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Postnatal weight gain induced by overfeeding pups and maternal high-fat diet during the lactation period modulates glucose metabolism and the production of pancreatic and gastrointestinal peptides

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ABSTRACT

The impact of rapid weight gain on glucose metabolism during the early postnatal period remains unclear. We investigated the influence of rapid weight gain under different nutritional conditions on glucose metabolism, focusing on the production of pancreatic and gastric peptides. On postnatal day (PND) 2, C57BL/6N pups were divided into three groups: control (C) pups whose dams were fed a control diet (10%kcal fat) and nursed 10 pups each; maternal high-fat diet (HFD) pups whose dams were fed an HFD (45%kcal fat) and nursed 10 pups each; and overfeeding (OF) pups whose dams were fed the control diet and nursed 4 pups each. Data were collected on PND 7, 14 and 21. The body weight gains of the HFD and OF pups were 1.2 times higher than that of the C pups. On PND 14, the HFD pups had higher blood glucose levels, but there were no significant differences in serum insulin levels between the HFD and C pups. The OF pups had higher blood glucose and serum insulin levels than that of the C pups. Insulin resistance was found in the HFD and OF pups. On PND 14, the content of incretins in the jejunum was increased in the OF pups, and acyl ghrelin in the stomach was upregulated in the HFD and OF pups. These results suggest that neonatal weight gain induced by overfeeding pups and maternal high-fat diet during the early postnatal period modulates the insulin sensitivity and the production of pancreatic and gastrointestinal peptides.

Introduction

The early postnatal period has been suggested to be a crucial window for programming of glucose metabolism that may influence later life. Rapid postnatal weight gain may cause impaired glucose tolerance and insulin sensitivity [6,35] in childhood, and thus represents a potential risk factor for Type 2 diabetes later in life. Rapid postnatal weight gain can be induced by maternal high fat diet [39] or overfeeding [23] during early postnatal period.

Gastrointestinal and pancreatic peptides play important roles in glucose and energy balance. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are synthesized by the endocrine cells in the small intestine [30]. The biological action of GLP-1 [16,26,29] and GIP [10], which stimulate glucose-dependent insulin secretion, has been well established by previous studies. In addition, each peptide is secreted in response to ingestion of nutrients, especially dietary carbohydrates and fat [2]. Ghrelin is produced predominantly by the stomach and stimulates growth hormone secretion, appetite, and fat accumulation [25,41]. Recent studies revealed an essential function of ghrelin in maintaining glucose homeostasis [27,28]. Ghrelin, which contains 28 amino acids, is *N*-octanoylated at Ser3 by ghrelin-*O*-acyltransferase (GOAT) [17,43], a unique modification that is necessary for ghrelin's activity. Ghrelin acylation can be influenced by changing the fatty-acid composition of the diet [24]. Although these peptides are important for maintaining glucose homeostasis,

Abbreviations: PND, postnatal day; GLP-1, glucagon-like peptide 1; GIP, glucose-dependent insulinotropic polypeptide; GOAT, ghrelin-*O*-acyltransferase; Pdx-1, pancreas duodenum homeobox-1; Ghrl, ghrelin; Ins, insulin; Gcg, glucagon; PUFA, polyunsaturated fatty acids.

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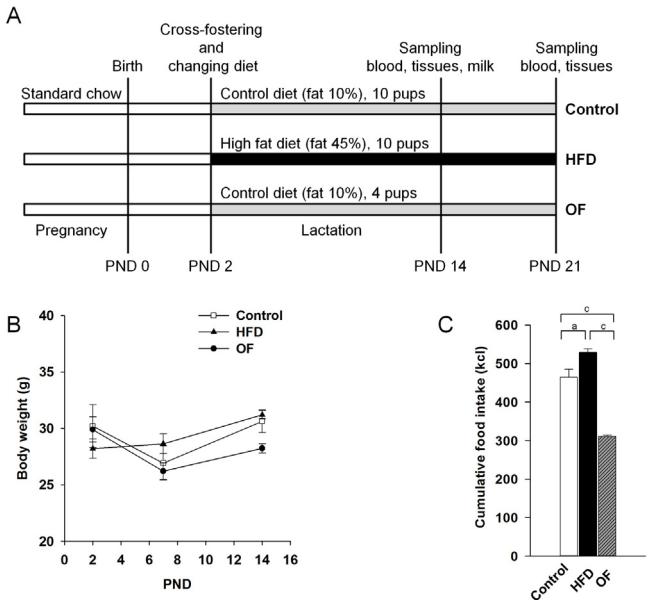

Fig. 1. Schematic of dietary intervention for the dams in three groups. Experimental procedures are indicated according to the age of the pups (A). Changes in body weight and cumulative food intake of dams in the Control, HFD, and OF groups during lactation. Body weight changes of the three groups on PND 2, 7, and 14 (A). Cumulative food intake (kcal) calculated from PND 2 to 14 (B). Data are means ± SE (Control, $n = 3$; HFD, $n = 3$; OF, $n = 4$). [a] $P < 0.05$, [c] $P < 0.001$.

we know relatively little about their production during the neonatal period.

Numerous recent studies have demonstrated the benefits of breastfeeding [1,13,33]. However, due to modern changes in diet, the fat composition of the maternal diet during lactation is high not only in western societies but also in Asia, new mothers consume far more fats and carbohydrates than they need [9]. To date, little is known about the effects of maternal diet on breast milk which directly change the neonatal growth and body composition. Neonatal rapid growth and childhood obesity are associated with the risk of later excessive weight gain and obesity [34,36,38]. In this study, using two different postnatal excessive weight gain mouse models (maternal high-fat diet and overfeeding), we investigated metabolic abnormalities related to childhood obesity and changes in gastrointestinal and pancreatic peptide synthesis. Moreover, we assessed in these animals how the composition of breast milk changes in response to maternal diet, and how these changes influence the offspring.

Materials and methods

Animals

C57BL/6N mice on day 15 of pregnancy were purchased from SLC Japan (Shizuoka, Japan). During pregnancy, the mice were housed individually under standardized environmental conditions (temperature of 24–25 °C, artificial lighting 0700–1900 h). Tap water and standard laboratory chow (CLEA Rodent Diet CE-2, Osaka, Japan) were freely available. All animal procedures were conducted in compliance with protocols approved by Japanese Physiological Society's guidelines for animal care, and were in accordance with the Animal Ethics Committee of National Cerebral and Cardiovascular Center Research Institute and Mie University.

Experimental protocol

Experimental design was shown in Fig. 1A. Dams were allowed to deliver spontaneously, and the day of birth was defined as postnatal day 0 (PND 0). Only dams with litter sizes between 6 and 9 were used in this study. Seventy-six pups were randomly distributed into three groups on PND 2, as follows: control pups (C pups, $n = 30$), whose dams were fed a control diet (3.85 kcal/g with 10% of total calories as fat consisting of soybean oil [5.6%] and lard [4.4%], and 20% as protein; formula D12450B, Research Diets Inc., New Brunswick, NJ, USA) and nursed 10 pups each; maternal high-fat diet pups (HFD pups, $n = 30$), whose dams were fed an HFD (4.73 kcal/g with 45% of total calories as fat consisting of soybean oil [5.6%] and lard [39.4%], and 20% as protein; formula D12451, Research Diets Inc.) and nursed 10 pups each; and overfeeding pups (OF pups, $n = 16$), whose dams were fed the control diet and nursed 4 pups each. The fatty-acid compositions of each diet were analyzed by gas chromatography, as described above, and are shown in Table 1. The sex ratios of pups were adjusted to almost 1:1. The C group included 15 males and 15 females; the HFD group included 15 males and 15 females; and the OF group included 10 males and 6 females. Pups were raised with foster mothers during nursing until PND 21. The dams and pups were weighed on PND 2, 7, 14, and 21. Measurements of body length were performed dorsally on pups from the tip of the nose to the base of the tail on PND 14 and 21. The food intakes of the dams were recorded weekly. Measurements of body length were performed dorsally on pups from the tip of the nose to the base of the tail on PND 14 and 21. Body weight gain was calculated as the difference between PND 2 and PND 14. Fat tissue weight was calculated as the sum of the subcutaneous fat weight and visceral fat weight of pups. Two dams and their pups from each group were euthanized on PND 14, and the others were euthanized on PND 21 between 1000 h and 1200 h under ad libitum feeding conditions to collect blood and tissues of the stomach, jejunum, ileum, and pancreas. On PND 14, stomach milk contents were weighed to assess pup milk intake. All samples were stored immediately at −80 °C until analysis.

Table 1
Fatty-acid composition of experimental diets (mg/100 kcal).

	Fatty acid	Control diet	High-fat diet
C10:0	Caprylic acid	0.80	2.70
C12:0	Lauric acid	1.71	4.97
C14:0	Myristic acid	9.50	50.83
C16:0	Palmitic acid	172.40	950.08
C16:1	Palmitoleic acid	7.52	66.69
C18:0	Stearic acid	81.17	473.79
C18:1	Oleic acid	316.56	1802.79
C18:2	Linoleic acid	449.07	1380.93
C18:3	α-Linolenic acid	41.84	84.57
C20:4	Arachidonic acid	1.36	10.63
C20:5	Eicosapentaenoic acid	0.00	0.00
C22:6	Docosahexaenoic acid	0.00	0.00

Assessment of insulin sensitivity and beta-cell function in the pups

On PND 14 and 21, blood was obtained from the fasted pups after the separation from their dams for 4 h to measure blood glucose and serum insulin levels. Each pancreas was also removed to assess the insulin content. The homeostasis model assessment (HOMA) is a method used quantify insulin resistance (-IR) and beta-cell function (-beta). HOMA-IR was calculated using the following formula: fasting insulin (μIU/ml) × fasting glucose (mg/dl)/405. HOMA-beta was calculated according the formula: [360 × fasting insulin (μIU/ml)]/[fasting glucose (mg/dl) − 63]. The conversion factor for insulin was 1 ng/ml = 26 μIU/ml.

Measurements of blood glucose and peptides

Blood glucose concentrations were measured using the One Touch Ultra Blood Glucose Monitoring system (LIFESCAN, Milpitas,