The Effect of Maternal Starvation on Plasma Insulin-Like Growth Factor I Concentrations in the Late Gestation Ovine Fetus

N. S. BASSETT, M. H. OLIVER, B. H. BREIER, AND P. D. GLUCKMAN

Developmental Physiology Laboratory, Paediatrics Department, School of Medicine, University of Auckland, Auckland, New Zealand

ABSTRACT. In view of the suggested relationship between substrate availability, fetal growth and circulating fetal IGF-I concentrations, we investigated the effect of maternal starvation on plasma IGF-I levels in the late gestation ovine fetus. Ten fetuses aged 125–130 d gestation were sampled daily from indwelling arterial catheters. Ewes were starved for 72 h. Starvation was terminated with an intravenous infusion of 10% glucose to the ewe. Food was then replaced 4 h later. Fetal IGF-I concentrations fell from 176.1 ± 15.2 ng/mL before starvation to 124.5 ± 10.3 ng/mL after 72 h starvation ($p < 0.05$, $n = 10$). The fall in IGF-I concentrations was reversed by 4 h of maternal glucose infusion. In five fetuses, where samples were obtained 24 h after terminating the starvation, fetal IGF-I concentrations were comparable to those seen before starvation (180.0 ± 37.7 ng/mL). This study demonstrates that acute maternal starvation causes a reversible decrease in fetal plasma IGF-I levels. These studies suggest that nutrient and in particular glucose availability is a significant determinant of fetal IGF-I secretion and support the hypothesis that IGF-I may play a role in the regulation of fetal growth. (*Pediatr Res* 27: 401–404, 1990)

Abbreviations

GH, growth hormone

The role of IGF in fetal life remains speculative. However, limited evidence suggests that IGF-I may have an important role in the regulation of fetal growth. IGF-I levels in umbilical cord blood at delivery have been shown to correlate with birth wt in a number of species including the rat (1, 2), rabbit (3), sheep (4), cow (5), and man (6–8). Studies in the fetal guinea pig (9) and fetal sheep (10, 11) have demonstrated that chronic alterations in placental function leading to fetal growth retardation result in decreases in fetal IGF-I levels. Such studies suggest that substrate availability may play a role in regulating fetal IGF-I secretion and that IGF-I may be an endocrine regulator of fetal growth. To further elucidate the role of nutritional factors in the acute regulation of fetal IGF secretion we investigated the effects of maternal starvation and refeeding on plasma IGF-I levels in the late gestation ovine fetus.

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Correspondence: P. D. Gluckman, Developmental Physiology Laboratory, Department of Paediatrics, School of Medicine, University of Auckland, Auckland, New Zealand.

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MATERIALS AND METHODS

Animals and surgical procedures. Romney ewes time mated with Suffolk rams provided fetuses of known gestational ages for these studies. At 125–130 d gestation (term = 145 ± 3 d) ewes were brought into the laboratory and allowed 4–5 to become acclimatized to laboratory conditions before undergoing surgery. Fetal vascular surgery was performed under sterile conditions using halothane anaesthetic. Vascular catheters were inserted into the fetal femoral artery and vein and into the maternal tarsal artery and vein to obtain regular blood samples. Fetus and mother were allowed 5 d postoperative recovery before commencing the experiment. Ewes were fed a mixture of chaffed hay, lucerne, and barley *ad libitum* with water freely available.

Experimental protocol. Blood samples were collected in sterile preheparinized syringes. Fetal and maternal blood samples were obtained for 2 d before commencing the starvation. Ewes were then starved for 72 h but with water *ad libitum*. Starvation was terminated with a maternal i.v. infusion of 10% glucose (0.5 mL/min). This was maintained for 4 h and then food was replaced and the ewe allowed to eat *ad libitum*. Glucose infusion was reduced to 2% (0.5 mL/h) and continued for another 12–18 h. This approach achieved an abrupt reversal in fetal substrate deprivation. Fetal and maternal blood samples were collected simultaneously twice daily. Plasma was separated by centrifugation and frozen at -20°C until assayed. This protocol was approved by the institutional Animal Ethical Committee.

Assays. Fetal and maternal plasma samples were analyzed for glucose by the glucose oxidase method using an autoanalyser (Beckman glucose analyser 2, Beckman Instruments Inc., Fullerton, CA). Fetal and maternal IGF-I concentrations were determined by RIA (12) after a modified acid ethanol extraction of the plasma. The principal modification was the addition of a cryoprecipitation step. This extraction technique has been developed and validated for fetal sheep plasma (13). In brief, 50 μL of fetal serum or plasma were diluted 1:10 with 450 μL of 0.01 M PBS (pH 7.6). To this 2 mL of acid-ethanol mixture (87.5% absolute ethanol, 12.5% 2 N hydrochloric acid vol/vol) were added, mixed thoroughly, and allowed to stand for 30 min at room temperature. Samples were then centrifuged at 36 000 rpm at 4°C for 30 min. Supernatants were decanted and 1 mL of 0.855 M Tris base added to neutralize the pH. Samples were then mixed and cryoprecipitation was achieved by incubation at -20°C for 1 h before centrifugation at 3600 rpm for 30 min at 4°C . The supernatant of each sample was then decanted and analyzed for IGF-I using an antibody (863/5) raised in New Zealand White rabbits to recombinant human N-terminal methylated (rh-met) IGF-I. This antibody was used at a final dilution of 1:75 000. The standard curve was generated from a postnatal calf serum pool. This was extracted in acid ethanol in the same manner as for fetal plasma samples. Results are expressed in ng of rh-met IGF-I (Dr. D. Burleigh, Pitman-Moore, Terre Haute,

IL, batch 742.44). Under these conditions, both fetal and maternal plasma extracts dilute in parallel with the standard curve. The minimum detectable concentration in this assay is 0.5 ng/mL with a half maximal displacement of 3 ng/mL. The interassay variation is 12.8% and the intraassay variation is 8.3%. This modified extraction methodology was validated by comparing results obtained in 12 maternal and 12 fetal plasma samples using both the modified extraction technique and G75 Sephadex chromatography under acid conditions (14). The results obtained by both methodologies were highly correlated ($r = 0.92$) and the slope of the linear regression was 1.14 (13).

Data analysis. Glucose and IGF-I measurements obtained for both the fetus and mother were meaned for each sampling time. Statistical differences from control values were determined by Student's *t* test after preliminary analysis of variance. Statistical differences from the 72-h samples were determined in the same manner. Data are expressed as mean $\pm$ SEM.

RESULTS

Maternal glucose concentrations fell from 61.7 ± 7.4 mg/dL during the prestarvation control period to 39.6 ± 3.9 mg/dL ($p < 0.05$) after 72 h starvation (Fig. 1). Glucose concentrations were then elevated after 4 h of glucose infusion to 184.0 ± 24.1 mg/dL and returned to normal 18–24 h later (63.4 ± 8.6 mg/dL). Fetal plasma glucose concentrations fell from 19.4 ± 2.0 mg/dL before starvation to 15.0 ± 1.2 mg/dL after 72 h of maternal starvation ($p < 0.05$, $n = 10$, Fig. 1). Fetal glucose returned to normal within 24 h of terminating the starvation (21.3 ± 2.5 mg/dL).

Fetal IGF-I concentrations fell from 176.1 ± 15.2 ng/mL before starvation to 124.5 ± 10.3 ng/mL ($n = 10$, $p < 0.05$, Fig. 2) after 72 h of starvation. After 4 h of maternal i.v. infusion of 10% glucose (0.5 mL/min), fetal IGF-I concentrations had increased to 149.2 ± 12.1 ng/mL. This was significantly elevated compared to IGF-I concentrations seen at 72 h starvation ($p < 0.05$). In five fetuses where samples were obtained 24 h after commencing the maternal glucose infusion fetal IGF-I concentrations had returned to prestarvation levels (180.0 ± 37.7 ng/mL). When the data were expressed as a percentage change from control values, fetal IGF-I fell to $76.4 \pm 9.45\%$ of basal values ($p < 0.05$) after 48 h starvation and to $73.8 \pm 9.45\%$ of basal values ($p < 0.05$) after 72 h starvation.

DISCUSSION

The evidence suggesting that the IGF play a role in the regulation of fetal growth has remained largely indirect and inferential (15–19). Plasma IGF-I concentrations have been shown to correlate with birth size in many species (2–8). Experimentally induced fetal growth retardation in several species has been associated with decreases in fetal IGF-I concentrations. Studies in the pregnant rat (20) and guinea pig (9) have demonstrated that chronic restriction of uterine blood flow results in a decrease in fetal IGF-I concentrations. Similarly in the fetal sheep a reduction in placental size by carunclectomy also results in a decrease in fetal IGF-I levels and in fetal wt (10, 11, 21). Such studies suggest that restrictions in fetal nutritional substrate and/or oxygen delivery that impair fetal growth also induce a fall in plasma IGF-I. It seems likely that these two observations are causally related. However, such inferences are based on manipulation that leads to chronic alterations in nutrient supply and placental function. In contrast, our study was designed to assess the role of nutrient supply in the acute regulation of fetal IGF-I secretion.

The regulation of circulating IGF-I secretion appears to differ between fetal and postnatal life. Postnatally growth hormone is the major regulator of plasma IGF-I concentrations (22–24). In addition, starvation reduces postnatal IGF-I secretion; an effect that appears to be regulated by alterations in GH receptors (25,

Fig. 1. Glucose concentrations in fetal and maternal plasma during maternal starvation. Mean fetal and maternal plasma glucose concentrations ($\pm$ SEM) are shown for 2 control d before starvation (–24 and 0 h), then at 24, 48 and 72 h after starvation, 4 h after terminating the starvation with a maternal glucose infusion (10% glucose, 0.5 mL/min) and 24 h later ($n = 10$). Statistical significance between sample times was determined by Student's *t* test after analysis of variance. The SEM falls within the symbols on all fetal measurements and is shown in only one direction for some points for clarity. * $p < 0.05$

Fig. 2. Fetal plasma IGF-I concentrations during maternal starvation. Mean fetal plasma IGF-I concentrations during the study. The mean for each sample time is indicated ($\pm$ SEM in one direction only). Statistical differences between sample times were determined by Student's *t* test after analysis of variance. * $p < 0.05$ compared to control values.

26). However, the concentration of GH receptors in the fetal liver are markedly reduced compared to in the postnatal sheep (27, 28) and growth hormone does not appear to play an important role in the regulation of fetal IGF-I release (29, 30). Thus, although undernutrition leads to a decrease in IGF-I levels both before and after birth it is possible that the processes involved are different. After birth at least part of the decline in plasma IGF-I associated with starvation has been associated with a loss

of hepatic GH receptors (25, 26) although postreceptor mechanisms have also been implicated (31). Given the very low number of GH receptors in the fetus it seems unlikely that changes in GH receptor concentrations mediate the effect of nutrition on fetal IGF-I release.

In our study, the starvation-induced fall in plasma IGF-I was reversed by i.v. glucose infusion alone. The very rapid rise in IGF-I on refeeding may simply reflect the i.v. route of nutrient administration compared to the slower rise seen on refeeding postnatally (25, 32). Alternatively it may reflect a different manner in which nutrient availability affects IGF-I secretion in the fetus compared to after birth; the latter being at least partially GH dependent. Our observations suggest that fetal glucose delivery is at least one important factor in the regulation of fetal IGF-I secretion. Whether such an effect of glucose is direct or is mediated via alterations in insulin release remains to be elucidated. Certainly insulin has generally been considered to be an important factor in the regulation of fetal growth. In addition, it has been suggested that its effects may be mediated through alterations in fetal IGF-I secretion (2, 19). Limited evidence has suggested that insulin may stimulate IGF-I release. Insulin has been reported to enhance IGF-I release by the perfused postnatal rat liver (33). Scanty data in the pig (34) and monkey (35) suggest that fetal hyperinsulinemia is associated with a slight elevation in fetal plasma IGF-I concentrations. Further, IGF-I concentrations are low at birth in infants with transient neonatal diabetes mellitus (36). In the pancreatectomized sheep fetus, IGF-I levels are low despite hyperglycemia and correlate with fetal insulin concentrations (37).

Fetal growth in late gestation is significantly affected by substrate (17, 18) and in particular glucose availability (38). Both chronic and acute nutritional limitation to the mother impair fetal growth. The effects of chronic maternal undernutrition on fetal growth have been reported in the rat (39), sheep (40), and inferred in man from famine studies (41, 42). Studies in the ovine fetus have demonstrated that complete starvation of the ewe results in a marked retardation in fetal growth rate after 3 d (40). These effects on both fetal growth rate and IGF-I concentrations suggest a possible causal relationship. Our observations demonstrate the importance of substrate availability as an acute regulator of fetal IGF-I release and provide further indirect evidence for the hypothesis that IGF-I is a determinant of fetal growth.

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Announcements

Seeking Acute Cases of Rheumatic Fever and Sydenham's Chorea

The Child Psychiatry Branch of the National Institute of Mental Health is seeking patients for a study of the course of psychologic symptoms accompanying Sydenham's chorea and rheumatic fever. Eligible patients should have had recent (within 2 mo) onset of rheumatic fever, continue to have symptoms, and be at least 6 y of age. This study will rate a variety of psychologic and psychiatric symptoms and link these symptoms to anti-CNS autoantibodies. The results will be important in defining the etiology of childhood-onset psychiatric disorders. Patients and their parents will be asked to travel at NIH expense to the NIMH in Bethesda, MD for an initial interview. Brief follow-up interviews will be conducted by telephone every 2 mo for 1 y. Serum samples (5 cc) will be obtained on four separate occasions. There will be no expense to the patient and no remuneration. *Please call* Dr. Susan Swedo at (301) 496-6081 *or write:* Dr. S. Swedo, Child Psychiatry Branch, NIMH, Bldg. 10, Room 6N240, 9000 Rockville Pike, Bethesda, MD 20892.

1990 ANNUAL MEETINGS

The American Pediatric Society, The Society for Pediatric Research and The Ambulatory Pediatric Association will meet May 7-11, 1990; Anaheim Hilton & Towers and Convention Center, Anaheim, CA.

Contact: **APS or SPR:** Association Headquarters, 2650 Yale Blvd., S.E., Suite 104, Albuquerque, NM 87106, (505) 764-9099 or 0068. **APA:** Ambulatory Pediatric Association, 6728 Old McLean Village, McLean, VA 22101, (703) 556-9222.

Special Program in Nutrition for a Health Heart Developed for Grade School Children and Their Parents

The J. David Gladstone Foundation of the University of California, San Francisco, has developed a heart healthy nutrition education curriculum for third grade students and their parents. This curriculum, called the Special Program in Nutrition (SPIN), consists of two manuals and a cookbook collection of low fat, low cholesterol recipes. The manuals contain an in-service program for teachers, 27 lessons for third grade students, seven lessons for fourth grade students, and a parents' program with 10 planned meetings.

The cost of the complete set is \$35.00; the two-volume curriculum and the cookbook may be purchased separately for \$30.00 and \$10.00, respectively. Add 6.5% California sales tax where applicable. *For further information contact:* The Gladstone Foundation Laboratories, 2550 23rd Street, P.O. Box 40608, San Francisco, CA 94140, (415) 826-7500.