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Effect of Maternal Food Restriction on the Evolution of Pregnancy in the Rat

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The present work analyzes the role of food and water intake, nitrogen balance, and *in vivo* D-glucose intestinal absorption in body weight gain during the second half of pregnancy in malnourished mother rats. Our results indicate that nitrogen balance and intestinal absorption of D-glucose were not changed as a result of food restriction, but important negative correlations between water intake and micturition and weight gain and micturition were found in experimental mothers. The retention of water seems to be one of the most important factor influencing body weight gain in malnourished mother rats in the second half of pregnancy.

Key words: Food Restriction, Pregnancy, Nitrogen Balance, Intestinal D-Glucose Absorption, Rats.

Numerous factors can modify the development of the mammalian fetus. Undernutrition during the perinatal period, in particular, can cause growth disturbances, especially in tissues that are undergoing rapid cellular multiplication (4, 8, 21).

Normal pregnant females of many species are known to require considerable amounts of extra energy for formation of conception products and for deposition of maternal fat. This additional energy is mainly obtained by a spontaneous increase of food intake; however, previous studies show that food consumption of normal pregnant rats is appreciably less near term than that of rats in early or mid-pregnancy (11, 16). In studies on the nitrogen requirements of normal pregnant rats it was found that a considerable amount of nitrogen is stored in the maternal body during the first half of pregnancy and used for fetal growth in late

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pregnancy (23). Nitrogen is stored during pregnancy in rats, because the urinary excretion of nitrogen decreases and consequently the nitrogen balance is positive (24).

Conversely, NAISMITH and MORGAN (22) showed both that early protein malnutrition in pregnant rats produced a decreased protein content in placenta and in some organs of the fetus and that administration of a protein-adequate diet to the mother during 5 days of gestation (days 6 to 10) restored all the alterations. Other investigators (12) demonstrated that maintaining pregnant rats on low-protein diets leads to deficiencies in both total protein intake and energy utilization.

Important anatomical and biochemical changes of gastrointestinal tract organs have been reported in mammals during the reproductive cycle (13). Glucose is the principal metabolic fuel for the growing embryo and the fetus (14). The application of low-carbohydrate diets during pregnancy produced important alterations in embryogenesis as well as fetal growth and development in rats (15).

In growing rats, protein malnutrition alters intestinal structure and function (9). In adult rats, partial food deprivation seems to enhance net sugar transport by increasing sugar entry through the apical membrane of enterocytes (18).

Our previous work (4, 5) showed that after the 8th day of gestation the body weight of malnourished rats was always lower than that of controls, but both control and malnourished rats showed a significant increase in body weight during the second half of pregnancy. Controls achieved this increase because they increased food intake, diminished urinary excretion of nitrogen, and suffered gastrointestinal tract modification, but what happens with these parameters in our model of malnutrition is not exactly known. The aim of the present study was to determine the role of nitrogen and intestinal glucose absorption in body weight gain during the second half of pregnancy in malnourished rat dams.

Materials and Methods

A total of 40 virgin female Wistar rats from the Functional Biology Department aged 67 to 77 days weighing 230 to 240 g were placed in groups of three in separate cages with one male per cage. The animals were kept under standard conditions of lighting and darkness (12 h each), temperature (23 ± 3 °C), and humidity (65 ± 1 %). The animals had free access to water and were fed a nonpurified diet (Panlab A04). The general composition of the diet was as follows, in %: Water, 12; protein, 18; fat, 3; fiber, 4.3; carbohydrates, 58.7; ashes, 8; Calcium, 1.28; Phosphorus, 0.65; NaCl, 0.6; Vitamin A, 8,500 I.U./kg; vitamin D_3, 2,000 I.U./kg; energetic value, 2,900 cal/kg.

Copulation was verified by daily vaginal smears (10.00 a.m.) for the presence of sperm (day 0 = day of copulation). Pregnant females were separated randomly into two groups: control (15 animals) and malnourished (20 animals). During pregnancy the malnourished dams were fed 14 g of diet daily (4, 21), while the control dams had free access to food. From the 11th day after the onset of the mating period until the 20th day, females (14 controls and 13 malnourished) were placed in metabolic cages. The lower number of control and experimental rats with respect to the original number of animals on copulation day is due to unsuccessful pregnancies. Daily body weight, food and water intake, defecation and micturition were recorded.

At 10.00 a.m. on the last day of pregnancy, after a 24-h fast, all the females (control and undernourished) were anaesthetized by sodium pentobarbital (40 mg/kg i.p., 10 mg/ml). *In vivo* intestinal absorption of D-glucose (11 mM) was measured as described (8). In order to control blood glucose levels two samples of blood were taken from the jugular vein, one before intestinal absorption of D-glucose and the other after. In the sample obtained before perfusion total protein was measured by using a Beckman multichannel Astra 8 apparatus (Beckman Instruments International, Switzerland).

The nitrogen content in feces was determined by the method of LOWRY *et al.* (17) in accordance with VÁZQUEZ-RODRÍGUEZ *et al.* (29), because they demonstrated the validity of such a method to analyze this parameter in feces.

Urea, creatinine, and total protein in urine were measured by using a Beckman multichannel Astra 8 apparatus. Nitrogen content of urine was calculated daily using the values of these parameters.

Table I. *Correlation coefficients in control and malnourished pregnant rats.*

Factors	Control	Malnourished
Food intake/water intake	0.894[d]	0.100[a]
Food intake/weight gain	0.781[c]	0.244[a]
Water intake/weight gain	0.888[d]	0.916[d]
Food intake/defecation	0.943[d]	0.447[a]
Water intake/miction	0.435[a]	−0.916[d]
Weight gain/defecation	0.741[c]	0.632[b]
Weight gain/miction	−0.754[c]	−0.964[d]

a = $p > 0.05$; b = $P < 0.05$; c = $P < 0.01$; d = $P < 0.001$

The nitrogen balance was determined in accordance with this formula: nitrogen balance = nitrogen intake − (urinary nitrogen + fecal nitrogen).

Student's «t» test with FISHER and YATES correction (6) was used to determine the differences between group means, and Pearson's correlation coefficients were used to examine the relationship between the following variables: maternal weight gain, food intake, water intake, micturition, and defecation.

Results

The evolution of maternal weight gain, food and water intake, defecation and micturition was monitored in metabolic cages during the last 10 day of pregnancy (fig. 1). The values were significantly higher in control with the exception of micturition values in the first days of the study.

The correlation coefficients of the variables analyzed in metabolic cages shows in control, with the exception of the correlation between water intake and micturition, all the other coefficients were very high and weight gain was negatively correlated with micturition (table I). In malnourished animals water intake and weight gain were correlated positively

Table II. *Body weight (BW) and morphometric parameters of small intestine in control and malnourished rats on the last day of pregnancy*
Mean ± S.E. In parentheses, number of animals.

Parameters	Control (n = 11)	Malnourished (n = 12)	p
Body weight (g)	300.58 ± 8.58	259.37 ± 5.89	< 0.001
Intestine wet weight (IWW)			
Absolute (g)	6.69 ± 0.25	6.04 ± 0.14	< 0.05
Relative (g/100 g BW)	2.23 ± 0.06	2.23 ± 0.07	N.S.
Intestine dry weight (g)	1.33 ± 0.06	1.02 ± 0.02	< 0.001
Intestine length (cm)*	118.50 ± 1.97	116.79 ± 1.64	N.S.
IWW/length (g/cm)	$(6 \pm 0.16) \times 10^{-2}$	$(5 \pm 0.15) \times 10^{-2}$	< 0.05

* Intestine stretch was measured under constant tension. N.S. = non significant.

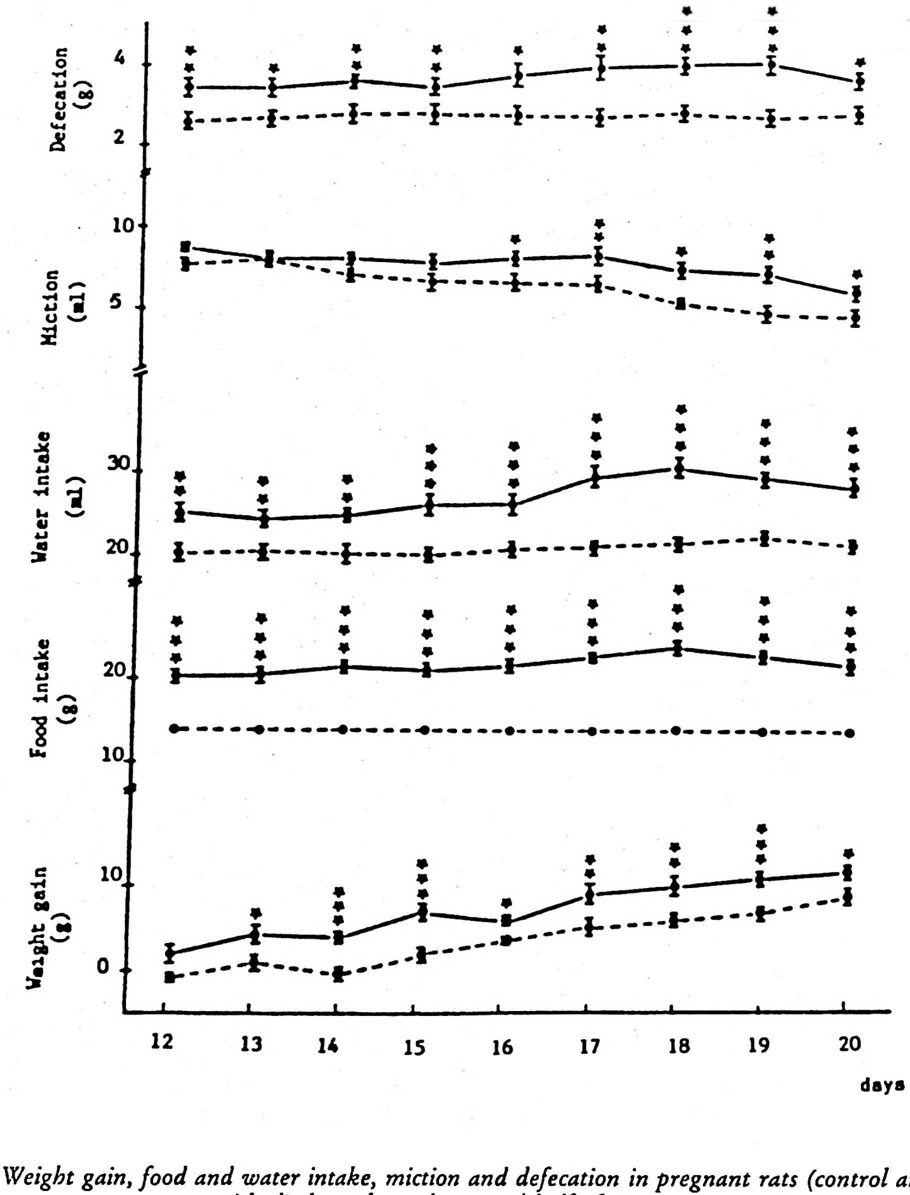

Fig. 1. *Weight gain, food and water intake, miction and defecation in pregnant rats (control and malnourished) throughout the second half of pregnancy.*
— Control, -- Malnourished. * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001.

with weight gain and defecation whereas water intake and weight gain were correlated negatively with micturition.

Intestinal morphometric values on the last day of pregnancy are shown in table II. The mean body weight for malnourished pregnant rats was significantly lower than that of the control. Similarly, mean absolute intestinal weight (wet and dry) was also significantly diminished in mal-

Table III. *In vivo intestinal absorption of D-glucose (mg of glucose/100 ml solution/cm of intestine/min) in control and malnourished fasted rats (24 h) on the last day of pregnancy.*
Mean ± S.E. In parentheses, number of animals.

First sample		
Control	4.72 ± 0.41	(10)
Malnourished	3.55 ± 0.54	(10) N.S.
Second sample		
Control	3.46 ± 0.26	(10)
Malnourished	2.75 ± 0.54	(12) N.S.
Third sample		
Control	2.69 ± 0.18	(10)
Malnourished	1.77 ± 0.24	(11)*

* $p < 0.01$. N.S. = non significant.

Table IV. *Blood values of total proteins before perfusion and glucose before (a) and after (b) in vivo perfusion of D-glucose in control and malnourished fasted rats (24 h) on the last day of pregnancy.*
Mean ± S.E. Number of animals, 10.

Parameters		Control	Malnourished
Total proteins (g/100 ml)		4.59±0.16	3.83±0.18***
Glucose (mg/100 ml)	a	66.70±3.79	56.00±4.82N.S.
	b	136.64±8.56	183.27±8.68***

*** $p < 0.001$; N.S. = non significant.

Table V. *Nitrogen (mg) balance in control and malnourished rats throughout the second half of pregnancy.*
Mean ± S.E. In parentheses the number of animals studied. A = Intake; B = Urinary; C = Fecal

Days	Parameters	Control	Malnourished
12	A	555.66 ± 18.50 (14)	380.80 ± 0.00 (14)***
	A-(B+C)	404.79 ± 34.03 (14)	281.57 ± 13.38 (11)**
13	A	566.41 ± 21.21 (14)	380.80 ± 0.00 (13)***
	A-(B+C)	468.30 ± 27.15 (14)	290.68 ± 6.95 (12)***
14	A	592.34 ± 14.91 (13)	380.80 ± 0.00 (13)***
	A-(B+C)	499.53 ± 18.54 (13)	258.20 ± 14.58 (12)***
15	A	574.50 ± 14.13 (14)	380.80 ± 0.00 (13)***
	A-(B+C)	435.17 ± 31.68 (12)	287.99 ± 0.00 (12)***
16	A	608.80 ± 10.01 (12)	380.00 ± 0.00 (13)***
	A-(B+C)	487.48 + 15.10 (13)	286.84 ± 11.76 (13)***
17	A	631.46 ± 11.11 (13)	380.80 ± 0.00 (13)***
	A-(B+C)	527.93 ± 11.53 (13)	289.51 ± 10.41 (13)***
18	A	661.87 ± 12.99 (13)	380.80 ± 0.00 (13)***
	A-(B+C)	561.75 ± 13.33 (13)	302.82 ± 9.70 (13)***
19	A	629.80 ± 15.11 (13)	380.80 ± 0.00 (13)***
	A-(B+C)	548.23 ± 14.99 (12)	311.18 ± 10.24 (12)***
20	A	604.47 ± 19.26 (13)	380.80 ± 0.00 (13)***
	A-(B+C)	537.03 ± 19.86 (12)	323.42 ± 7.48 (12)***

** $p < 0.01$; *** $p < 0.001$.

nourished mothers. The mean intestinal length was similar in both groups.

In vivo intestinal absorption of D-glucose (table III) was always higher in control mothers and in the last sample was significantly higher, and decreased both in control and undernourished mothers with time.

However, blood glucose levels after intestinal absorption was significantly higher in undernourished mothers. Basal values of proteins were significantly reduced in malnourished mothers (table IV).

Daily nitrogen balance of control and malnourished pregnant rats, showed an increased balance from the 1st day of the study until the end, but in controls this increase was more significant. Daily nitrogen intake was significantly higher in controls (table V).

Discussion

In the second half of pregnancy, control and malnourished mother rats increased body weight as nitrogen balance was positive and increased throughout pregnancy. Controls showed a positive nitrogen balance because they increased food intake, there being a significant positive correlation between weight gain and both water and food intake. A decreased micturition and urinary excretion of nitrogen were also observed. These results are in agreement with those of other authors (7, 12, 24). In malnourished rats, the weight gain during pregnancy is more difficult to explain, because food intake was the same throughout pregnancy and water intake did not change significantly. In addition, there is no correlation between weight gain and food and water intake. This may be explained by the significant negative correlation between water intake with micturition and weight gain with micturition. These results point to a possible water retention. Some authors (28) have shown an increase in the hydration of fat-free body weight in malnutrition suffering children. On the other hand, blood urea levels were significantly increased in malnourished dams compared with controls (2). ADELMAN (1), in moderately uremic rats, found an abnormal body composition with a retention of water and pointed out that the changes observed seemed to be primarily a consequence of uremia *per se* rather than undernutrition. Perhaps the high urea level found in the malnourished pregnant rats (2) can be explained by water retention.

In accordance with previous results (24), urinary excretion of nitrogen decreased throughout pregnancy.

The change in intestinal weight in malnourished pregnant rats (table II) parallels changes in body weight. Intestine length did not change in response to malnutrition. However, *in vivo* intestinal absorption of D-glucose after a 24-h fast decreased in the three samples of malnourished rats and in the last sample showed marked differences. These results are in disagreement with findings by other authors (3, 18), who reported in semi-starved non pregnant rats, that during the period of restricted food intake, in which the animals lost weight, the absorption capacity of the small intestine was greatly increased in order to maintain homeostasis. However other authors have reported that the results are not different when the animals are fasted as 1 day fasted rats showed a reduced sugar and amino acid transport activity (20). Our experimental conditions summarize both semi-starvation and 24-h fasting and measured *in vivo* intestinal glucose absorption, whereas others (3, 18) studied this absorption *in vitro*.

On the last day of pregnancy and after 24-h of fasting, blood glucose levels decreased in malnourished mother rats (table IV), but after *in vivo* perfusion of D-glucose, this value was higher in malnourished mothers than in controls. This could be due to the fact that malnutrition induces decreases in insulin release, which promote catabolism of different tissues, especially fat. On the other hand, low levels of insulin facilitate blood glucose increase and glucose by fetus, because fetal glucose uptake is a function of maternal glucose concentration (10). In this sense some authors have shown that malnutrition decreases blood insulin levels in pregnant rats (20, 27).

Plasma proteins are primarily synthesized in the liver and increase in quantity during pregnancy (26). A severe food restriction during pregnancy, as in this experiment, reduces liver hypertrophy, hyperplasia and glucose storage (25). Because of this, proteins are broken down by the mothers's liver to produce glucose (19), thus decreasing protein serum levels. Our results are in agreement with this.

The influence of lipidic metabolism and hormones in our malnourished mother rats during pregnancy, is not known. Further studies are necessary to analyze these parameters and others implicated in the evolution of pregnancy and food restriction during this important phase for the offspring.

The retention of water seems to be the most important factor influencing body weight gain in malnourished mother rats in the second half of pregnancy.

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Resumen

Se analiza el efecto de la ingesta de comida y agua, el balance de nitrógeno y la absorción intestinal de D-glucosa *in vivo* en la ganancia de peso corporal de ratas madres malnutridas, durante la segunda mitad de la preñez. Los resultados indican que el balance de nitrógeno y la absorción intestinal de D-glucosa no experimentan cambios como resultado de la restricción alimentaria, aunque las madres experimentales muestran una correlación negativa entre la ingesta de agua y la micción, y la ganancia de peso y la micción, por lo que la retención de agua parece ser el factor más importante que influye en la ganancia de peso corporal en ratas madres malnutridas en la segunda mitad de la preñez.

Palabras clave: Restricción alimentaria, Preñez, Balance de nitrógeno, Absorción intestinal de D-glucosa, Rata.

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