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EFFECT OF MODERATE MATERNAL MALNUTRITION ON THE LEVELS OF ALKALINE RIBONUCLEASE ACTIVITY IN THE HUMAN PLACENTA†

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Ribonuclease (RNAse) activity was measured in placentas from a sample of 49 urban Guatemalan women from low (30) and high (19) socioeconomic status (SES). Low SES women had a larger proportion (40%) of high levels of RNAse (> 60,000 units/g) than women from the high SES group (5%). A trend to inverse association ($p \cdot 0.06$) was also observed between amount of supplemented food energy during pregnancy and RNAse activity in rural populations covered by two food supplementation programs (protein energy and energy). Two indicators of maternal nutrition, third trimester weight and height, also showed inverse associations with placental RNAse activity. It is inferred from these results that improved maternal nutrition decreases placental RNAse activity. Moreover, high levels of placental RNAse activity were associated, up to 36 months of age, with higher proportions of: physical growth retardation in weight, height and head circumference; below-average psychological test performance; and infant deaths. These results deserve consideration from both the physiological and public health points of view.

INTRODUCTION

It has been shown that in rural populations with moderate malnutrition, food supplementation during pregnancy produces increments in birth weight (Lechtig *et al.*, 1975b), and in placenta weight (Lechtig *et al.*, 1975 c). As yet, the mechanisms by which a moderate degree of maternal undernutrition affects fetal growth have not been elucidated. Recent data support the possibility that maternal undernutrition may primarily affect placental function and secondarily fetal growth. Placentas from low socioeconomic groups from urban or rural communities are characterized by small weights (Lechtig *et al.*, 1975c), reduced DNA content (Winick, 1969), reduced proportion of ribosomal RNA aggregated in polysomes (Laga, Driscoll and Munro, 1972b) and reduced peripheral villi surface area (Lechtig *et al.*, 1975c; Laga, Driscoll and Munro, 1972a). In addition, protein malnutrition in the rat reduces the rate of transfer of glucose (Rosso, 1974) and certain amino acids (Rosso, 1975a). All these findings suggest a reduction in overall metabolic function of the placenta and a reduced ability to carry out transfer of nutrients.

Some of these biochemical changes in the placenta may provide useful markers to determine whether fetal nutritional status during pregnancy is adequate. In addition, the activity of alkaline ribonuclease pH 7.8 (RNAse) in placentas from women belonging to a low socioeconomic group in Ecuador has been found to be elevated (Velasco *et al.*, 1976), but the causes and implications of such a change in placental RNA metabolism are still unclear. Elevated RNAse activity has been described in placenta and other tissues associated with a reduced concentration of cellular RNA (Rosso, 1975b; Rosso and Winick, 1975; Kraft and Shortman, 1970) and such a reduced concentration of RNA would imply a reduced capacity to synthesize protein. In the placenta, reduced protein synthesis may affect nutrient transport by reducing the availability of carrier and/or the activity of enzymes involved in transfer mechanisms. However, regardless of the biochemical changes responsible for an elevated RNAse activity, such an

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elevation could prove to be a valuable marker of fetal nutritional status during pregnancy. Two specific questions are addressed by this study: First, does moderate maternal malnutrition produce an increment of the activity of placental RNAse? Second, does increased RNAse activity have any correlation with postnatal growth and development?

EFFECT OF MODERATE MATERNAL MALNUTRITION ON THE ACTIVITY OF PLACENTAL RNAse

In order to answer the first question, two studies in urban and rural areas, were carried out. Their design and results have been described previously (Lechtig *et al.*, 1975c). In both studies placentas were collected immediately after birth, refrigerated (4–8° C) weighed (± 1 g) and the portion (approximately 20 g) used for the present study was ground and immediately frozen. Placentas were maintained frozen for approximately three years until analysis at the Institute of Human Nutrition, Columbia University, New York.

RNAse activity was determined as previously reported (Velasco *et al.*, 1976) in a sample of a 15 percent homogenate using a personal modification of the method of Roth (1968) and expressed as unit (U) per 30 minutes, calculated arbitrarily by assuming that a change of 1.0 U of Optical Density was equal to 100 U of RNAse activity. The reliability of this determination as measured by test-retest correlations between different samples of the same placenta was 0.95.

Urban Study

Two groups of pregnant women of high and low socioeconomic status (high SES and low SES, respectively) were studied (Lechtig *et al.*, 1975c).

Figure 1 shows that placental RNAse activity was significantly lower in the high SES group than in the low SES group. Based on the values observed in the high SES group, placental RNAse values equal to or higher than 60,000 units/gram were defined as "high" RNAse values. The limit value was obtained by adding two standard deviations to the mean value observed in the high SES group. The value obained (59,313 units/g) was rounded to 60,000 units/g. The basic assumption is that this value covers most of the mothers who have an adequate nutritional status.

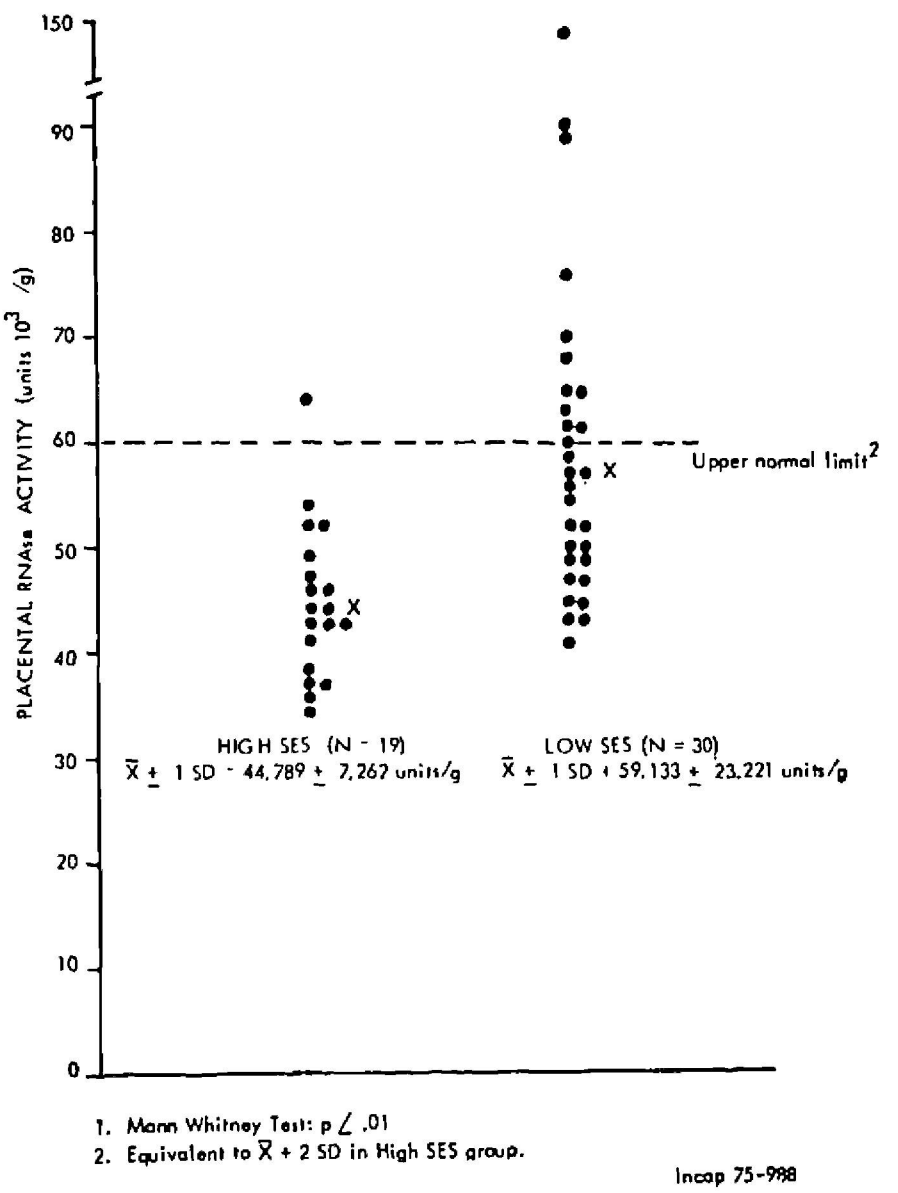


FIGURE 1 Placental RNAse activity according to level of socioeconomic status (SES)[1] in urban Guatemalan women.

As seen in Figure 1, 12 (40.0 percent) of the 30 cases in the low SES group presented high placental RNAse values while only one (5.3 percent) of the 19 high SES cases presented high values of RNAse. Previous reports on the same study sample showed that the differences in SES reflect differences in nutritional status as measured by anthropometric and biochemical indicators (Lechtig *et al.*, 1975c). Consequently, the finding that RNAse activity is higher in low as opposed to high SES mothers is compatible with the hypothesis that moderate maternal malnutrition is associated with an increment of placental RNAse activity.

Rural Study

The study population was made up of pregnant women from four villages presently involved in the INCAP study of nutrition and mental development (Lechtig *et al.*, 1975b; Klein, Habicht and Yarbrough, 1973).

In two of the villages a protein-energy preparation called *atole* (a gruel) was distributed; the other two villages received an energy supplement

called *fresco* (a refreshing cool drink). The energy concentration of the fresco being around one third that of the atole. Lastly, both preparations provided similar quantities of the vitamins and minerals which might be deficient in the diets of this population (Lechtig *et al.*, 1975b).

In the following analyses, supplement intake will be expressed as energy to facilitate comparisons between the fresco and the atole groups. It should be stressed that in the atole group 41.8 KJ (10,000 Cals) of supplemented energy are accompanied by 675 grams of supplemented protein. Table I shows that there existed a trend to inverse association between the amount of supplemented energy during pregnancy and placental RNAse activity in both the fresco and the atole groups. Although this association was weak, the negative trend was seen in both groups. Similar results were observed when this analysis was repeated computing rank correlations. Also, the slope values of the association did not change significantly after controlling for maternal home diet, height, head circumference, first trimester weight, days of disease during pregnancy, socioeconomic score within the villages, parity, gestational age and placental weight. The fact that energy is the main limiting factor in the diet of this population may explain the lack of significant slope differences between the atole and fresco groups (Lechtig *et al.*, 1975b).

Combined Urban and Rural Studies

Table II shows that two indicators of maternal nutrition: third trimester weight, an approximate indicator of nutritional status during pregnancy;

TABLE I

Correlation values between caloric supplementation during pregnancy and placental RNAse activity in rural Guatemalan women

Study group	Correlation (r)	Slope (RNAse units per 10,000 supplemented calories)	n	p <	Supplemented calories during pregnancy		RNAse units/g placenta	
					$\bar{X}$	SD	$\bar{X}$	SD
Fresco	—0.318	—2800	27	0.10	27866	23753	63407	20500
Atole	—0.105	—1000	29	0.40	30172	20411	62690	20088
Total	—0.217	—2000	56	0.10	29061	21913	63036	20105
Total[a]	—0.262	—2200	56	0.06	—	—	—	—

[a]Partial correlation after controlling, in a multiple regression predicting RNAse activity, for maternal diet and morbidity during pregnancy; maternal height, head circumference, first trimester weight and socioeconomic score; parity, gestational age and placental weight.

TABLE II

Relationship between indicators of maternal nutritional status and placental RNAse activity in urban and rural Guatemalan women

Indicator of maternal nutrition	Sample	Correlation (r)	Slope (b)	n	p value (less than)
			(RnAse units/kg)		
Third trimester weight	Urban	—0.284	—520	44	0.05
	Rural	—0.158	—410	39	0.30
	Total	—0.246	—420	83	0.01
			(RNAse units/cm)		
Height	Urban	—0.189	—434	44	0.20
	Rural	—0.167	—694	54	0.20
	Total	- 0.185	—449	98	0.03

and height, a possible reflector of maternal nutritional history, were also inversely related to placental RNAse activity.

Obviously, it is always possible that genetic factors may be producing both low height or low weight and high placental RNAse activity. However, this possibility appears to be remote since in the two studies presented above (which represent two independent comparisons), the slope values were very similar.

Different sanitary conditions or different height may have played a role in the differences in RNAse activity observed in the urban study. However, these factors obviously cannot explain differences in RNAse activity associated with the amount of supplemented calories given during pregnancy in the rural study. The possibility remains that low height produced low dietary intake and, therefore, increased the RNAse placental activity. If this were the case, maternal dietary intake would still be an indispensable component of the causal chain influencing placental RNAse activity. However, this alternative explanation seems unacceptable since no differences in height and home diet were observed in the rural group between the low and high supplemented groups (Lechtig *et al.*, 1975c). Therefore, a conservative interpretation of these data is that in these populations moderate maternal malnutrition causes increased placental RNAse activity.

PLACENTAL RNAse ACTIVITY AND POSTNATAL GROWTH AND DEVELOPMENT

An attempt to answer this question was made by studying the rural population group where follow-up data up to 36 months of age were available.

The independent variable for these analyses was placental RNAse activity and the dependent variables were: proportion of children with physical growth retardation at birth, 12, 24 and 36 months of age; proportion of children scoring below the average in psychological tests (Klein *et al.*, 1975) at 6, 15, 24 and 36 months of age, and proportion of infant deaths.

An inverse and consistent relationship between placental RNAse activity and birth weight in the urban, rural, and total samples can be observed in Table III and in Figure 2, the slope values being very similar in the urban and rural groups.

It is evident that there was a trend toward a higher proportion of low birth weight (LBW) babies in the groups with high RNAse values.

A strong positive relationship was previously reported between placental weight and birth weight in the same sample (Lechtig *et al.*, 1975c). In order to learn what proportion of the association between indicators of maternal nutrition and birth weight could be explained by both placental weight and RNAse activity, the reduction in the association between maternal nutrition and birth weight produced after controlling for the influence of these two factors was estimated (Figure 3). Clearly, a marked reduction in the explained variance of birth weight and in the slope values can be observed in all three analyses (see differences between left, intermediate and right bars of Figure 3) indicating that most of the association between indicators of maternal nutrition and birth weight could be explained by placental weight and RNAse activity. In the light of studies in animals (Hill *et al.*, 1971) the present data suggest that in humans the increment in placental weight and the decrement in placental RNAse activity are important mechanisms of the effect of improved maternal nutritional status on birth weight.

Figure 4 summarizes the main relationship between level of activity of placental RNAse and proportion of cases with physical growth re-

TABLE III

Correlations between placental RNAse activity and birth weight in infants in rural and urban Guatemala

Study group	Correlation (r)	Slope (g birth weight per 1,000 units of RNAse) (b)	n	p value (less than)
Rural	—0.247	—6.2	53	0.10
Urban	—0.260	—5.6	48	0.10
Total	—0.223	—5.0	101	0.05

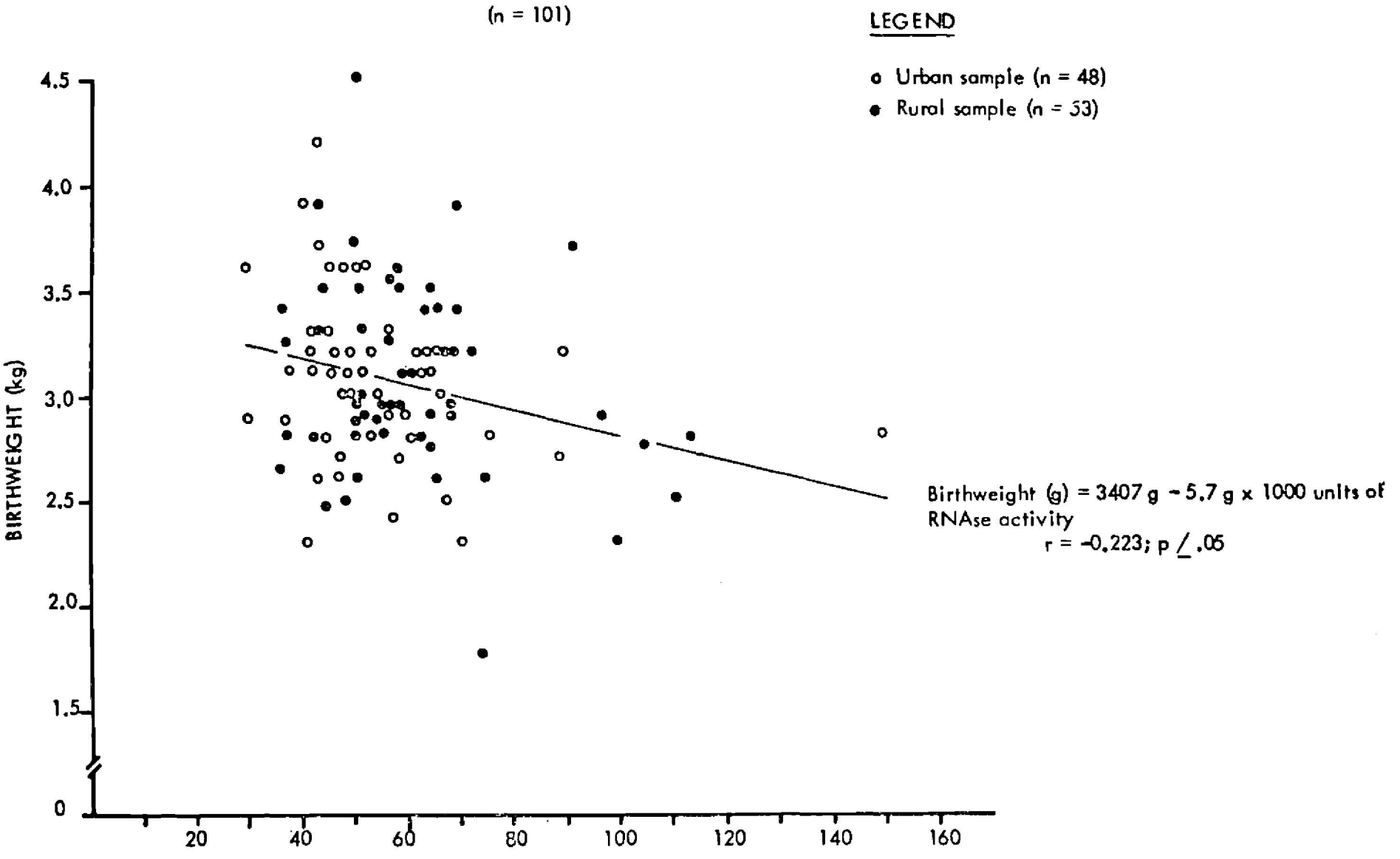


FIGURE 2 Relationship between placental RNAse activity and birth weight in the combined samples of urban and rural Guatemalan mothers and infants.

tardation (PGR) defined as the lower tercile of the population distribution in attained weight, height and head circumference at 12, 24 and 36 months of age. All these children were below the 10th percentile of Denver standards (Hansman, 1970) in all measurements. Since we do not believe that these population groups differ in genetic potential (Habicht *et al.*, 1974) we regard this deficit as true retardation. It can be observed in Figure 4 that in all the comparisons made there was a trend to higher proportions of PGR children in the high RNAse groups and that the nine correlations computed using continuous variables were negative. Therefore, it was concluded from these data that high levels of placental RNAse activity were associated with higher proportions of children with PGR in weight, height and head circumference up to 36 months of age.

Figure 5 presents the relationship between levels of placental RNAse activity and the proportion of cases (scoring below the average SBA) in psychological tests administered at 6, 15, 24 and 36 months of age (Klein *et al.*, 1975). A dichotomous variable was chosen for these analyses due to the small number of cases available in the lowest tercile of the population. A trend is clear towards a higher proportion of SBA cases in the high RNAse group. The partial correlations after controlling for a composite indicator of family social and economic status were essentially unchanged: −0.186, −0.065, −0.334 and −0.185 at 6, 15, 24 and 36 months of age, respectively. Similar results were observed after controlling for birth weight and placenta weight.

The small number of deaths (six deaths) did not permit analyses with discrete variables to be carried out. However, it is remarkable that the correlations between placental RNAse activity and infant mortality (alive = 1, dead = 2) were 0.103 (p = NS), 0.358 ($p < 0.01$) and 0.269 ($p < 0.01$) for the urban, rural and total samples, respectively. The observation that all six infant deaths had placental RNAse values higher than 50,000 units per g and that only 67 percent of those who survived were over this value, provides further insight into the different distributions of RNAse values in infants who survived and those who died.

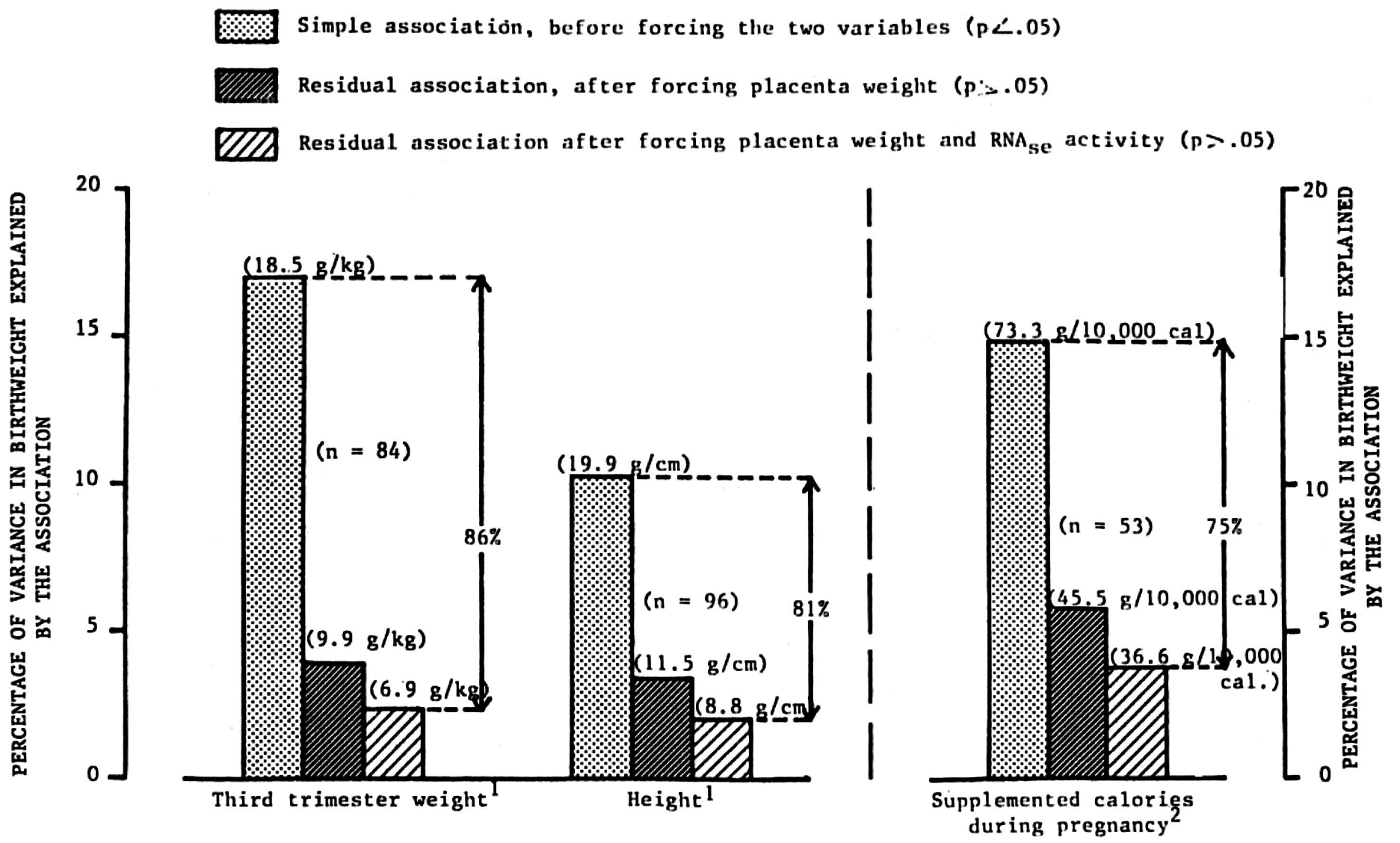
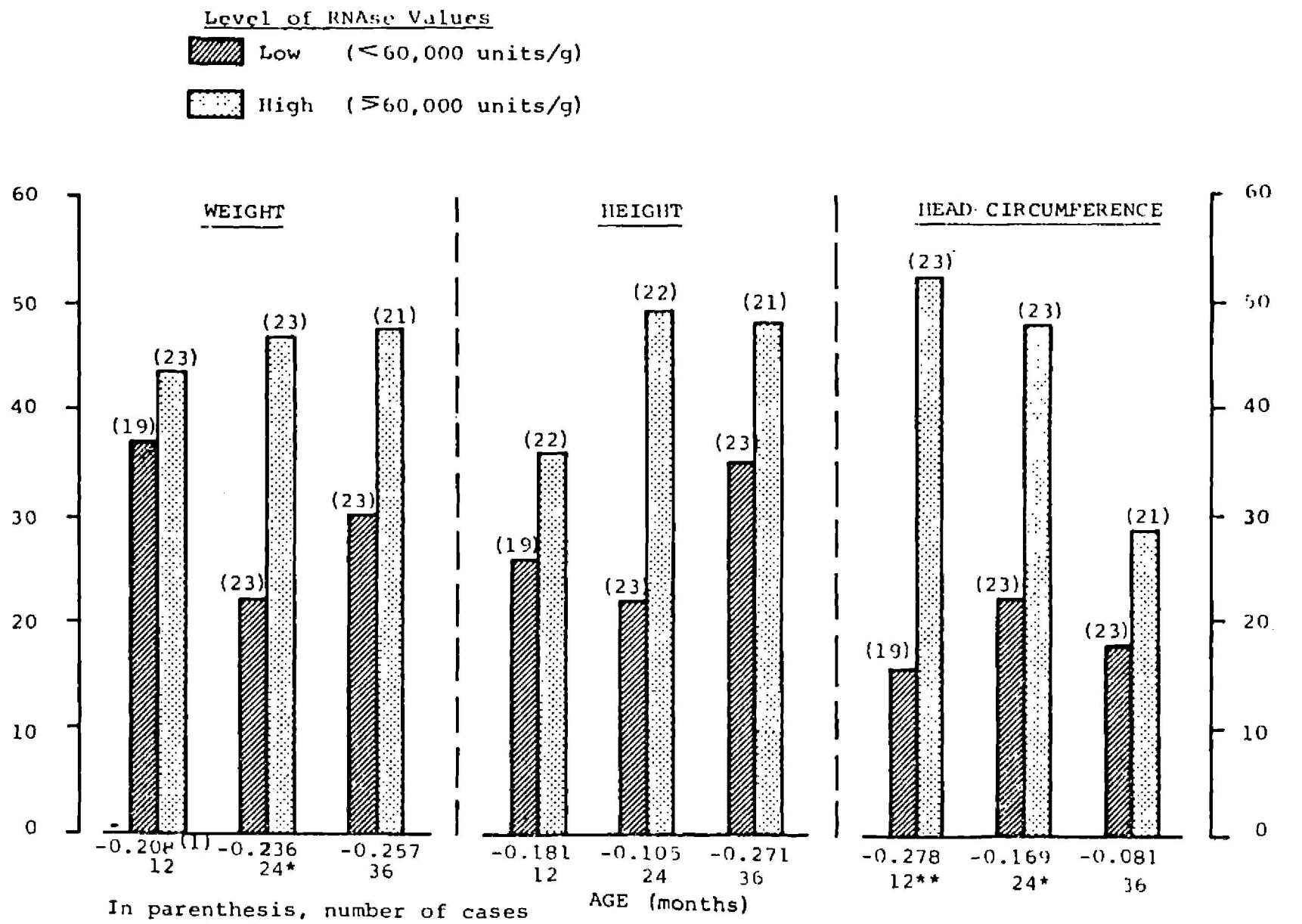


FIGURE 3 Effect of forcing placenta weight and RNAse activity on the association between indicators of maternal nutritional status and birth weight.

DISCUSSION

Consistent with previous reports (Velasco *et al.*, 1976) levels of placental RNAse activity were found to be higher in women from low as opposed to high socioeconomic groups. The supplementation study done in rural areas demonstrated that both protein-energy and energy supplementation alone during pregnancy influenced placental levels of RNAse. In both the urban and rural studies the most important factors capable of affecting the relationship between maternal nutrition and levels of RNAse activity were controlled, either through the study design or data analysis. These studies also show that high levels of placental RNAse activity were associated up to 36 months of age with a higher prevalence of physical growth retardation, poor psychological test performance and possibly higher rates of infant mortality. Obviously, further study will be required to explore the causal nature of these associations. It should be noted for example that postnatal nutrient intake and morbidity may be important confounding factors whose influence on the above mentioned relationships should be assessed through additional studies with larger sample size. In addition, it is hard to imagine what changes in the placenta, that may influence subsequent development, are reflected by, or occur concomitantly with an elevation in RNAse activity.

On the other hand, if additional studies con firm the consistency of these associations, their pu blic health implications may be very important since nutritional inverventions may help to reduce the prevalence of developmental retardation and infant mortality in poor populations (Chase, 1969; Lechtig *et al.*, 1975a). Placental RNAse activity may be a marker to identify babies at high risk of developmental retardation and therefore, it may be useful in increasing the efficiency of programs aimed at decreasing infant impaired functioning and mortality (Lechtig *et al.*, 1976).



In parenthesis, number of cases
t-test:*p ∠.05; **p ∠ .01
(1) Correlation values with continuous variables.

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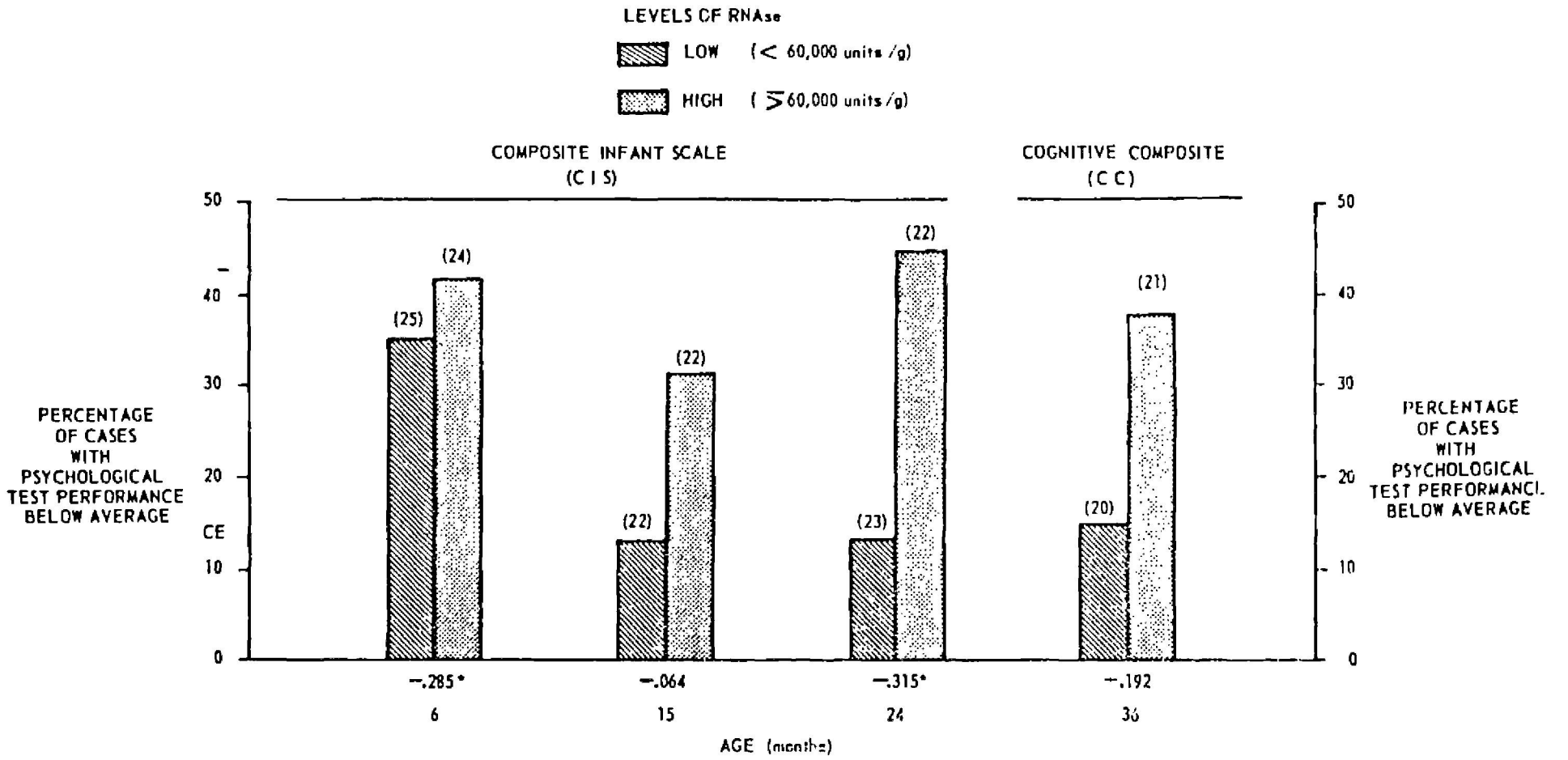
FIGURE 4 Proportion of cases with physical growth ratardation at 12, 24, and 36 months of age per level of placental RNAse values.



In parenthesis, number of cases in each RNAse level.
* $p < .05$; ** $p < .01$.
(1) Average of population distribution from January 1969 to November 1974.
(2) Correlation values with continuous variables.

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FIGURE 5 Percentage of cases with psychological test performance below average[1] according to RNAse level.

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