Implication of Maternal Nitrogen Balance in the Regulation of Circulating Levels of Insulin-Like Growth Factor-I in Human Pregnancy

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Abstract. Nutrition plays an important role in regulating the circulating levels of insulin-like growth factor-I (IGF-I). We have demonstrated that reduced nitrogen balance is related to changes in serum IGF-I levels but not serum growth hormone levels in pregnant rats. In the present study, we investigated the effects of changes in nitrogen balance on serum IGF-I levels in normal and malnourished pregnant women (defined as having negative nitrogen balance). Forty-eight pregnant women (threatened miscarriage and premature labor, 39; pre-eclampsia, 3; hyperemesis, 3; ileus, 2; bleeding from the colon, 1) and 19 non-pregnant women admitted in Kobe University Hospital were enrolled in this study. Blood samples of normal pregnant controls were also obtained from 172 healthy pregnant women attending the outpatient clinic. Serum IGF-I levels and nitrogen balance were measured serially in 9 pregnant women with threatened miscarriage and premature labor and 9 malnourished pregnant women out of 48 pregnant women. Serum IGF-I and urinary nitrogen levels were measured by radioimmunoassay with acid-ethanol extraction and the Dumas method, respectively. Nitrogen balance was expressed as the difference between daily nitrogen intake and nitrogen excretion assessed by urinary nitrogen levels. Serum IGF-I levels in normal pregnant controls significantly increased in the third trimester of pregnancy compared with non-pregnant controls. No difference in serum IGF-I levels in any trimester of pregnancy was observed between normal pregnant controls and pregnant women with threatened miscarriage and premature labor. There was no significant difference in nitrogen balance between the pregnant women with threatened miscarriage and premature labor and non-pregnant controls. In the longitudinal study, no correlation was found between the changes in serum IGF-I levels and those in nitrogen balance in the 9 pregnant women with threatened premature labor (daily nitrogen balance > 0 g/day) on the basis of linear regression analysis. On the other hand, the changes in serum IGF-I levels in the 9 malnourished pregnant women were significantly correlated with those in nitrogen balance ($y = 1.72x + 17.5$; $r = 0.60$; $P < 0.05$: linear regression analysis). These results indicate that maternal nutritional states have a major effect on serum IGF-I levels in malnourished pregnant women, but not in pregnant women with daily nitrogen balance > 0 g/day. Serum IGF-I levels can be a potent index of nutritional states under malnutrition during human pregnancy.

Key words: Human pregnancy, Circulating IGF-I, Nitrogen balance, Maternal nutritional status, Nutrition
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NUTRITION plays an important role in regulating circulating levels of insulin-like growth factor-I (IGF-I) [1]. IGF-I mediates the anabolic effects of growth

hormone (GH) [2–4]. Circulating levels of IGF-I decrease in fasting volunteers [5] and malnourished patients [6] accompanied by a reduction in nitrogen retention, whereas serum levels of GH increase during starvation [7]. Fasting also inhibits the GH-stimulated increase in circulating levels of IGF-I in GH-deficient patients [8]. These findings have shown that a nutritional state plays a dominant role in the regulation of circulating IGF-I levels in the malnourished state.

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In normal pregnancy, variant GH secreted from the placenta regulates the circulating levels of IGF-I [9], which increase in the third trimester of pregnancy [10–12]. In malnourished pregnant women, nutritional states are likely to affect circulating levels of IGF-I because the protein catabolism of malnutrition is responsible for acquired GH resistance [13]. We have already demonstrated that reduced nitrogen balance is related to changes in circulating levels of IGF-I in pregnant rats [14]. Limited information, however, exists regarding the effect of circulating IGF-I levels on maternal nutritional states in malnourished pregnant women.

We designed the present study to clarify the effects of changes in nitrogen balance on circulating levels of IGF-I in normal and malnourished pregnant women defined as having a daily nitrogen balance of less than 0 g/day.

Materials and Methods

Subjects

Forty-eight women with singleton pregnancies,

under the care of the Department of Obstetrics and Gynecology at Kobe University Hospital, were enrolled in the study. The clinical indications for admission to hospital were as follows: threatened miscarriage and premature labor ($n = 39$); pre-eclampsia defined by the criteria of Davey and MacGillivray [15] ($n = 3$); hyperemesis ($n = 3$); ileus ($n = 2$) and bleeding from the colon ($n = 1$).

Serum levels of IGF-I and nitrogen balance were measured longitudinally in 9 patients with threatened premature labor and 9 malnourished ones out of the 48 pregnant women. The clinical characteristics and reasons for admission of 9 malnourished pregnant women are shown in Table 1. Malnutrition was attributable to hyperemesis, ileus, fasting due to bleeding from the colon or pre-eclampsia. Nineteen age matched non-pregnant women admitted with sterility participated in this study as non-pregnant controls. None of the women were taking medication known to interfere with IGF-I metabolism. During hospitalization, each woman had a regular diet with nitrogen content of 11.2 to 14.4 g/day (Table 2). Pregnant women before 27 weeks of pregnancy, as well as non-pregnant women, were served standard diet 1. Pregnant women after 28 weeks of pregnancy and

Table 1. Clinical characteristics and reasons for admission of malnourished pregnant women

Case	Age	Diagnosis	Gestational weeks (weeks)	Length of hospital admission (days)	Body mass index (kg/m^2)	Baseline nitrogen balance (mg/day)	Serum total protein level (g/dl)	Serum albumin level (g/dl)	Baseline IGF-I level (ng/ml)
1	23	Hyperemesis	8	12	16.2	– 3.3	4.7	2.8	84.6
2	24	Hyperemesis	10	9	20.1	– 2.9	4.8	2.9	92.3
3	30	Hyperemesis	11	31	18.5	– 4.2	4.7	2.7	60.6
4	31	Fasting due to ileus	18	24	21.9	– 3.6	4.4	2.5	68.2
5	25	Fasting due to ileus	19	37	22.9	– 3.2	4.5	2.6	75.4
6	28	Fasting due to bleeding from the colon	28	22	20.0	– 3.7	4.4	2.9	54.4
7	26	Pre-eclampsia	35	10	25.2	– 0.9	4.6	2.5	155
8	29	Pre-eclampsia	36	13	24.6	– 4.5	4.4	2.3	103
9	26	Pre-eclampsia	36	8	24.3	– 10.1	4.2	2.2	75.7

Table 2. Nutritional composition of regular diets

	Energy (K cal)	Carbohydrate (g)	Protein (g)	Fat (g)	Nitrogen (g)
Standard diet 1	1900	300	70	45	11.2
Standard diet 2	2100	300	80	65	12.8
Protein-rich diet	1800	230	90	60	14.4

pregnant women with pre-eclampsia were served standard diet 2 and the protein-rich diet, respectively. Water or low caloric carbonated beverage containing no measurable amounts of protein was made freely available. Daily nitrogen intake during hospitalization was assessed from written dietary records. Blood samples were obtained from 172 age matched, healthy pregnant women in the outpatient clinic as normal pregnant controls. All pregnant women and non-pregnant controls provided their informed consent after the purpose of the study was explained to them.

Experimental design

Blood and urine samples from hospitalized patients were obtained on admission after an overnight fast. In the longitudinal study, serum IGF-I levels and nitrogen balance at the first sample collection were regarded as the baseline levels. Subsequent sample collections were carried out at 3–14 day intervals until the end of the study period. Blood samples from the normal pregnant controls were obtained between 09:00 and 13:00 in the outpatient clinic. All blood samples were collected into plain tubes. Sera were obtained at room temperature by centrifugation and stored at -20°C until assayed. Daily 24-hour urine samples were obtained from hospitalized patients for urinary nitrogen measurement. The Institutional Review Board has approved this study design.

IGF-I measurement

The iodination of IGF-I with [^{125}I] was performed as previously described [14]. Serum levels of IGF-I were measured by a radioimmunoassay according to the method of Hintz *et al.* [16] with Seppak C18 cartridge chromatography [17].

Nitrogen balance determination

Urinary nitrogen was measured by the Dumas method [18]. Nitrogen balance was expressed as the difference between daily nitrogen intake and daily nitrogen excretion determined from urinary nitrogen levels using a previously reported method [5].

Statistical analyses

Data were analyzed using the unpaired Student's *t*-test or simple regression analysis. Statistical significance was accepted at the < 0.05 level.

Results

Serum levels of IGF-I in normal pregnant controls significantly increased in the third trimester of pregnancy ($n = 68$: 214.3 ± 96.4 ng/ml, mean $\pm$ S.D.) but not in the first ($n = 43$: 139.4 ± 53.7 ng/ml) and second trimester ($n = 61$: 175.6 ± 70.0 ng/ml) compared with those in non-pregnant controls ($n = 19$: 141.4 ± 63.3 ng/ml). Serum levels of IGF-I in 39 pregnant women admitted with threatened miscarriage and premature labor also increased in the third trimester ($n = 19$: 226.3 ± 136.8 ng/ml) but not in the first ($n = 8$: 117.6 ± 84.3 ng/ml) and second trimester ($n = 12$: 138.3 ± 68.7 ng/ml) compared with those in non-pregnant controls. No significant difference in serum IGF-I levels in any trimester was observed between normal pregnant controls and pregnant women admitted with threatened miscarriage and premature labor. Nitrogen balance in pregnant women with threatened miscarriage and premature labor is illustrated in Fig. 1. Mean values of nitrogen balance in each gestational month (from 0.5 g/day to

Fig. 1. Change in nitrogen balance during pregnancy. Columns are expressed as mean values in each gestational month for 3–7 pregnant women with threatened miscarriage or premature labor and 19 non-pregnant controls. Each bar represents the standard deviation.

5.2 g/day) did not change throughout pregnancy. No significant difference in nitrogen balance between the pregnant women admitted with threatened miscarriage and premature labor and non-pregnant controls (2.2 ± 2.1 g/day) was observed.

In the longitudinal study, no correlation was found between the changes in IGF-I levels and those in nitrogen balance in the 9 pregnant women with threatened premature labor with a daily nitrogen balance

Fig. 2. Association between the change in serum IGF-I levels and nitrogen balance in 9 pregnant women threatened premature labor (nitrogen balance > 0 g/day). Changes are expressed as differences from the baseline levels. There is no correlation between two variables.

> 0 g/day using linear regression analysis (Fig. 2). The changes in IGF-I levels and nitrogen balance in one of the women are shown in Fig. 3 as an example. Serum IGF-I levels were markedly higher than the baseline level (84.0 ng/ml) after 28 weeks of pregnancy, and they were almost twice (175.0 ng/ml) the baseline level at 32 weeks of pregnancy. In contrast, there was no difference in nitrogen balance levels between 22–32 weeks of pregnancy.

The changes in IGF-I levels and nitrogen balance in 3 out of 9 malnourished pregnant women are shown in Fig. 4. In case 3, a nadir level of IGF-I (55.7–80.9 ng/ml) with negative nitrogen balance was

Fig. 3. Change in serum IGF-I levels and nitrogen balance in a pregnant woman with threatened premature labor.

Fig. 4. Change in serum IGF-I levels and nitrogen balance in the malnourished pregnant women (cases 3, 6 and 7).

observed until 13 weeks of pregnancy. Serum IGF-I levels were dramatically elevated (166.6 ng/ml at 15 weeks) because of an increase in nitrogen balance caused by intravenous hyperalimentation (IVH) at 14 weeks. Case 6 did not eat for several days at 27 weeks because of bleeding from the colon. Serum IGF-I levels, therefore, decreased to 36.4 ng/ml at 28 weeks, but they rose after 29 weeks when the woman recommenced eating with a corresponding improvement in nitrogen balance. In case 7, a progressive loss of nitrogen by proteinuria (1.0 g/day) was observed after 35 weeks (-15.4 g/day and -41.4 g/day at 36 and 37 weeks, respectively). Circulating IGF-I levels, therefore, gradually declined until delivery. The changes in IGF-I levels were significantly correlated with those in nitrogen balance in the 9 malnourished pregnant women with a daily nitrogen balance < 0 g/day ($r = 0.60$, $P < 0.05$; linear regression analysis) (Fig. 5).

Fig. 5. Association between the change in serum IGF-I levels and nitrogen balance in the 9 malnourished pregnant women (nitrogen balance < 0 g/day). Changes are expressed as differences from the baseline levels. There is a significant correlation between these two variables ($y = 1.72x + 17.5$; $r = 0.60$; $P < 0.05$; linear regression analysis).

Discussion

In the present study, we have shown that there was no significant difference in nitrogen balance between pregnant and non-pregnant women, whereas pregnant women retained nitrogen in the fetus and their reproductive tissues. The reason for the absence of difference in nitrogen balance may be attributable to the error of estimating nitrogen intake by written dietary record. In this study, mean nitrogen retention in non-pregnant controls was 2.2 g/day, which means that the value in the third trimester was expected to be between 0.9 and 2.2 g/day. Nitrogen balance levels in pregnant women in this study are consistent with the findings of Johnstone *et al.* [19] who reported a mean apparent nitrogen retention of 1.2 g/day between 30–34 weeks of pregnancy. This value was equal to the estimated daily nitrogen retention for growth of the fetus and reproductive tissues during this period, which means that pregnant women are unable to store protein. Previous reports have estimated the total amount of surplus nitrogen stored by pregnant women during the entire pregnancy [20] and the third trimester of pregnancy [21] to be 120–150 g. However, particular attention was not paid in these studies to the pregnant woman diet.

Estimating maternal nitrogen balance in humans will always be subject to error, given that fetal nitrogen content is difficult to measure because no non-invasive method exists. In pregnant rats, we have previously determined maternal nitrogen balance using measurement of fetus nitrogen content [14]. The gestational period in rats, however, is markedly shorter than that in humans. Further investigation of maternal nitrogen balance using pregnant animals, which have a longer gestational period, is required.

In the longitudinal study, we have demonstrated for the first time that changes in nitrogen balance in malnourished pregnant women are correlated with changes in circulating IGF-I levels. There was no such association in pregnant women who were normally nourished. These results support the view that maternal nutritional status affects circulating levels of IGF-I in malnourished pregnant women. Our previous findings in pregnant rats [14] correspond with the hypothesis presented in this study. In pregnant women who are normally nourished, it seems likely that circulating variant GH regulates the circulating levels of IGF-I because it is regarded as a

dominant regulator of circulating levels of IGF-I in human pregnancy [9].

To our knowledge, circulating levels of variant GH in malnourished pregnant women remain to be elucidated. Moreover, little information is available as to the effects of nutritional status on the synthesis and secretion of variant GH by the trophoblast [22]. GH resistance is a commonly recognized feature of protein catabolic status [13]. It seems likely that circulating levels of variant GH in malnourished pregnant women are elevated compared with those in normal pregnant women, and that variant GH has little effect on the circulating levels of IGF-I.

Several investigators have argued that serum specific protease activity for IGF binding protein-3 (IGFBP-3) is noted during human pregnancy [23, 24]. IGFBP-3 is responsible for the turnover of circulating IGF-I because the ternary complex formation consisting of IGF-I, IGFBP-3 and acid labile subunit extends the half-life of IGF-I peptide in the circulation [25–29]. Serum protease activity for IGFBP-3, therefore, may be an alternative factor responsible for the regulation of IGF-I availability during human pregnancy. Malnutrition during severe illness in humans has been noted to induce the activity of serum protease for IGFBP-3 [30]. These findings suggest that serum protease activity for IGFBP-3 in malnourished pregnant women may play a role in the change in circulating levels of IGF-I.

Throughout the longitudinal study, fetal weight

was estimated regularly using ultrasonography. Interestingly, intrauterine growth restriction was not detected in any of the malnourished pregnant woman, even though the daily maternal nitrogen balance in malnourished pregnant women was < 0 g/day. This supports the hypothesis that reduced maternal IGF-I levels may contribute to the inhibition of maternal anabolism to maintain fetal growth in malnourished pregnant women. Further investigation in a large population of malnourished pregnant women is required to verify this hypothesis.

In conclusion, the present study provides, for the first time, evidence for an association between nutritional status and circulating levels of IGF-I in malnourished pregnant women. Serum IGF-I levels may be used as a potential index of nutritional status in malnourished pregnant women.

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