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Supplemental arginine above the requirement during suckling causes obesity and insulin resistance in rats

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ABSTRACT

Nutrition in early life is important in determining susceptibility to adult obesity, and arginine may promote growth acceleration in infants. We hypothesized that maternal arginine supplementation may promote growth in their pups and contribute to obesity and alteration of the metabolic system in later life. Dams and pups of Wistar rats were given a normal diet (15% protein) as a control (CN) or a normal diet with 2% arginine (ARG). Altered profiles of free amino acids in breast milk were observed in that the concentrations of threonine and glycine were lower in the ARG dams compared with the CN dams. The offspring of the CN and ARG dams were further subdivided into normal-diet (CN-CN and ARG-CN) groups and a high fat–diet groups (CN-HF and ARG-HF). In response to the high fat–diet feeding, the visceral fat deposits were significantly increased in the ARG-HF group (although not compared with the CN-HF group); no difference was observed between the CN-CN and ARG-CN groups. The blood glucose and insulin levels after glucose loading were significantly higher in the ARG-HF group compared with the CN-HF group. The results suggest that the offspring of dams supplemented with arginine during lactation acquired increased susceptibility to a high-fat diet, resulting in visceral obesity and insulin resistance. The lower supply of threonine and glycine to pups may be one of the contributing causes to the programming of lifelong obesity risk in offspring. Our findings also indicated that maternal arginine supplementation during suckling causes obesity and insulin resistance in rats.

1. Introduction

Metabolic syndrome—which includes central obesity, insulin resistance, and hypertension—has a multifactorial etiology that involves a series of complex interactions between individual dietary habits and the genetic background. The current prevalence of metabolic syndrome is a consequence of pervasive obesity. Increasing evidence indicates that the risk of obesity can be developmentally induced during the prenatal or postnatal period by unbalanced maternal nutrition. As such, alterations of nutrient delivery during early periods can significantly impact an individual's suscep-

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CT, computed tomography; IGF-1, insulin like growth factor–1; OGTT, oral glucose tolerance test; TG, triglyceride.

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tibility to obesity, diabetes, and cardiovascular diseases later in life.

The nursing period is an important critical window in the determination of the future metabolic system. Babies born small who experience rapid catch-up growth have been shown to tend to develop obesity in later life [1]. In addition, rapid weight gain during the first 3 months of life is associated with central adiposity and reduced insulin sensitivity in early adulthood compared with slower weight gain [2]. Rapid early growth may thus be linked to adult obesity.

Singhal et al [3,4] suggested that low birth weight with rapid catch-up growth in infants is associated with the feeding of formula milk. The disposition to obesity during the suckling period has also been observed in animal models. In rats, the adult offspring of mothers that were overnourished while suckling are susceptible to obesity despite a normal postweaning diet [5,6]. Overnutrition during preweaning also induced adiposity in young-adult baboons [7]. It was demonstrated that dietary arginine supplementation stimulated protein synthesis and accretion in the skeletal muscle of neonatal pigs [8,9].

Arginine is necessary for the optimal growth of young mammals including rats, pigs, and dogs [10–12]. It is one of the major sources of tissue proteins and the precursor of nitric oxide, urea, polyamines, proline, glutamate, and agmatine [13]. Arginine is a well-known growth hormone stimulator, and growth hormone is an important modulator of linear growth. However, little is known about the influence of arginine supplementation in early life on metabolic systems in later life. We hypothesized that excessive maternal arginine intake during the suckling period may promote early growth and cause metabolic changes in rat offspring. In the present study, we thus examined the effect of arginine supplementation on susceptibility to obesity and insulin resistance in rat offspring, as well as its effect on the composition of breast milk. Our findings demonstrated that excessive arginine supplementation during the suckling period resulted in visceral obesity with insulin resistance in offspring. This result suggests that additional care may be required regarding the arginine intake level in lactating women.

2. Methods and materials

2.1. *Animals*

Eight pregnant (14 days) Wistar rats were purchased from Charles River Laboratories (Kanagawa, Japan). Rats were fed a commercial diet (MF-2, Oriental BioService) until delivery. After delivery, we randomly divided the dams and pups into 2 groups; the first was given a normal diet (15% protein diet) as a control group (CN, 4 dams in the group), and the second group was given a normal diet supplemented with 2% arginine as the arginine-treated group (ARG, 4 dams per). All litters were culled to 8 pups on postnatal day 4. The mean body weight of the pups kept alive was similar in both litters. The dams and their pups were maintained under these conditions until weaning on day 21. After weaning, only the male offspring were studied and were placed in individual wire mesh cages (KN-615, Natsume Seisakusho. Tokyo). The size of cage was 750 mm wide × 210 mm long × 170 mm high.

From 3 weeks of age, all animals were given a normal diet (15% protein diet). At 6 weeks of age, the offspring in the CN and ARG groups were further divided into a normal-diet group (15% protein, 12.5% energy from fat) and a high-fat diet group (15% protein, 35% energy from fat). The composition of each purified diet is described in Table 1. The number of rats in each group was 9-10. The offspring were weighed once a week. Their food intake was measured every day.

An oral glucose tolerance test (OGTT) was performed when the pups reached 11 weeks of age, as follows. After a 16-hour overnight fast, the rats were administered glucose (2 g kg^{-1}) orally. Blood was collected from the tail vein in heparinized tubes chilled on ice. Blood glucose levels were measured using a commercial kit (Wako Pure Chemical Industries, Osaka, Japan), and insulin was measured using an insulin measurement kit (Morinaga Institute of Biological Science, Yokohama, Japan) following the manufacturer's instructions. All samples were run in duplicate.

At 12 weeks of age, the rats were anesthetized with pentobarbital (intraperitoneal, 30 mg·kg^{-1}; Abbott, North Chicago, IL, USA) after a 1-hour fast, and blood was taken from the carotid artery. All rats were euthanized by exsanguination under pentobarbital anesthesia immediately after the blood sampling. The liver, longissimus muscles, and mesentery fats were excised, snap-frozen in liquid nitrogen, and stored at –80°C until the analyses. All animal experiments were carried out in accord with the guidelines of the Animal Usage Committee of the Faculty of Agriculture, University of Tokyo, and were approved by the committee (Permission No. P09-375).

2.2. *Computed tomography scan analysis (body composition)*

For the computed tomography (CT) analysis of body fat mass, 11-week-old rats were anesthetized with isoflurane (3%-4%, 5 L/min; Dainippon Sumitomo Pharma, Osaka, Japan) and then

Table 1 – Semipurified ingredient composition of the diets fed to the rats

	15% protein	15% protein with 2% arginine	High-fat diet
	g/kg		
Energy from fat	12.5%	12.5%	35.0%
Corn starch	620.5	600.5	506.7
L-Arginine	–	20.0	–
Lard	–	–	113.8
Casein [a]	175.0	175.0	175.0
DL-Methionine	2.5	2.5	2.5
Soy bean oil	20.0	20.0	20.0
Vitamin mixture [b]	10.0	10.0	10.0
Mineral mixture [b]	40.0	40.0	40.0
Cellulose powder	100.0	100.0	100.0
Choline chloride	2.0	2.0	2.0

[a] Crude protein 85.71%.
[b] AIN-76 prescription (Oriental Bio Service, Inc, Tokyo, Japan).