

Short Report

# Effect of Meal Time Restriction and Protein Nutrition during Pregnancy and Lactation on the Maternal Body and Growth of Offspring

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## Abstract

Few studies have investigated nutrition consumption and meal time restriction related to maternal dietary intake. We studied the influence of meal time restriction during pregnancy and lactation on the maternal body and on growth of offspring in rats. Pregnant Sprague-Dawley rats aged 7-10 weeks were fed either of the AIN-93G based diets containing 20% or 30% protein (w/w); the control groups were fed *ad libitum* and the two meal-time restricted groups, an 8 hour group fed for 8 hours per day (8h) and a 4 hour group fed for 4 hours per day (4h) were fed the AIN-936 based diets. Food intake and body weight of dams tended to be higher in *ad libitum* groups compared with the 4 h and 8 h groups. Birth body weight of pups was not significantly different among all groups. The final body weight of the *ad libitum* groups tended to be higher than that of the 4 h and 8 h groups. We reported that meal time restriction during pregnancy and lactation on the maternal body had an influence on the growth of offspring in rats.

## 1. Introduction

An adequate supply of nutrition during pregnancy and lactation is important for fetal and postnatal development as well as for milk production by the mother<sup>1)</sup>. Studies of macronutrient (carbohydrate, fat, and protein) consumption or meal time restriction during pregnancy and lactation by mothers<sup>2-4)</sup> and dams<sup>5-7)</sup> report that maternal dietary intake during pregnancy and lactation affects growth and the life span of the child. In addition, maternal dietary intake is associated with an increased risk of lifestyle-related diseases such as obesity, hypertension, diabetes, or carcinogenesis after adulthood<sup>8)</sup>. However, few studies have investigated nutrition consumption and meal time restriction related to maternal dietary intake. In the present study, we studied the effect of protein nutrition and meal time restriction of dams during pregnancy and lactation on the growth of their offspring.

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## 2. Methods

### 2.1 Experimental design

Pregnant Sprague-Dawley rats (Japan CLER, Tokyo, Japan) aged 7-10 weeks were received on day 1 of pregnancy. They were housed in individual plastic cages in a room maintained at 22-25°C with a 12 hour light: dark cycle (artificial lighting from 8:00 to 20:00). Dams and pups had free access to water and food. Dams were weighed three times a week. Pups were weighed three times a week throughout lactation and every day after weaning. The food intake of dams and pups were measured every day to determine their daily intake during the experimental period. The composition of diets shown in Table 1 was based on the AIN-93G diet<sup>9)</sup>. Casein was added to the 30% protein diet and substituted for  $\alpha$ -cornstarch and sucrose.

Dams were randomly assigned to one of two experimental diets (20% or 30% protein diet (w/w)). Each group was divided into three groups: a 24 h group fed *ad libitum* and two meal-time restricted groups, an 8 h group (fed from 8:00 to 12:00 and from 16:00 to 20:00) and a 4 h group (fed from 16:00 to 20:00). Dams were fed from day 1 of pregnancy to day 21 of lactation. Within 24 h of birth, pups were weighed and culled to ten each, then nursed by their dams. On days 7 and 21 after birth, two pups were euthanized in each group. On day 21, the remaining pups were weaned, housed individually, and provided the experimental diet. The pups were euthanized on day 49 (week 7) after birth (day 28 after weaning).

The animal experimental protocols were approved by the Animal Experimentation Committee of Kawasaki University of Medical Welfare, and the study was conducted in accordance with the animal experimentation guidelines of Kawasaki University of Medical Welfare (16-006, 17-001).

Table 1 Composition of the experimental diets

Ingredients	20% protein diets	30% protein diets
Sucrose	22.8	19.4
$\alpha$ - Starch	45.5	38.9
Casein	20.0	30.0
Rapeseed oil	2.2	2.2
Cellulose	5.0	5.0
Mineral mix <sup>1)</sup>	3.5	3.5
Vitamin mix <sup>2)</sup>	1.0	1.0
Total	100.0	100.0
Energy ratio (kcal %)		
Protein	21.4	32.2
Fat	5.3	5.3
Carbohydrate	73.2	62.5

<sup>1)</sup>AIN-93G Mineral mixture. <sup>2)</sup>AIN-93G Vitamin mixture.

### 2.2 Biochemical assays

Blood was collected from the heart of each rat under pentobarbital-induced anesthesia after 12 h of fasting at the end of the experimental period. The collected blood was centrifuged, and then serum was collected and stored at -30°C until assayed. Liver, kidney and perirenal, abdominal, and genital fat tissues were removed from pups and weighed.

Serum glucose and triglyceride concentrations were measured using enzyme methods (Glucose: Glucose CII-Test Wako, Triglyceride: Triglyceride E-Test Wako, Wako Pure Chemical, Osaka, Japan). Serum total protein<sup>10)</sup> and albumin<sup>11)</sup> were measured using a previously described method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by SRL, Inc. Tokyo, Japan.

### 2.3 Statistical analysis

The experimental data are expressed as the mean  $\pm$  standard error (S.E.). Statistical analyses were performed using IBM SPSS Statistics 22 (IBM Japan, Ltd., Tokyo). Tukey's multiple range test was performed to test the significance of inter-group differences after conducting a one-way analysis of variance. Differences between groups were considered statistically significant when  $p < 0.05$ .

## 3. Results

### 3.1 Food intake and body weight change of dams

The food intake and body weight change of dams throughout pregnancy and lactation are shown in Figure 1. Food intake and body weight tended to be higher in the 24 h (*ad libitum*) groups compared with the 4 h and 8 h groups.

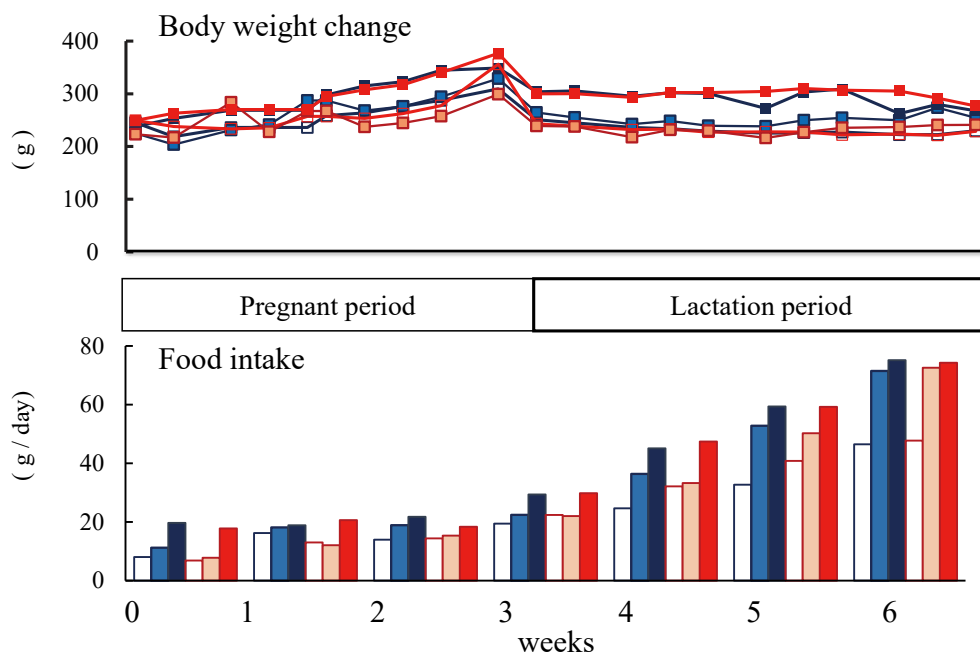


Figure 1 Average intake of experimental diet per week and body weight change of dams. □ ; 20% protein diet and meal time restriction for 4 hours, ■ ; 20% protein diet and meal time restriction for 8 hours, ■ ; 20% protein diet and *ad libitum*, □ ; 30% protein diet and meal time restriction for 4 hours, ■ ; 30% protein diet and meal time restriction for 8 hours, ■ ; 30% protein diet and *ad libitum*.

### 3.2 Food intake, birth and final body weights, body fat weight and organ weights of pups

The mean amount of food intake by pups per week after weaning is shown in Figure 2. Food intake in all groups increased, and was significantly higher in the *ad libitum* groups than in the 4 and/or 8 h groups. However, during the experimental period, no significant differences were observed between the 20% and 30% protein diets. Birth and final body weights and body fat weight of pups are shown in Figure 3. Birth body weight was not significantly different among all groups. The final body weight of the *ad libitum* groups tended to be higher than that of the 4 h and 8 h groups. In the 20% protein diet groups, the final body weight was significantly higher in the *ad libitum* groups and 8 h groups than in the 4 h group. In the 30% protein diet groups, the final body weight was significantly higher in the *ad libitum* group than in the 4 h and 8 h groups. Body fat weight of both protein diets tended to be higher in the *ad libitum* groups than in the 4 h and 8 h groups. In the 20% protein diet groups, the body fat weight was significantly higher in the

*ad libitum* and 8 h groups compared with the 4 h group. In the 30% protein diet groups, the body fat weight was significantly different among all groups. The weights of livers and kidneys in the 20% and 30% protein diet groups tended to increase as the allowed meal time increased (Table 2). In the 20% protein diet groups,

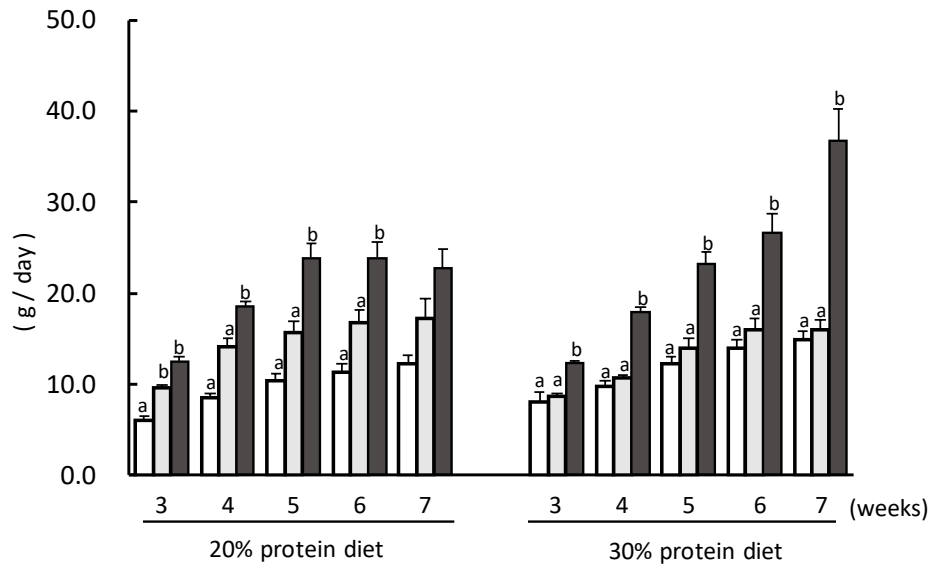


Figure 2 Average intake of experimental diet of pups per week. Each value is the mean  $\pm$  S.E.  $\square$ ; meal time restriction for 4 hours,  $\square$ ; meal time restriction for 8 hours,  $\blacksquare$ ; *ad libitum*. Values not sharing a common letter are significantly different within the same protein diets ( $p < 0.05$ ).

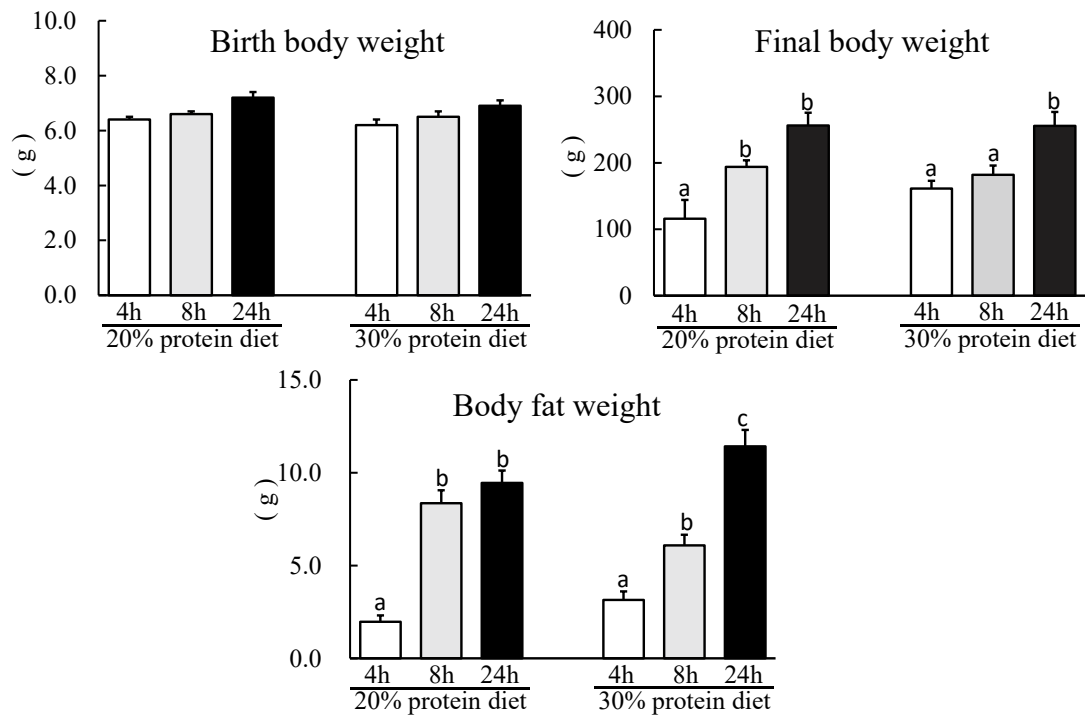


Figure 3 Birth body weight, final body weight and body fat weight of pups. The final body weight was measured on day 49 (week 7) after birth (day 28 after weaning). Each value is the mean  $\pm$  S.E. 4h; meal time restriction for 4 hours, 8h; meal time restriction for 8 hours, 24h; *ad libitum*. Values not sharing a common letter are significantly different within the same protein diets ( $p < 0.05$ ).

the liver and kidney weights were significantly higher in the *ad libitum* and 8 h groups compared with the 4 h group. In the 4 h groups, the liver and kidney weights were significantly higher in the 30% protein diet groups compared with the 20% protein diet groups.

### 3.3 Serum biochemical assays in pups

Serum glucose, total protein and albumin concentrations and ALT and ASL activities were not significantly different between all groups (Table 3). The serum triglyceride concentration tended to be higher in the 8 h groups than in the 4 h and *ad libitum* groups.

Table 2 Liver and kidney weights of pups

		Liver ( g )	Kidney ( g )
20% protein diet	4h	4.64 ± 0.39 <sup>a</sup>	1.04 ± 0.07 <sup>a</sup>
	8h	8.61 ± 0.68 <sup>b</sup>	1.76 ± 0.09 <sup>b</sup>
	24h	9.41 ± 0.57 <sup>b</sup>	2.05 ± 0.11 <sup>b</sup>
30% protein diet	4h	6.84 ± 0.59 <sup>#</sup>	1.61 ± 0.13 <sup>#</sup>
	8h	7.86 ± 0.59	1.65 ± 0.13
	24h	9.61 ± 1.04	2.10 ± 0.17

Each value is the mean ± S.E. 4h; meal time restriction for 4 hours, 8h; meal time restriction for 8 hours, 24h; *ad libitum*. Values not sharing a common letter are significantly different within the same protein diets ( $p < 0.05$ ). A statistically significant difference # $p < 0.05$ , vs 20% protein diet.

Table 3 Serum glucose, triglyceride, total protein and albumin concentrations and ALT and AST activities of pups

	Glucose (mg / 100 mL)	Triglyceride (mg / 100 mL)	Total protein (g / 100 mL)	Albumin (g / 100 mL)	ALT <sup>1)</sup> (IU / L)	AST <sup>2)</sup> (IU / L)	
20% protein diet	4h	133.2 ± 14.4	64.2 ± 10.3 <sup>a</sup>	6.7 ± 0.2	4.2 ± 0.2	17.7 ± 1.0	89.0 ± 4.8
	8h	155.8 ± 16.9	131.1 ± 30.5 <sup>b</sup>	7.7 ± 0.4	4.8 ± 0.4	21.7 ± 1.9	121.3 ± 9.5
	24h	130.0 ± 10.9	74.8 ± 7.2 <sup>ab</sup>	7.3 ± 0.2	4.4 ± 0.4	20.2 ± 2.9	108.5 ± 12.8
30% protein diet	4h	135.4 ± 15.5	55.4 ± 5.9	6.8 ± 0.2	4.8 ± 0.5	22.2 ± 0.8	136.7 ± 12.7
	8h	135.4 ± 16.5	91.2 ± 16.0	7.4 ± 0.6	4.1 ± 0.2	26.2 ± 1.3	142.2 ± 24.2
	24h	102.2 ± 7.2	57.8 ± 7.6	6.8 ± 0.2	4.3 ± 0.2	40.7 ± 12.0	159.2 ± 28.3

Each value is the mean ± S.E. 4h; meal time restriction for 4 hours, 8h; meal time restriction for 8 hours, 24h; *ad libitum*. Values not sharing a common letter are significantly different within the same protein diets ( $p < 0.05$ ).

<sup>1)</sup>Alanine aminotransferase. <sup>2)</sup>Aspartate aminotransferase.

## 4. Discussion

Previous human<sup>9)</sup> and animal<sup>12)</sup> studies reported that dietary habits and lifestyle during pregnancy and the lactation of mothers affected the birth weight of children and their morbidity after adulthood. In this study, we examined the effect of meal time restriction and high-protein diet during pregnancy and lactation on the growth of pups.

Meal time restriction, but not dietary protein content, affected the food intake and body weight gain of dams during pregnancy and lactation. Moreover, the birth weight of pups tended to increase as the meal time became longer, regardless of the dietary protein content. Anderson et al.<sup>13)</sup> reported that the birth body

weight of pups from dams with a restricted diet during pregnancy was significantly lower than groups fed *ad libitum*. Therefore, the difference in food intake because of meal time restriction during the pregnancy and lactation of dams affected the birth weight of pups. The final body weight of pups was significantly higher in the *ad libitum* and 8 h groups compared with the 4 h groups in those fed a 20% protein diet, and in the *ad libitum* group compared with the 4 and 8 h groups fed a 30% protein diet. Furthermore, the total fat weight increased as the meal time became longer. A study that fed the active period, inactive period and *ad libitum* reported no difference in food consumption among the three groups, and the body weight was significantly higher during the inactive period than in the *ad libitum* group and during the active period<sup>14</sup>. However, in the current study, food intake, body weight, and total fat weight were significantly higher in the *ad libitum* groups than in the 4 and 8 h groups. This difference might be because rats in the previous study were larger than those in the current study and were forced to exercise. Furthermore, the food intake of pups was low in the current study because the meal time restriction was initiated just after weaning.

Kidney weights were significantly higher in the *ad libitum* and 8 h groups compared with the 4 h groups fed a 20% protein diet. The longer the meal time, the higher the kidney weight in those fed a 30% protein diet. A previous study reported that a high protein diet during the neonatal period or growth period increased the kidney weight<sup>15,16</sup>. Furthermore, the excessive consumption of protein reduced the excretion of protein from the kidney causing the glomerulus to be enlarged and increasing the weight of the kidney. In the current study, kidney weights increased in the 30% protein diet group as the meal time became longer, suggesting the glomerulus was also enlarged in our experimental groups.

Serum albumin and total protein concentrations were not significantly affected by dietary protein content or meal time because the protein supply in blood to the whole body was sufficient. ALT and AST activities, which are indicators of liver function, tended to be higher in the 30% protein group than in the 20% protein group<sup>17</sup>. A previous study reported that a high protein diet places a burden on the liver and kidney, which might have occurred in the current study causing a functional decline.

Serum glucose and triglyceride concentrations of pups were the highest in the 8 h groups compared with the other groups in the 20% and 30% protein groups. A study that compared the active and inactive periods and *ad libitum* fed group reported that food intake during the inactive period caused a significant increase or decrease in blood glucose levels, potentially by altering blood glucose regulatory mechanisms. In the current study, the 8 h group, which had 8 h meal times during an inactive period, might have had reduced control of blood glucose levels leading to increased insulin resistance and/or decreased insulin sensitivity. Storage of glucose in the liver and muscle as glycogen is limited, and excessive glucose is catabolized to triglyceride that accumulates in body fat. Therefore, triglyceride is closely related to glucose metabolism. Another study reported that an irregular meal time disturbed the rhythm of the liver circadian clock genes and caused lipid metabolism disorders such as hypertriglyceridemia and hyper cholesterol<sup>18</sup>. In the current study, the irregular meal time of 8 h might explain the high levels of serum glucose and triglyceride. However, blood glucose and triglyceride levels in the 4 h groups were lower than in the 8 h groups because it was not possible to consume a sufficient amount of food in 4 h groups.

In summary, pups born from dams with different meal time restrictions and high protein diets throughout pregnancy and lactation were bred under the same dietary conditions as their dams. The birth body weight of pups was not affected by the protein content of the diet consumed by dams, but was affected by meal time restriction. The birth body weight of pups born from dams whose meal time was longer tended to be higher, because the dams ate more in those groups. This suggested that differences in the meal time of dams affected the growth of the fetus. Moreover, the growth of pups after weaning was affected by the meal time of dams and themselves. At this time point, although the protein content in the diet of pups did not affect their growth, the results indicated that those fed a 30% protein diet, which contained more than the normal diet, had altered liver function and metabolism. This study indicates that the lifestyle and eating habits of a dam during pregnancy and lactation affects its fetus, and if the pups have a similar eating habit

to the dam, their growth may be affected.

## 5. Conflict of interest

The authors declare that there is no conflict of interest.

## 6. Acknowledgements

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