and usage of fatty acids via activation of the AMPK pathway [21], we examined the level of ACC phosphorylation. There was no significant difference between LPC and LPQ offspring at postnatal week 3 (Fig. 3A). At postnatal week 23, a significant increase in phosphorylated ACC protein abundance was found in LPQ offspring

Table 3
Plasma parameters of offspring

Group	Week 3			Week 23		
	CC	LPC	LPQ	CC	LPC	LPQ
Glc (mg/dl)	153.7±3.6	149.9±3.6	154.0±5.1	142.4±4.1	148.2±4.7	149.8±4.8
T-cho (mg/dl)	115.9±5.4	115.9±2.2	111.8±4.0	68.2±5.4	75.5±5.6	84.2±10.0
Tg (mg/dl)	148.0±20.0	124.5±19.1	88.2±12.7[a]	141.0±22.7	145.2±12.9	201.5±32.1
BUN (mg/dl)	13.5±0.8	13.6±0.8	14.1±0.9	13.8±0.8	15.1±0.6	15.4±0.7
Alb (mg/dl)	3.2±0.0	3.3±0.0	3.2±0.1	3.9±0.1	3.9±0.1	3.9±0.1
Insulin (ng/ml)	2.40±0.43	1.68±0.28	2.80±0.53	3.03±0.43	2.73±0.21	4.33±0.37[a,b]
Adiponectin (ng/ml)	ND	ND	ND	251.7±31.8	305.7±43.7	474.5±56.2[a,b]

Values are means±S.E. (n=7–13). ND, not determined.
[a] P<.05 compared with CC.
[b] P<.05 compared with LPC.

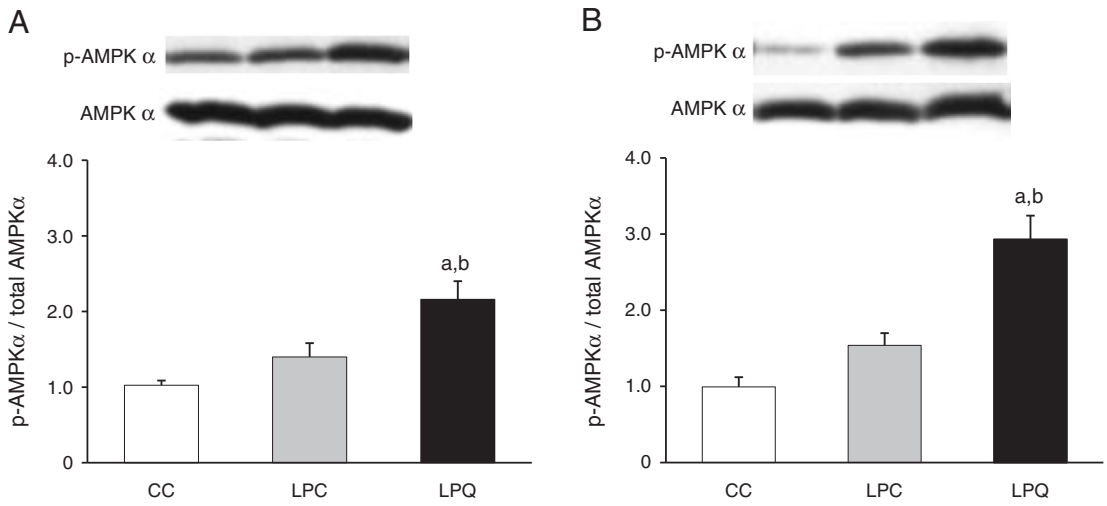

Fig. 2. Protein abundance of total and phosphorylated AMPK in the livers of offspring at postnatal weeks 3 (A) and 23 (B). Values are mean±S.E. (n=8–13). [a]P<.05 compared with CC. [b]P<.05 compared with LPC.

compared with that in CC and LPC offspring (Fig. 3B), indicating that quercetin intake during lactation was associated with inactivation of ACC in the liver.

3.5. Effect of the quercetin diet on the phosphorylation of eNOS protein and urinary NOx content

Because AMPK is reported to be associated with the phosphorylation of eNOS, resulting in an increased release of NO [7,8,22], we examined the expression and phosphorylation of eNOS. At postnatal week 3, there was no significant difference in the expression or phosphorylation of eNOS among CC, LPC and LPQ offspring (Fig. 4A). On the other hand, at week 23, a significant increase in the protein abundance of phosphorylated eNOS (P<.05, CC, 1.029±0.094, p-eNOS/β-actin ratio, n=8; LPC, 0.902±0.065, n=10; LPQ, 1.367 ±0.126, n=10) and total eNOS (P<.05, CC, 1.061±0.033, total eNOS/β-actin ratio, n=8; LPC, 0.890±0.047, n=10; LPQ, 1.096 ±0.63, n=11) was found in LPQ offspring compared with that in CC and LPC offspring, respectively, and p-eNOS/total eNOS level in LPQ was higher than that in CC (Fig. 4B). In addition, the levels of 24-h urinary NOx in LPQ offspring at postnatal week 22 increased significantly compared with that in LPC, and there was no significant difference between CC and LPQ (Fig. 5).

3.6. Effect of the quercetin diet on the phosphorylation of mTOR protein

Since AMPK phosphorylation is associated with mTOR phosphorylation [23], we examined whether the level of mTOR phosphorylation was altered in the liver. There was no significant difference among the three groups in the level of phosphorylation of mTOR protein in the liver at week 23; the levels of p-mTOR/β-actin ratio were 0.996±0.022, 1.007±0.057 and 1.062±0.116 in the CC (n=8), LPC (n=9) and LPQ (n=10) groups, respectively.

4. Discussion

Maternal undernutrition or dietary protein restriction during pregnancy is believed to be associated with long-term metabolic

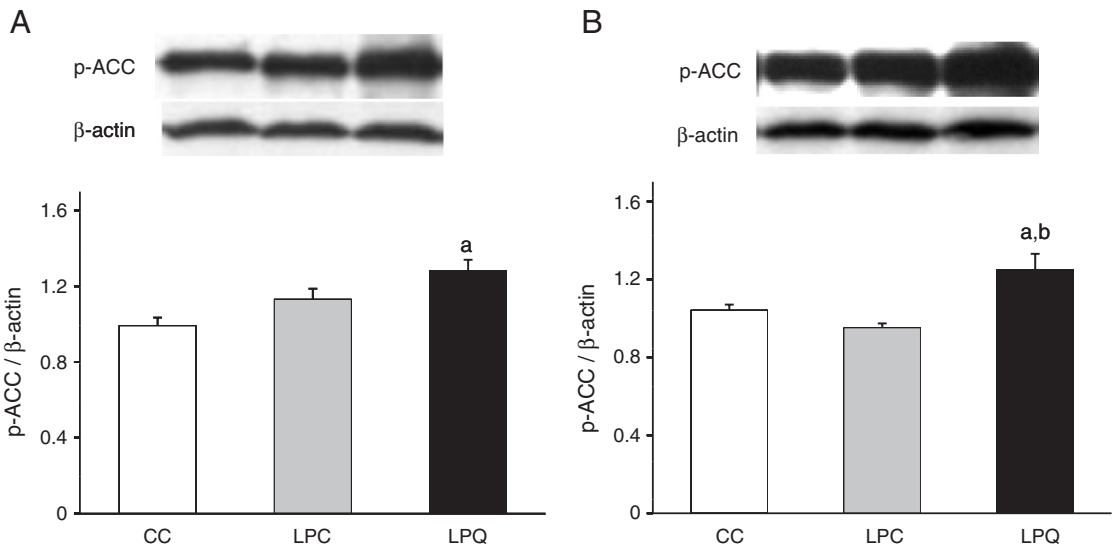

Fig. 3. Protein abundance of phosphorylated ACC in the livers of offspring at postnatal weeks 3 (A) and 23 (B). Values are mean±S.E. (n=8–12). [a]P<.05 compared with CC. [b]P<.05 compared with LPC.

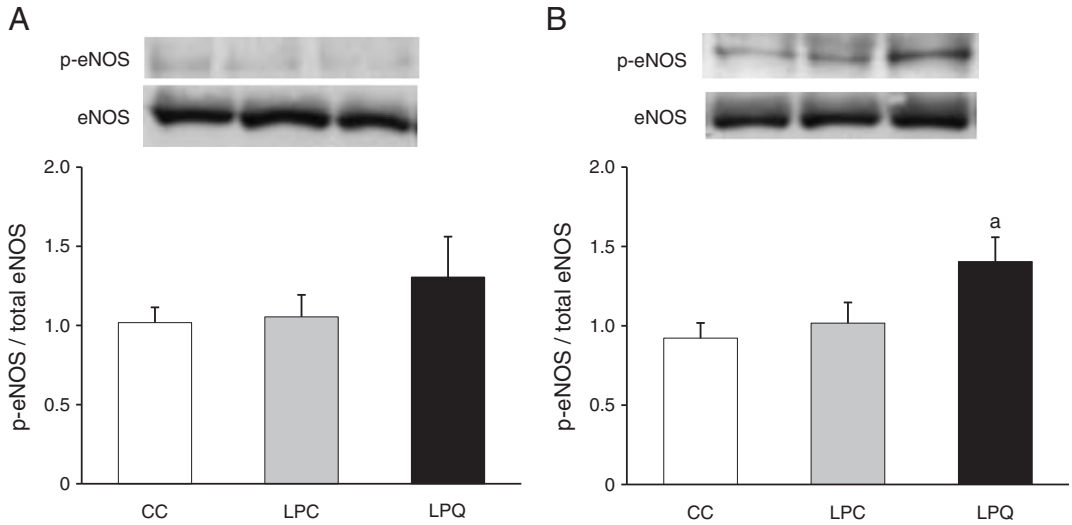

Fig. 4. Protein abundance of phosphorylated eNOS in the livers of offspring at postnatal weeks 3 (A) and 23 (B). Values are mean±S.E. (n=7–10). [a]P<.05 compared with CC.

consequences such as obesity, diabetes and hypertension. However, there is limited information about the long-term effects of the intake of polyphenolic compounds such as quercetin and resveratrol on hepatic energy metabolic pathways in adult offspring programmed by maternal protein restriction. The major findings of the present study are as follows: in the livers of offspring programmed by maternal protein restriction, when quercetin was given during lactation, (a) quercetin intake during lactation up-regulated AMPK phosphorylation, and (b) ACC and eNOS phosphorylation increased significantly.

In this study, we demonstrated an increase in the abundance of phosphorylated AMPK protein in the livers of adult offspring of protein-restricted dams at postnatal week 3 when quercetin was given during lactation, indicating that AMPK is activated in the liver. AMPK functions as a sensor of cellular energy status in most tissues and organs, including the liver, and is activated by increases in the cellular AMP:adenosine triphosphate ratio caused by metabolic stresses [24]. Polyphenolic compounds found in natural sources, including quercetin, resveratrol and epigallocatechin gallate, are involved in the activation of AMPK [25,26]. For instance, quercetin increased the phosphorylation of AMPK in mammalian cells [13,15,27]. Lu et al. [14] reported that quercetin significantly activated AMPK in the brains of old mice fed a high-cholesterol diet. Therefore, we concluded that at postnatal week 3, AMPK in the livers of offspring of protein-restricted dams is activated by quercetin intake during lactation. It is interesting that a significant increase in the phosphorylation of AMPK was found in LPQ offspring even at week 23, despite the fact that no quercetin was given after weaning. This suggests that AMPK activation by quercetin intake during lactation might be preserved, leading to the modulation of the AMPK pathway in the livers of adult offspring.

AMPK is known to phosphorylate a number of metabolic enzymes associated with lipid metabolism in the liver; for instance, ACC, a key enzyme in fatty acid synthesis, is phosphorylated and inactivated by AMPK [5]. In this study, we found that the abundance of phosphorylated ACC protein was increased in the livers of adult offspring of protein-restricted dams at weeks 3 and 23, suggesting that ACC may be inactivated. Moreover, Tg levels in the liver of LPQ offspring at week 23 tended to be lower than in LPC, suggesting it to be due to the phosphorylation of AMPK. Taken together, these findings suggest that the up-regulated activation of AMPK may play a role in the modulation of lipid metabolism in the livers of adult offspring of protein-restricted mothers given quercetin.

In this study, we showed that at week 23 but not at week 3, eNOS phosphorylation increased significantly in the livers of adult offspring programmed by maternal protein restriction with quercetin. In addition, the increased NOx level in the 24-h urine of LPQ offspring might reflect the up-regulated eNOS phosphorylation in the liver. Endothelial dysfunction is associated with a decrease in activity and expression of eNOS in the aortas of offspring of nutritionally restricted dams [28–30]. We also demonstrated that maternal protein restriction during gestation and lactation down-regulated NO production and eNOS phosphorylation [31]. Quercetin increases eNOS activity and prevents endothelial dysfunction [17]; however, whether quercetin intake during lactation directly activated eNOS in adult offspring in this study is unclear. Several studies have reported that AMPK phosphorylates and activates eNOS [7,8,32,33]. Davis et al. [34] reported that AMPK phosphorylation increases by metformin, a drug used for the treatment of type 2 diabetes, increasing eNOS activation and NO bioactivity *in vivo*. Therefore, we hypothesized that eNOS is activated, at least in part, through the phosphorylation of the AMPK signaling cascade and that NO production is enhanced in the liver.

Previous studies have described an increase in islet cell vulnerability in adult rat offspring of protein-restricted dams [35,36]. Theys et al. [37] reported lower plasma insulin levels and islet dysfunction in offspring exposed to maternal protein restriction during pregnancy and suckling. However, quercetin potentiated insulin secretion and protected β-cells against oxidative damage [38,39]. Here, we

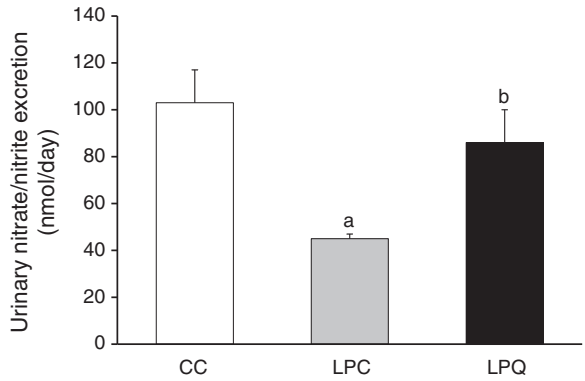

Fig. 5. Urinary NOx excretion. Values are mean±S.E. (n=7–10). [a]P<.05 compared with CC. [b]P<.05 compared with LPC.

demonstrate that plasma insulin levels increased significantly in LPQ adult offspring but decreased in LPC offspring at week 23. Thus, it is plausible that quercetin intake during lactation improves pancreatic insulin secretion in the islets of adult offspring of dams fed a low-protein diet during pregnancy.

In conclusion, we show here that quercetin intake during lactation up-regulates the activation of AMPK in adult offspring of protein-restricted dams and modulates the AMPK pathway in the liver. Although the reasons for the effects of quercetin intake during lactation on the AMPK pathway in the livers remain unclear, the feeding of quercetin, an AMPK activator, to protein-restricted dams during lactation is likely to up-regulate AMPK activation and may, at least in part, lead to long-term alterations of AMPK pathway in the livers of adult offspring of protein-restricted dams.

Acknowledgment

The authors thank Keiko Tamakuma for her technical assistance.

References

[1] Barker DJ, Bagby SP, Hanson MA. Mechanisms of disease: in utero programming in the pathogenesis of hypertension. Nat Clin Pract 2006;2:700–7.
[2] Curhan GC, Willett WC, Rimm EB, et al. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. Circulation 1996;94:3246–50.
[3] McMullen S, Langley-Evans SC. Maternal low-protein diet in rat pregnancy programs blood pressure through sex-specific mechanisms. Am J Physiol Regul Integr Comp Physiol 2005;288:R85–90.
[4] Warner MJ, Ozanne SE. Mechanisms involved in the developmental programming of adulthood disease. Biochem J 2010;427:333–47.
[5] Hardie DG, Pan DA. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. Biochem Soc Trans 2002;30:1064–70.
[6] Saha AK, Ruderman NB. Malonyl-CoA and AMP-activated protein kinase: an expanding partnership. Mol Cell Biochem 2003;253:65–70.
[7] Zhang Y, Lee TS, Kolb EM, et al. AMP-activated protein kinase is involved in endothelial NO synthase activation in response to shear stress. Arterioscler Thromb Vasc Biol 2006;26:1281–7.
[8] Chen Z, Peng IC, Sun W, et al. AMP-activated protein kinase functionally phosphorylates endothelial nitric oxide synthase Ser633. Circ Res 2009;104:496–505.
[9] Bauer C, Walcher F, Kalweit U, et al. Role of nitric oxide in the regulation of the hepatic microcirculation in vivo. J Hepatol 1997;27:1089–95.
[10] Schoen JM, Wang HH, Minuk GY, et al. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. Nitric Oxide 2001;5:453–64.
[11] García-Trevijano ER, Martínez-Chantar ML, Latasa MU, et al. NO sensitizes rat hepatocytes to proliferation by modifying *S*-adenosylmethionine levels. Gastroenterology 2002;122:1355–63.
[12] Ahn J, Lee H, Kim S, et al. The anti-obesity effect of quercetin is mediated by the AMPK and MAPK signaling pathways. Biochem Biophys Res Commun 2008;373:545–9.
[13] Lee YK, Park SY, Kim YM, et al. AMP kinase/cyclooxygenase-2 pathway regulates proliferation and apoptosis of cancer cells treated with quercetin. Exp Mol Med 2009;41:201–7.
[14] Lu J, Wu DM, Zheng YL, et al. Quercetin activates AMP-activated protein kinase by reducing PP2C expression protecting old mouse brain against high cholesterol-induced neurotoxicity. J Pathol 2010;222:199–212.
[15] Jung JH, Lee JO, Kim JH, et al. Quercetin suppresses HeLa cell viability via AMPK-induced HSP70 and EGFR down-regulation. J Cell Physiol 2010;223:408–14.
[16] Khoo NK, White CR, Pozzo-Miller L, et al. Dietary flavonoid quercetin stimulates vasorelaxation in aortic vessels. Free Radic Biol Med 2010;49:339–47.
[17] Sánchez M, Galisteo M, Vera R, et al. Quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats. J Hypertens 2006;24:75–84.
[18] Wlodek ME, Mibus A, Tan A, et al. Normal lactational environment restores nephron endowment and prevents hypertension after placental restriction in the rat. J Am Soc Nephrol 2007;18:1688–96.
[19] Harwood M, Danielewska-Nikiel B, Borzelleca JF, et al. A critical review of the data related to the safety of quercetin and lack of evidence of in vivo toxicity, including lack of genotoxic/carcinogenic properties. Food Chem Toxicol 2007;45:2179–205.
[20] Stoewsand GS, Anderson JL, Boyd JN, et al. Quercetin: a mutagen, not a carcinogen, in Fischer rats. J Toxicol Environ Health 1984;14:105–14.
[21] Ha J, Daniel S, Broyles SS, et al. Critical phosphorylation sites for acetyl-CoA carboxylase activity. J Biol Chem 1994;269:22162–8.
[22] Calvert JW, Gundewar S, Jha S, et al. Acute metformin therapy confers cardioprotection against myocardial infarction via AMPK-eNOS-mediated signaling. Diabetes 2008;57:696–705.
[23] Chotechuang N, Azzout-Marniche D, Bos C, et al. mTOR, AMPK, and GCN2 coordinate the adaptation of hepatic energy metabolic pathways in response to protein intake in the rat. Am J Physiol Endocrinol Metab 2009;297:E1313–23.
[24] Towler MC, Hardie DG. AMP-activated protein kinase in metabolic control and insulin signaling. Circ Res 2007;100:328–41.
[25] Hwang JT, Kwon DY, Yoon SH. AMP-activated protein kinase: a potential target for the diseases prevention by natural occurring polyphenols. N Biotechnol 2009;26:17–22.
[26] Reiter CE, Kim JA, Quon MJ. Green tea polyphenol epigallocatechin gallate reduces endothelin-1 expression and secretion in vascular endothelial cells: roles for AMP-activated protein kinase, Akt, and FOXO1. Endocrinology 2010;151:103–14.
[27] Suchankova G, Nelson LE, Gerhart-Hines Z, et al. Concurrent regulation of AMP-activated protein kinase and SIRT1 in mammalian cells. Biochem Biophys Res Commun 2009;378:836–41.
[28] Franco Mdo C, Arruda RM, Dantas AP, et al. Intrauterine undernutrition: expression and activity of the endothelial nitric oxide synthase in male and female adult offspring. Cardiovasc Res 2002;56:145–53.
[29] Brawley L, Itoh S, Torrens C, et al. Dietary protein restriction in pregnancy induces hypertension and vascular defects in rat male offspring. Pediatr Res 2003;54:83–90.
[30] Alexander BT. Fetal programming of hypertension. Am J Physiol Regul Integr Comp Physiol 2006;290:R1–10.
[31] Sato S, Mukai Y, Norikura T. Maternal low-protein diet suppresses vascular and renal endothelial nitric oxide synthase phosphorylation in rat offspring independent of a postnatal fructose diet. J Dev Orig Health Dis 2011;2:168–75.
[32] Kahn BB, Alquier T, Carling D, et al. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 2005;1:15–25.
[33] Vázquez-Chantada M, Ariz U, Varela-Rey M, et al. Evidence for LKB1/AMP-activated protein kinase/ endothelial nitric oxide synthase cascade regulated by hepatocyte growth factor, S-adenosylmethionine, and nitric oxide in hepatocyte proliferation. Hepatology 2009;49:608–17.
[34] Davis BJ, Xie Z, Viollet B, et al. Activation of the AMP-activated kinase by antidiabetes drug metformin stimulates nitric oxide synthesis in vivo by promoting the association of heat shock protein 90 and endothelial nitric oxide synthase. Diabetes 2006;55:496–505.
[35] Merezak S, Reusens B, Renard A, et al. Effect of maternal low-protein diet and taurine on the vulnerability of adult Wistar rat islets to cytokines. Diabetologia 2004;47:669–75.
[36] Goosse K, Bouckenooghe T, Balteau M, et al. Implication of nitric oxide in the increased islet-cells vulnerability of adult progeny from protein-restricted mothers and its prevention by taurine. J Endocrinol 2009;200:177–87.
[37] Theys N, Clippe A, Bouckenooghe T, et al. Early low protein diet aggravates unbalance between antioxidant enzymes leading to islet dysfunction. PLoS One 2009;4:e6110.
[38] Coskun O, Kanter M, Korkmaz A, et al. Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and beta-cell damage in rat pancreas. Pharmacol Res 2005;51:117–23.
[39] Youl E, Bardy G, Magous R, et al. Quercetin potentiates insulin secretion and protects INS-1 pancreatic β-cells against oxidative damage via the ERK1/2 pathway. Br J Pharmacol 2010;161:799–814.