Obligatory Nitrogen Losses in Pregnant Rats with Special Reference to Estimation of Net Protein Utilization

Sadaichi SAKAMOTO, Masahiro MORI,* Masaharu OHNACA, and Yoshiaki NIIYAMA

Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima 770, Japan
*Department of Domestic Science, Kinran Junior College, Suita 565, Japan
(Received July 15, 1992)

Summary For estimation of net protein utilization of dietary proteins during pregnancy, obligatory nitrogen losses were measured in protein-deficient rats in which pregnancy was maintained by administration of ovarian steroids. On shift from normal to protein-free diet, urinary nitrogen, expressed as mg/day or mg/100 g BW per day, decreased initially rapidly and then gradually during the first two weeks in both pregnant and nonpregnant rats. However, urinary endogenous nitrogen increased during the final week of pregnancy, whereas it continued to decrease in nonpregnant controls. The endogenous urinary nitrogen excretions during early-mid and late pregnancies were significantly higher in pregnant rats (666 mg/15 days and 234 mg/6 days, respectively) than in nonpregnant animals (585 mg/15 days and 153 mg/6 days, respectively), indicating pregnancy-induced protein hypercatabolism. The metabolic fecal nitrogen excretions in pregnant and nonpregnant rats were comparable. In pregnant rats, a protein-free diet resulted in decrease of basal energy expenditure, from 24 kcal/day on day 1 to about 15 kcal/day on days 16, 19 and 22 of pregnancy. Thus, the ratio of endogenous urinary nitrogen to basal energy expenditure increased in late pregnancy, indicating that “the law of a constant relationship of minimal nitrogen and energy output” is not applicable to the pregnant animals. We discuss which values for obligatory nitrogen loss should be used for estimating the net utilization efficiency of dietary proteins in pregnancy.

Key Words endogenous urinary nitrogen, metabolic fecal nitrogen, endogenous nitrogen/BMR, pregnancy induced hypercatabolism, NPU, protein-free diet

Obligatory urinary and fecal excretions of nitrogen have been determined in various animals, including humans, to determine the minimum nitrogen requirements and/or to assess the net utilization of dietary proteins (NPU) (1). Results
indicated that urinary excretion of endogenous nitrogen expressed in relation to basal metabolism, is relatively constant in different animal species (2, 3). These observations have led to proposition of "the law of a constant relationship of minimal nitrogen and energy output" (4).

Obligatory nitrogen losses in pregnant women have not been determined because administration of a protein-free diet may have adverse effects on mothers and their babies. Moreover, no data are available on obligatory nitrogen excretions in pregnant laboratory animals, because administration of a protein-free diet results in abortion (5). However, we (6) and others (7, 8) found that pregnancy could be maintained in these animals by daily injections of ovarian steroids. Thus, we examined the obligatory nitrogen losses in pregnant rats maintained on steroids to test the above law and to obtain fundamental values for NPU estimation.

MATERIALS AND METHODS

Virgin Sprague-Dawley strain rats, weighing about 190 g, were mated with males of the same strain. Day 1 of pregnancy was determined by daily examination of vaginal smears.

Animals were fed on protein-free diet ad libitum throughout pregnancy. To prevent the abortion due to undernutrition, the animals received daily s.c. injections of 0.5 μg of estrone and 4 mg of progesterone from day 3 of pregnancy until the day before autopsy. Nonpregnant rats receiving protein-free diet and steroids were used as controls.

Animals were housed individually in metabolic cages at 23±1 °C with a 12 h-12 h light-dark cycle. Urine and feces were collected during the entire experimental period for nitrogen analysis. Food intake and body weight were measured daily.

On days 16, 19 and 22 of pregnancy, basal energy expenditure was measured by indirect calorimetry. For this, animals were placed in a metabolic chamber maintained at 30 °C and their expired air was aspirated into a paramagnetic O₂ analyzer (Beckman Model E2) and an infra-red CO₂ analyzer (Horiba LIA-2B) at a rate of 300 ml/min.

On days 16 and 22 of pregnancy, animals were killed by decapitation and the products of conception were removed and weighed. Nitrogen in the urine and feces was determined by the semimicro-Kjeldahl method. Creatine and creatinine in urine were determined by the alkaline-picrate method.

RESULTS

Table 1 shows the total food intakes in 21 days and the changes in body weight of pregnant and nonpregnant animals during the experiment. Pregnant and nonpregnant rats ate totals of 188 and 169 g of diet, respectively. The body weights of both pregnant and nonpregnant animals decreased steadily during the first two weeks. The body weight of pregnant rats increased during the last week of
Table 1. Food intakes and body weights.

|                | Food intake | Body weight on |
|----------------|-------------|----------------|
|                | Total (g/21 days) | Daily (g) | Day 1 (g) | Day 16 (g) | Day 19 (g) | Day 22 (g) |
| Pregnant (8)1  | 188±242     | 8.9         | 192±8     | 164±4a      | 174±6b     | 194±12b    |
| Nonpregnant (6) | 169±41     | 8.0         | 191±6     | 159±2       | 153±4      | 146±3      |

1 Figures in parentheses indicate numbers of rats. 2 Mean±SD. a and b Significantly different from values for nonpregnant rats at levels of 5 and 0.1%, respectively.

Table 2. Reproductive performances of rats given protein-free diet and ovarian steroids.

|                | Weight of products of conception1 (g) | Weight2 of | Litter size |
|----------------|---------------------------------------|------------|-------------|
|                |                                       | Fetus (g)  | Placenta (mg) |           |
| Day 16 (11)2  | 10.6±1.34                              | 0.30±0.06  | 167         | 9.7±1.6    |
| Day 22 (8)    | 38.5±4.0                               | 2.66±0.41  | 278         | 10.0±1.9   |

1 Products of conception consist of the uterus, placenta, fetuses and amniotic fluid. 2 Weight of one fetus or placenta. 3 Figures in parentheses indicate numbers of rats. 4 Mean±SD.

Table 3. Changes in urinary endogenous nitrogen excretion during pregnancy.

| Days of pregnancy | 1–4 | 4–7 | 7–10 | 10–13 | 13–16 | 16–19 | 19–22 |
|-------------------|-----|-----|------|-------|-------|-------|-------|
| (mg/day)          |     |     |      |       |       |       |       |
| Pregnant (8)1     | 65±82 | 46±8 | 42±10 | 36±4 | 33±4 | 38±4a | 40±5b |
| Nonpregnant (6)   | 57±12 | 38±10 | 38±7 | 32±8 | 30±2 | 27±6 | 25±5 |
| (mg/100 g BW per day) |       |       |      |       |       |       |       |
| Pregnant          | 35±4 | 26±5 | 25±6 | 22±2 | 20±2 | 22±2a | 22±4a |
| Nonpregnant       | 30±6 | 22±6 | 22±4 | 19±4 | 19±1 | 17±4 | 16±3 |

1 Figures in parentheses indicate numbers of rats. 2 Mean±SD. a and b Significantly different from values for nonpregnant rats at levels of 1 and 0.1%, respectively.

pregnancy, while that of nonpregnant rats at the same time continued to decrease.

Reproductive performances on days 16 and 22 of pregnancy are shown in Table 2. Rats maintained pregnancy with the aid of exogenous steroids, although they received protein-free diet. However, fetal growth was about 50% of that of rats receiving a normal diet (6).

Table 3 shows the changes in urinary endogenous nitrogen output during pregnancy, expressed as mg/day and mg/100 g body weight per day, respectively. Absolute amounts of nitrogen output decreased gradually during the first two
Table 4. Obligatory nitrogen losses during pregnancy.

|                | Days 1–16 | Days 16–22 | Days 1–22 |
|----------------|-----------|------------|-----------|
|                | Urine (mg/15 days) | Feces | Urine (mg/6 days) | Feces | Urine (mg/21 days) | Feces | Sum |
| Pregnant (8)   | 666±201²  | 255±45    | 234±21ᵃ    | 72±13   | 901±77ᵇ   | 337±31 | 1,238±67ᵇ |
| Nonpregnant (6)| 585±99    | 249±30    | 153±27     | 63±24   | 738±123   | 312±51 | 1,050±168 |

¹Figures in parentheses indicate numbers of rats. ²Mean±SD. ᵃ and ᵇ Significantly different from values for nonpregnant rats at levels of 0.1 and 5%, respectively.

Table 5. Changes in creatinine output during pregnancy.

|                | Days of pregnancy |
|----------------|-------------------|
|                | 1–4               | -7    | -10  | -13  | -16  | -19  | -22  |
| (mg/day)       |                   |       |      |      |      |      |      |
| Pregnant (8)   | 7.05±0.30ᵃ        | 6.91±0.43 | 6.63±0.47 | 6.41±0.48 | 6.35±0.54 | 6.54±0.72ᵃ | 5.85±0.76 |
| Nonpregnant (6)| 6.37±0.68         | 6.59±0.36 | 6.65±0.41 | 6.27±0.46 | 6.32±0.23 | 5.67±0.51 | 5.61±0.66 |
| (mg/100 g BW per day) |            |       |      |      |      |      |      |
| Pregnant       | 3.81±0.16ᵃ       | 3.94±0.29 | 3.95±0.19 | 3.92±0.32 | 3.88±0.31 | 3.91±0.39 | 3.19±0.46ᵃ |
| Nonpregnant    | 3.42±0.37        | 3.68±0.26 | 3.84±0.23 | 3.78±0.27 | 3.95±0.12 | 3.66±0.41 | 3.77±0.48 |

¹Figures in parentheses indicate numbers of rats. ²Mean±SD. ᵃSignificantly different from the value for nonpregnant rats at a level of 5%.
weeks, irrespective of whether the animals were pregnant or not. However, nitrogen output increased during the final week of pregnancy, whereas it continued to decrease in the nonpregnant controls. Changes in relative amounts of urinary endogenous nitrogen were similar with those in absolute amounts in both groups. The possibilities that increased urinary nitrogen in late pregnancy was due to proteinuria or genital bleeding near term were excluded by checking urinary proteins.

Obligatory losses of urinary and fecal nitrogen in two periods of pregnancy (days 1 to 16 and 16 to 22) are shown in Table 4. Urinary losses in early-mid and late pregnancies were larger than those in the corresponding periods in control rats. The total urinary losses in pregnant and nonpregnant rats were 901 and 738 mg/21 days, respectively, suggesting that pregnancy resulted in increased protein catabolism. On the other hand, fecal nitrogen loss in pregnant rats was comparable with that in nonpregnant rats, amounting to totals of 337 and 312 mg/21 days, respectively.

Changes in urinary creatinine output during pregnancy are shown in Table 5. Absolute amounts of creatinine excretion (mg/day) decreased slightly and progressively in pregnant and control animals, whereas relative amounts (mg/100 g body weight per day) were fairly constant throughout this period in both groups. Daily creatine excretion in the urine was increased rapidly and linearly from 1.5 mg on day 1 to 6.5 mg on day 22 during pregnancy (detailed data not shown).

Table 6 shows the basal metabolic rate (BMR) and the ratio of urinary endogenous nitrogen to BMR in rats during late pregnancy. Basal energy expenditure of pregnant rats fed protein-free diet was about 15 kcal/day in late pregnancy. As the urinary level of endogenous nitrogen increased in late pregnancy, amounting to 33, 38 and 40 mg/day on days 16, 19 and 22, respectively, the ratio of endogenous nitrogen to the BMR increased in late pregnancy from 2.2 on day 16 to 2.7 at term.

---

Table 6. Minimal nitrogen and energy outputs of rats during late pregnancy.

|                  | BW (g) | BMR (kcal/d) | EN (mg/d) | Ratio (mg/kcal) |
|------------------|--------|--------------|-----------|-----------------|
| Pregnant         |        |              |           |                 |
| Day 1 (7)        | 184±6  | 24.0±1.0     | —         | —               |
| Day 16 (8)       | 160±3  | 15.2±1.1     | 33±4      | 2.2±0.4         |
| Day 19 (8)       | 172±7  | 14.8±1.2     | 38±4      | 2.6±0.3         |
| Day 22 (8)       | 186±11 | 14.9±0.6     | 40±5*     | 2.7±0.3*        |
| Nonpregnant      |        |              |           |                 |
| Day 16 (6)       | 159±2  | 18.0±3.1     | 30±2      | 1.7±0.5         |
| Day 22 (6)       | 146±3  | 14.3±1.5     | 25±5      | 1.7±0.3         |

1 Basal metabolic rate. 2 Urinary endogenous nitrogen. 3 Figures in parentheses indicate numbers of rats. 4 Mean±SD. 5 Significantly different from the value on day 22 for nonpregnant rats at a level of 0.1%.
In contrast, this ratio did not change in nonpregnant animals. The ratio in early-mid pregnancy was fairly constant (about 1.7, detailed data not shown) and was comparable with that of controls.

DISCUSSION

Amounts of obligatory nitrogen loss are important for estimating both the minimal nitrogen requirements of animals and the true digestibility, or NPU, of a dietary protein (9,10). Accordingly, these losses have been studied extensively in humans and various animals. However, no data are available for pregnant animals, because of nutritional abortion resulting from administration of a protein-free diet (5). Daily administration of ovarian steroids to protein-deficient rats, however, allows the maintenance of pregnancy (6-8) and so measurement of obligatory nitrogen losses during pregnancy.

In the present study as well as in earlier studies in which obligatory nitrogen losses were examined, animals were fed a protein-free diet ad libitum and became anorexic (11). On the contrary, in most of experiments in humans, sufficient dietary energy was provided to maintain the body weight. Differences in energy consumption between animals and humans pose the problem of whether minimal nitrogen outputs of animals with decreased energy intakes can be regarded as "obligatory losses" (12). In this connection, it is noteworthy that studies on rats, dogs and humans showed that reduction in the amount of protein-free diet by 50% of a normal diet liberally consumed did not affect nitrogen excretion (1). The intake of protein-free diet by our rats was more than half of that of normal diet consumed by rats during pregnancy (Table 1) (6). Thus, it seemed reasonable to consider nitrogen output as obligatory loss.

As shown in Table 3, urinary endogenous nitrogen output was higher in pregnancy, particularly in late pregnancy than in nonpregnancy. The reason for this difference is not obvious, but is probably due to pregnancy-induced hypercatabolism. Increases in endogenous nitrogen excretion near term may be partly due to decreased energy intakes at term.

Terroine and Sorg-Matter first showed that in warm-blooded adult animals, the endogenous nitrogen level was proportional to the BMR (2). This finding has been confirmed by many workers and has led to "the law of a constant relationship of minimal nitrogen and energy output" (4). In pregnant rats, however, the ratio of urinary endogenous nitrogen to the BMR increased near term from 2.2 on day 16 to 2.7 on day 22, whereas the ratio in nonpregnant controls in the corresponding period remained lower and constant at about 1.7 (Table 6). Preliminary experiments showed that the ratios in early-mid pregnancy were lower than those in late pregnancy and were comparable to those of controls (data not shown). From the present results, we concluded that the above law derived from observations on nonpregnant animals is not applicable to pregnant animals, indicating that estimation of the minimal nitrogen requirement by multiplying the constant ratio of
endogenous nitrogen/BMR by the measured BMR is not correct for pregnant females (9, 10).

What figures of obligatory nitrogen losses should be used for estimating the NPU of dietary proteins in pregnancy? In nonpregnant rats the urinary excretion of endogenous nitrogen changes exponentially in three phases (1). The initial, second and terminal phases coincide with the catabolisms of labile, mobilizable and "fixed" proteins, respectively, and the durations of these phases are about 1 week, 1 month and 40 to 200 days, respectively. The amount of urinary nitrogen at the steady level in the late second phase has so far been regarded as endogenous nitrogen loss and used for NPU estimation. In pregnant rats, however, urinary endogenous nitrogen did not reach a steady level because pregnancy terminates at 3 weeks and the level increased in late pregnancy as shown in Table 3. Thus, it is reasonable to use the total amounts of obligatory nitrogen loss during the 21-day pregnancy period (Table 4) for estimating the NPU of dietary proteins. We are now estimating the net utilization efficiencies of various proteins in pregnant rats.

REFERENCES

1) Peret, J., and Jacquot, R. (1972): Nitrogen excretion on complete fasting and on a nitrogen-free diet—Endogenous nitrogen, in Protein and Amino Acid Functions, ed. by Bigwood, E. J., Pergamon Press, Oxford, pp. 73–118.
2) Terroine, E. F., and Sorg-Matter, H. (1928): Influence of the quantity of heat output on the endogenous nitrogen metabolism. Compt. Rend., 186, 1017–1019.
3) Hawley, E. E., Murlin, J. R., Nasset, E. S., and Szymansky, T. A. (1948): Biological values of six partially-purified proteins. J. Nutr., 36, 153–169.
4) Terroine, E. F., and Sorg-Matter, H. (1927): The quantitative law of the nitrogen minimum of homotherms. Arch. Int. Physiol., 29, 121–132.
5) Nelson, M. M., and Evans, H. M. (1953): Relation of dietary protein level to reproduction in the rat. J. Nutr., 51, 71–84.
6) Endo, S., Niiyama, Y., Kamori, K., and Inoue, G. (1974): Effect of protein deprivation during pregnancy on nucleic acid and protein syntheses in fetal rat brain and liver. Nutr. Rep. Int., 10, 209–218.
7) Nelson, M. M., and Evans, H. M. (1954): Maintenance of pregnancy in the absence of dietary protein with estrone and progesterone. Endocrinology, 55, 543–549.
8) Hazelwood, R. L., and Nelson, M. M. (1965): Steroid maintenance of pregnancy in rats in the absence of dietary protein. Endocrinology, 77, 999–1013.
9) Smuts, D. B. (1934): The relation between the basal metabolism and the endogenous nitrogen metabolism, with particular reference to the estimation of the maintenance requirement of protein. J. Nutr., 9, 403–433.
10) Report of a Joint FAO/WHO ad hoc Expert Committee (1973): Energy and Protein Requirements. WHO Techn. Rep. Ser. No. 522.
11) Hitier, Y., Champigny, O., Homayoon, P., and Bourde, G. (1982): Circadian feeding pattern in pregnant rats fed three levels of protein. Ann. Nutr. Metab., 26, 129–137.
12) Does feeding a protein-free diet accurately estimate endogenous protein and amino acid recovery? (1990) Nutr. Rev., 48, 293–294.

Vol. 38, No. 5, 1992