Nutrition 29 (2013) 688–692

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

A maternal diet rich in fish oil may improve cardiac Akt-related signaling in the offspring of diabetic mother rats

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ARTICLE INFO

Article history:
Received 14 May 2012
Accepted 24 November 2012

Keywords:
Fish oil
Heart
Infants
Diabetic mother
Insulin signaling
MAP kinase signaling

ABSTRACT

Objective: Newborns of diabetic mothers have abnormal circulatory organs, so in this study, we explore insulin signaling in the newborn rat heart.
Methods: Pregnant rats were divided into streptozotocin-induced diabetic groups (DM) and control groups (CM). Rats were fed lard (21% fat), fish oil (21% fat), or a control diet (7% fat). To examine changes in insulin signaling in the hearts of infants of diabetic mothers (IDM) in relation to diet, we isolated the hearts from the IDM and control infants and determined the phosphorylation levels of Akt308, Akt473, p38, c-jun-NH2-terminal protein kinase (JNK), and extracellular signal-regulated protein kinase (ERK), and the expression levels of phosphoinositide-dependent protein kainase1 (PDK1) and mammalian target of rapamycin (mTOR).
Results: The mean blood glucose levels in the DM group and their infants were significantly higher than those in the CM group ($P < 0.05$) and their infants ($P < 0.05$), but the mean blood glucose levels of all infants was normal on postnatal d 4. Phosphorylation levels of Akt $^{Thr\ 308}$ ($P < 0.05$) and Akt $^{Ser\ 473}$ and the expression levels of PDK1 and mTOR were lower in infants of diabetic mothers (IDM) than in control infants. The phosphorylation level of Akt $^{Ser\ 473}$ and the expression level of mTOR increased in IDM fed the fish oil diet compared with those fed the lard diet ($P < 0.05$).
Conclusion: A maternal diet rich in fish oil improves cardiac Akt-related signaling in the offspring of diabetic rats.

Introduction

Diabetes mellitus is one of the most common medical complications of pregnancy. Approximately 7% of all pregnancies are affected by diabetes, of which 90% are classified as gestational diabetes mellitus [1]. The prevalence of gestational diabetes is increasing worldwide, and recent studies have suggested that increases in gestational diabetes may lead to an intergenerational cycle of increasing obesity and glucose intolerance [2,3]. In addition to effects on the mother, the infants of diabetic mothers (IDM) are at high risk for increased perinatal morbidity and mortality [4]. Newborn offspring of diabetic mothers also exhibit an elevated risk for cardiomyopathy [4]. It is thought that hypertrophic cardiomyopathy is found in 40% of IDM [5]. Other works, such as by Reller et al., has suggested that the degree of cardiomyopathy in IDM can vary from mild to severe, depending on blood glucose level [6,7]. Why gestational diabetes may cause cardiomyopathy in infants is not known.

Recent data have suggested that nutrition during fetal and neonatal life can have profound effects on the lifespan, fetal growth, glucose and fat metabolism, and development of the cardiovascular system [8–10]. Indeed, previous work from our laboratory has demonstrated that a high-fat lard diet during pregnancy is associated with hyperglycemia and stillbirth in the diabetic rat [11]. Other studies have shown that a high-fat lard diet has been linked to the development of obesity, insulin resistance, and type 2 diabetes, as well as impairment of the glucose signaling system in beta cells [12–14]. As in other

R. N-K. and A. N. designed the research; A. N., S. K., E. B., K. K., and T. K conducted the research; R. N-K. and A. N. analyzed the data; R. N-K. and A. N. wrote the paper; and A. N. had primary responsibility for the final content. All authors read and approved the final manuscript.

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http://dx.doi.org/10.1016/j.nut.2012.11.017

insulin-sensitive tissues, insulin signaling via the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway is thought to play a key role in cardiac glucose uptake [15]. How gestational diabetes may affect Akt-related signaling has, to our knowledge, not been investigated.

In contrast twitho that seen with high-fat lard diets, the consumption of fish oil containing n-3 polyunsaturated fatty acids can act to decrease blood glucose and triglyceride levels [16–18]. Retrospective studies from the early 1980s have shown an inverse relationship between coronary heart disease and the consumption of fish containing n-3 polyunsaturated fatty acids [19,20]. Recent interventional studies add credence to this work and have demonstrated that the ingestion of n-3 polyunsaturated fatty acids is associated with diminished rates of myocardial infarction and sudden death [21,22]. Whether the ingestion of diet rich in fish oil improves blood glucose levels and Akt-related signaling in the IDM has to our knowledge, not been investigated.

Materials and methods

Animal, diet, and samples

Animals were treated in accordance with the Guidelines for the Care and Use of Experimental Animals (Prime Minister's Office, Notification No. 6). This study was approved by the animal committee of Takasaki University of Health and Welfare (No. 9001). Pregnant 15-wk-old Wistar rats (d 1 of gestation, 230 g to 250 g) purchased from Nippon Clea Co. (Tokyo, Japan) were divided into two groups: diabetic mothers (DM, n = 15) and control mothers (CM, n = 15). Diabetes was induced by intravenous injection of streptozotocin (STZ, 60 mg/kg; Wako, Tokyo, Japan) in 0.05 M citrate buffer (pH 4.5) on d 2 of gestation. The rats were fed with AIN 93G diet (control diet, -C) [23] and modified AIN 93G diets *(high-fat lard diet, -L and high-fat fish-oil diet, -F)*(Nippon Clea Co., Tokyo, Japan), which were stored at −20°C and replaced daily. The modified AIN 93G diets added 140 g/kg lard oil and 140 g/kg fish oil (NOF Corporation, Tokyo, Japan), respectively, to the AIN 93G diet. The DM and CM mother rats were designated DM-C (n = 5), DM-L (n = 5), DM-F (n = 5), CM-C (n = 5), CM-L (n = 5), and CM-F (n = 5) by diet. The female infants of each mother rats were D-C (n = 10), D-L (n = 10), D-F (n = 10), C-C (n = 10), C-L (n = 10), and C-F (n = 10). The compositions of the three diets are summarized in Table 1. All groups were individually housed in plastic cages with woodchip bedding at 25°C, with a humidity of 60% ± 5% and a 12 hr light/dark cycle. At the appropriate age, the total body weights of five suckling infants from each litter or mother were recorded. Subsequently, the rats were lightly anesthetized (isoflurane), killed by guillotine, and their hearts were rapidly removed and rinsed in ice cold 0.9% NaCl.

Blood chemistry analysis

Blood was collected from the tip of the tail from the pregnant rats and infants on d 1, 4, 8, 15, 22, 29, 36, 43, and 50. Blood glucose was measured by the glucose oxidase method using a glucose analyser (Wako, Tokyo, Japan). On postnatal d 4, serum taken by blood samples from the heart was used for triglyceride analysis.

Table 1
Composition of experimental diets*

	Control	Lard	Fish oil
	g/kg diet		
Cornstarch	397.8	389.5	389.5
Casein	200	200	200
Dextrinized cornstarch	132		
Lard oil		140	
Fish oil			140
Sucrose	100	100	100
Soybean oil	70	70	70
Fiber	05	50	50
Mineral mix†	35	35	35
Vitamin mix†	10	10	10
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	2.5
tert-Butylhydroquinone (TBHQ), mg	14.0	14.0	14.0

* Animals were fed isocaloric semisynthetic diet containing identical dietary constituents differing only in the composition of dietary oil.

† Mineral mix and vitamin mix were prepared according to the AIN-93 [23].

Serum was obtained by centrifugation at 3000 × *g* at 4°C for 10 min. Serum triglycerides were measured using a triglyceride E-test kit (Wako, Tokyo, Japan).

Immunoblotting

Total protein from cardiac muscles was prepared as previously described [24], with slight modifications. The total protein was separated by 12.5% SDS-PAGE and transferred to a nitrocellulose membrane (Hybond-ECL; GE Healthcare, Bucks, UK, Ltd) using the Genie Electrophoretic Transfer system (Idea Scientific Company, Minneapolis, MN, USA) according to the manufacturer's instructions. Western blotting was performed according to the method of Rice et al. [24]. Primary antibodies against Akt (#9272), phospho-Akt (Akt ^{Thr308}; pAkt-Thr308, #9275), phospho-Akt (Akt ^{Ser473}) (pAkt-Ser473, #9271), 3-phosphoinositide-dependent protein kinase-1 (PDK1; #3062), mammalian target of rapamycin (mTOR; #2972), extracellular signal-regulated protein kinase (ERK)1/2-mitogen-activated protein kinase (MAPK; Thr 202/Tyr204, #9106S), Phospho ERK1/2-MAPK (Thr 202/Tyr204, #4377S), JNK (#9252), Phospho JNK Thr (183/Tyr185, #9251S), p38 MAPK (#9212), Phospho p38 MAPK (Thr 180/ Tyr 182, #9216L), glyceraldehyde 3-phosphate dehydrogenase (GAPDH, #2118), secondary antibodies conjugated with horseradish peroxidase (HRP) (antirabbit, #7074, or antimouse, #7076) were purchased from Cell Signaling Technology (Beverly, MA). The membrane was developed using ECL detection reagents (GE Healthcare, Piscataway, NJ, USA), and quantities determined by densitometry using an LAS3000 (Fujifilm, Tokyo, Japan) with Scion Image software. The same membrane was stripped and reprobed with other antibodies to assess the equal loading of protein. All other chemicals were purchased from Sigma (St. Louis, MO, USA).

Statistical analysis

All data are expressed as mean values and standard error of mean; differences among the three groups were compared using two-way analysis of variance (ANOVA) with a post hoc Tukey's test. $P < 0.05$ was considered statistically significant. The statistical analysis was performed using SPSS software version 14.0 (SPSS, Tokyo, Japan).

Results

Blood glucose and triglycerides

Table 2 lists the mean blood glucose levels over time in mothers and infants. The mean blood glucose level of the mothers was higher in DM-C, DM-L, and DM-F than in CM-C, CM-L, and CM-F groups. In the diabetic mothers, the mean blood glucose level was lower in DM-F compared with DM-L. There were no differences in blood glucose levels among the control mother rats. The mean blood glucose level was significantly higher in IDM than in the control group in the infants on postnatal d 1. In the IDM on postnatal d 1, the mean blood glucose level was in order of D-L > D-F > D-C. On postnatal d 4, there were no significant differences in the mean blood glucose level among the groups. In IDM, the triglyceride level was in order of D-F < D-C < D-L. In the control group, there were no significant differences in the triglyceride level among the diets.

Effects of diabetes on Akt-related signaling

The phosphorylation level of Akt ^{Thr308} was lower in D-C, D-L, and D-F compared with C-C, C-L, and C-F. There were no significant differences in phosphorylation levels of Akt ^{Thr308} among the IDM. In the control group, the phosphorylation level of Akt ^{Thr308} of C-L and C-F was significantly lower than that observed in the C-C (Fig. 1A). The expression level of PDK1 was lower in D-C and D-F compared with C-C and C-F, respectively. There were no significant differences in the expression level of PDK1 among D-C, D-L, and D-F. In the control group, the expression level of PDK1 of C-L and C-F was significantly lower than that for C-C (Fig. 1B). The phosphorylation level of Akt ^{Ser473} was lower in D-C and D-L compared with C-C and C-L, respectively. In the IDM, the phosphorylation level of Akt ^{Ser473} in D-F was higher than that in D-C and D-L. In the control group, the phosphorylation level of