Undernutrition in Pregnant Rats Induces Glucose Intolerance with Enhanced Expression of Inflammation-Related Genes in Peripheral Leukocytes of the Offspring

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Summary Impaired glucose tolerance (IGT) induces chronic inflammation and subsequent development of complications triggered by arteriosclerosis. Moreover, undernutrition in pregnant rodents can induce IGT in their offspring. Here, we assessed whether undernutrition in pregnant rats would induce chronic inflammation in their offspring by measuring the expression levels of inflammation-related genes in peripheral blood leukocytes. Pregnant Wistar rats were divided into two groups: the control group received an American Institute of Nutrition Rodent diet (AIN-93G) ad libitum, and the undernutrition group had their diet restricted by 50% (w/w) compared with the control group from day 10 of pregnancy until birth of the offspring. Subsequently, mothers and pups were allowed to access the AIN-93G diet freely. At day 35 after birth, male pups were fasted for 4 h and subsequently orally administered with glucose solution (2 g/kg body weight). Blood glucose area under the curve (AUC) after glucose loading was significantly greater in the undernutrition group than the control group. The mRNA levels for inflammatory cytokines were increased by glucose loading especially in the undernutrition group. Expressions of genes encoding S100A9 and cell adhesion molecule CD11b were increased by glucose loading in the undernutrition group. Thus, undernutrition of pregnant rats during mid to late gestation induced the expression of inflammation-related genes in peripheral blood leukocytes of their offspring, with the development of IGT and impaired insulin secretion.

Key Words fetal undernutrition, chronic inflammation, cytokine, impaired glucose tolerance, leukocyte adhesion molecule

The intrauterine environment is concerned not only with programing organ development but can also be involved with establishing the progression of metabolic diseases in later life of the offspring. This is known as the developmental origins of health and disease (DOHaD) hypothesis. A cohort study of people born around the time of the Dutch famine in 1944–45 demonstrated that subjects exposed to undernutrition in utero had a higher incidence of glucose intolerance (IGT) as adults (1). In addition, a meta-analysis covering 14 studies reported that neonates with a low birth weight (<2,500 g) had a higher incidence of type 2 diabetes (T2DM) as adults (2). Studies using animal models have shown that 50% caloric restriction during the fetal- to the suckling-period induced metabolic abnormalities (3–5). A model of intrauterine growth retardation (IUGR), established by ligation of the uterine arteries on day 19 of gestation, showed reduction of pancreatic β-cell mass and insulin content in the β-cells by 35–40% in neonates and by 40–45% in 3-mo-old offspring (6). In addition, it was reported that rats subjected to IUGR exhibited an impairment of first-phase insulin secretion during the 1-wk suckling period (7).

Recent epidemiological studies have indicated that postprandial hyperglycemia, caused by blunted first-phase insulin secretion, induced the development of atherosclerosis-related diseases as well as T2DM. Large-scale longitudinal studies such as the Diabetes Epidemiology Collaborative analysis Of Diagnostic criteria in Europe and the Funagata Diabetes Study in Japan have suggested that postprandial hyperglycemia—rather than glycated hemoglobin (HbA1c) levels and/or fasting glucose levels—was strongly positively correlated with the subsequent development of arteriosclerotic cardiovascular disease (CVD) (8). Several intervention studies have demonstrated that treatment with the α-glucosidase inhibitor (α-GI) acarbose, which reduces postprandial hyperglycemia by inhibiting disaccharidase activities in the brush border of the small intestine, to subjects with IGT or T2DM, reduced the development of T2DM and adverse cardiovascular events (9–11).

Many studies have suggested that higher circulating concentrations of inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α are associated with the development of arteriosclerosis-
related diseases in subjects with metabolic disorders (12). These inflammatory cytokines are expressed in neutrophils and monocytes and induce arteriosclerosis by activating them (13). This activation is a cause of vascular injuries through the production of reactive oxygen species and the release of proteases such as neutrophil elastase (14). In addition, the activation status of innate immune cells caused by hyperglycemia, as reflected by the increased levels of the immune cell-surface marker CD11b, was increased in subjects with T2DM (15). Integrins, which are adhesion molecules and expressed by innate immune leukocytes, play critical roles in the interaction with integrin ligands on vascular endothelium. Activated neutrophils enhance the initial step of the development of arteriosclerosis, which involves injury to blood vessels caused by reactive oxygen species and proteases including elastase secreted from the neutrophils, and led to atherosclerosis by the release of low-density lipoprotein from macrophages (16).

We have previously demonstrated that postprandial hyperglycemia enhanced the expression of genes encoding IL-1β and TNF-α in peripheral leukocytes in rats with moderate hyperglycemia induced by streptozotocin, and this induction was inhibited by the α-GI drug miglitol (17). In addition, we have demonstrated that reduction of postprandial hyperglycemia by switching the α-GI from acarbose to miglitol, which reduces earlier postprandial hyperglycemia better than acarbose, reduced the expression of genes encoding IL-1β and TNF-α in peripheral leukocytes from Japanese patients with T2DM (18). These results indicate that postprandial hyperglycemia and/or IGT might induce arteriosclerosis-related diseases by inducing expression of inflammation-related genes in leukocytes such as neutrophils and monocytes. However, it is unclear whether IGT caused by fetal undernutrition induces the expression levels of inflammation-related genes including inflammatory cytokines and integrins. Here, we examined whether undernutrition in pregnant rats subjected to 50% food restriction from day 10 of gestation until birth would increase the expression levels of inflammation-related genes in the peripheral leukocytes of the offspring.

**MATERIALS AND METHODS**

**Animals.** Four pregnant Wistar rats were purchased
at day 8 of gestation from Japan SLC, Inc. (Shizuoka, Japan). Rats were divided into two groups at day 10 of gestation by a randomized allocation: the control group had free access to American Institution of Nutrition rodent diet (AIN)-93G (Oriental Yeast Co., Ltd., Tokyo, Japan), and the undernutrition group was restricted to 50% (w/w) of the AIN-93G diet against the food intake of control rats from day 10 of gestation until birth. The number of pups was adjusted to 10 per litter at 4 d after birth. Pups whose body weight are far from the median were excluded from the group. Both control and undernutrition group pups were reared by their dams after birth and had free access to AIN-93G diet. At 27 d after birth, all pups were weaned. All animals were allowed free access to tap water and maintained at a stable temperature (22 ± 2°C) and humidity (55 ± 5%) under a 12-h light : dark cycle (lights on 07:00–19:00) according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka (Approval No. 165108).

Oral glucose tolerance test (OGTT). At 35 d after birth, the control (n=6) and undernutrition (n=7) group male offspring were selected with average weight, fasted for 4 h and administered orally with a glucose solution (2 g/kg body weight). Blood glucose concentrations were measured using a blood glucose meter (Gluestest mint; Sanwa Kagaku Kenkyusyo Co., Ltd., Aichi, Japan) just before and at 15, 30, 60, 120, and 180 min after glucose loading. Serum was collected from the tail vein at the same time points, and serum insulin concentrations were measured using Rat Insulin ELISA kits (Shibayagi, Gunma, Japan).

RNA analysis. We performed quantitative reverse transcription polymerase chain reaction (RT-qPCR) to evaluate gene expression levels in the two groups. Blood samples were collected from the tail vein just before and at 15, 30, 60, 120, and 180 min after glucose loading. Serum was collected from the tail vein at the same time points, and serum insulin concentrations were measured using Rat Insulin ELISA kits (Shibayagi, Gunma, Japan).

Fig. 1. Changes in blood glucose levels and serum insulin levels and areas under the curve (AUC) over time following an OGTT in the control and undernutrition groups of rats. (a) Blood glucose. (b) Serum insulin. Data are expressed as the means±SE for 6–7 animals per group. Changes in the concentrations of glucose and insulin were analyzed by one-way ANOVA followed by Tukey’s multiple comparison test. AB, ab Values not sharing a common superscript are significantly different from one another within the same group (p<0.05). Statistical significance of the difference between the control and undernutrition groups for the AUCs of blood glucose and serum insulin concentrations was determined by Student’s t-test. *Significantly different from control rats at p<0.01.
RESULTS

Effects of energy restriction of pregnant mothers on the body weight of their offspring

Body weight of male offspring from the energy restricted mothers (5.9 ± 0.2 g, n = 14) was lower than control (6.5 ± 0.2 g, n = 8) at day 4. Body weight of the 35-d-old male offspring was still lower in the undernutrition group (86.1 ± 1.8 g, n = 7) than in control group (99.2 ± 3.6 g, n = 6).

Effects on energy restriction of pregnant mothers on blood glucose and serum insulin concentrations following an OGTT in the offspring

There were significant time-related changes in blood glucose concentration in both control and undernutrition groups. The blood glucose concentration after oral glucose loading in the control group showed a peak at 15 min, and then decreased rapidly and returned to the fasting level at 60 min. In the undernutrition group, blood glucose concentration peaked at 15–30 min after glucose loading and returned to the fasting level at 120–180 min. The AUC of blood glucose concentration over time was significantly (by 22%, p < 0.05) higher in the undernutrition group than in control group (Fig. 1a).

A significant time-related change was observed only in control group. Serum insulin concentrations in the control group increased at 15 min after glucose loading, however, in the undernutrition group, a time-related change was not observed after glucose loading. The AUC of the insulin concentration over time tended to be lower in the undernutrition group than in the control group (Fig. 1b).

Effects of energy restriction of pregnant mothers on the expression of inflammation-related genes in peripheral blood leukocytes of their offspring

We performed RT-qPCR using total RNA of peripheral leukocytes obtained just before and at 180 min after glucose loading. Expression of genes encoding inflammatory cytokines including TNF-α, IL-1β and cytokine-like factors such as S100 proteins was higher at 180 min after glucose loading than just before glucose loading. Undernutrition during fetal stage also increased the mRNA levels of inflammatory cytokines. The mRNA
levels of S100A9 were synergistically affected by undernutrition during fetal stage and glucose load, and were significantly higher in the undernutrition group at 180 min after glucose loading than in the control group at the same time period (Fig. 2).

Effects of energy restriction of pregnant mothers on the expression of mRNAs for integrins in peripheral blood leukocytes of their offspring

We next determined the expression levels of mRNAs for integrins in peripheral leukocytes from the control and undernutrition groups. Undernutrition during fetal stage had a significant effect on the mRNA levels of CD11a, CD11b, CD11c and CD68. The mRNA levels of CD11b were increased by 2-fold after glucose loading in the undernutrition group of offspring (Fig. 3).

DISCUSSION

In this study, we have demonstrated that a 50% restriction of maternal dietary intake during mid to late gestation of female rats resulted in IGT in the offspring. The results of the present study were supported by previous studies using animal models showing that undernutrition caused by 50% food restriction in pregnant rodents induced IGT in the developing and/or adult stages and reduced pancreatic β-cell mass and insulin content at postnatal day 1 of the pups (20). We found that serum insulin concentration was unchanged following oral glucose load in the offspring of the undernutrition group of rats at 35 d of age. These results suggest that undernutrition during the pregnancy in the rats induces impaired insulin secretion from the weaning period, although it is unclear at present whether insulin resistance is also involved in the IGT in the offspring of the undernutrition group.

In this study, we showed that prolongation of postprandial hyperglycemia caused by fetal undernutrition enhanced the mRNA levels of inflammatory cytokine even with a modest increase in blood glucose peak. We also found that undernutrition of the mother during pregnancy led to an increase in the expressions of inflammatory cytokines such as TNF-α in peripheral leukocytes of the offspring. A previous study demonstrated that a high-fat diet intake induced IGT and enhanced the mRNA levels of TNF-α and IL-1β in peripheral leukocytes of rats (21). IL-1β mRNA expression in peripheral leukocytes was reported to be higher in patients with T2DM than in healthy subjects (22). In addition, studies in rodent models have demonstrated that pronounced or moderate postprandial hyperglycemia was associated with the enhanced expression of inflammation-related genes including those encoding TNF-α, IL-1β, S100A8, and S100A9 (17, 23, 24). The S100A8/A9 heterodimer is also known as a ligand of the receptor for advanced glycation end products (RAGE) and is associated with atherogenesis (25). It is likely that these inflammatory cytokines and S100 proteins should trigger the production of adhesion molecules, such as E-selectin, intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1, which enhance the attachment of leukocytes to blood vessels (26–28).

We found in this study that energy restriction in preg-
Fetal Malnutrition Induces Glucose Intolerance in the Offspring in Rats

The authors no conflicts of interest directly relevant to the content of this article.

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