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Tissue and Serum Concentrations of Somatomedin-C/Insulin-Like Growth Factor I in Fetal Rats Made Growth Retarded by Uterine Artery Ligation

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ABSTRACT. To study the role of somatomedin-C/insulin-like growth factor I (Sm-C/IGF I) in fetal growth, intrauterine growth retardation was induced by uterine artery ligation on day 17 of gestation in pregnant rats. Fetuses of the nonligated horns served as appropriately grown controls. On day 21 of gestation, fetal serum, liver, and lung were obtained and analyzed for Sm-C/IGF I by radioimmunoassay. Serum insulin was determined by radioimmunoassay and serum glucose by a glucose oxidase method. Fetal weight, serum concentrations of glucose, insulin and Sm-C/IGF I, and liver Sm-C/IGF I concentrations were reduced in fetuses from uterine artery ligated horns, as compared to those from nonligated control horns. Fetal weight was correlated with serum glucose ($r = 0.703$; $p < 0.001$), liver Sm-C/IGF I ($r = 0.682$; $p < 0.001$), and serum Sm-C/IGF I ($r = 0.452$; $p < 0.001$). Stepwise linear regression demonstrated that these three factors in combination correlated highly with fetal weight ($r = 0.836$). No correlation was found for serum insulin or lung Sm-C/IGF I and fetal weight. Serum insulin concentrations correlated with serum, but not liver, Sm-C/IGF I concentrations, making a direct effect of insulin on Sm-C/IGF I synthesis appear unlikely. However, serum glucose concentrations correlated with liver ($r = 0.404$; $p < 0.001$) and with serum Sm-C/IGF I ($r = 0.308$; $p < 0.002$) concentrations, implicating fetal glucose delivery in the regulation of Sm-C/IGF I synthesis. Taken together, these data suggest that Sm-C/IGF I synthesis in the fetus is influenced by nutrition and that Sm-C/IGF I plays a mediating role in the control of growth. (*Pediatr Res* 20: 126–130, 1986)

Abbreviations

Sm-C, somatomedin C
IGF, insulin-like growth factor
MSA, multiplication stimulating activity
IUGR, intrauterine growth retardation
RIA, radioimmunoassay

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The somatomedins, Sm-C/IGF I and IGF II/MSA, are peptide mitogens which share structural homology with insulin (1, 2). They are thought to be critical to postnatal growth (1, 3), and more recently have been implicated in fetal growth (4, 5). The factors that regulate somatomedins in the fetus are not fully defined, but do not appear to be the same as those which modulate somatomedins postnatally. Nutrition has a major regulatory influence on blood Sm-C/IGF I concentrations in adult man (6–9) and in neonatal and postweanling rats (10, 11). In the fetus, however, the interrelationships between nutritional status, growth, and Sm-C/IGF I remain to be clarified.

In this study we employed the uterine artery ligation method of Wigglesworth (12) to induce fetal growth failure. Because nutritional deficiency secondary to impaired transport of nutrients is a major component of the growth failure which occurs in this model (13), study of these growth retarded fetuses offers the opportunity to assess the physiological relationship of Sm-C/IGF I both to intrauterine growth and to nutritional deficiency. Tissue (liver and lung) and serum Sm-C/IGF I concentrations, as well as glucose and insulin concentrations were assessed in this study. We found that a reduction in glucose and Sm-C/IGF I concentrations in both liver and serum accounts in large part for the growth retardation of fetuses subjected to uterine artery ligation.

MATERIALS AND METHODS

Animals and procedure. Pregnant Sprague-Dawley rats of known gestation weighing between 250 and 300 g were purchased from the Charles River Laboratories (Wilmington, MA) and allowed free access to a stock diet. Rats were mated between 1600 and 0700 h, and day 0 of pregnancy was considered to begin at 0700 h. Intrauterine growth retardation was induced by the procedure of Wigglesworth (12). Briefly, on day 17 of gestation a laparotomy was performed under sterile conditions with ketamine anesthesia (20 mg intraperitoneally). The uterus was exposed and a 3–0 silk ligature was placed around the uterine artery supplying the uterine horn with the larger number of fetuses. The vasculature of the opposite uterine horn was not touched and the fetuses in the unperturbed horn served as controls. The uterus was returned to the abdominal cavity, the incision was closed, and the pregnancy was allowed to continue until the day of sacrifice. Rats recovered quickly after surgery, usually within 30–60 min, and continued to gain weight at the rate of about 10 g/day.

On day 21 of gestation under light ketamine anesthesia (10 mg intraperitoneally) a small incision was made in the uterus (leaving the uteroplacental circulation intact) such that a fetal arm could be exposed without delivering the fetus. A deep incision was made in the axillary region and blood was collected

by capillary action into a 100-μl glass tube. Serum was separated into three aliquots and stored at −70° C until the time of analysis (within 8 wk). After exsanguination, each fetus was weighed. Liver and lung were dissected, blotted, immediately immersed in liquid nitrogen, and stored at −70° C until analysis. A number indicating position within the uterine horn was assigned to each fetus (including resorbed fetuses), such that position 1 designated the fetus nearest the ovary.

Assays. Serum glucose concentration was assayed by the glucose oxidase method (14). Insulin was analyzed using a RIA kit purchased from Amersham (Arlington Heights, IL). If the volume of serum was inadequate for individual insulin analysis (after allotting serum for other assays), equal volumes of serum from littermates of the same uterine horn were pooled.

Sm-C/IGF I was estimated in serum by RIA using a published modification (15) of the original reported procedure (16). Use of this assay for rat serum has previously been validated (17, 18). Prior to assay, all sera were diluted 1:1 with glycine-glycine HCl buffer (ionic strength = 0.1) in order to achieve a final pH of 3.6 and incubated at 37° C for 24 h. This acid incubation procedure maximizes the amount of measurable, immunoreactive Sm-C/IGF I by antigenic sites of Sm-C/IGF I free from binding proteins (19). Neutralization of acid-incubated sera was accomplished with 1 M NaOH. Tissue (liver and lung) Sm-C/IGF I concentrations were estimated by the same assay after extraction using a validated modification (20) of the original reported procedure (18). In several cases, lung tissues were pooled with littermates of the same horn prior to analysis. Results were calculated using a human serum standard (15) and expressed as U/ml for serum and U/g wet weight for tissues. Adult rat serum is 7 to 15-fold more potent than adult human serum in this assay (18).

Statistics. Statistical analysis was initially performed by analysis of variance to determine the influence of uterine artery ligation and uterine position on the measured variables. Thereafter the unpaired t test was performed to test differences between individual fetal variables in ligated and control horns at the same uterine position.

Since pooling of samples was required for determination of serum insulin and lung Sm-C/IGF I concentrations, differences between horn means (rather than individual fetal concentrations) were assessed. Finally, correlation coefficients and stepwise linear regressions were performed among variables using Statistical Analysis Systems (Cary, NC) programs. Statistical significance is assigned to p values < 0.05.

RESULTS

A total of 28 pregnant rats underwent uterine artery ligation. Thirteen rats were excluded from the study because uterine artery ligation resulted in no viable fetuses in the ligated horn. As expected with this procedure, the number of fetuses in each uterine artery ligated horn was reduced (2.73 ± 0.31, mean ± SEM; $n = 15$) compared to the control horns (4.13 ± 0.34; $n = 15$; $p < 0.05$). A total of 101 viable fetuses resulted, 90 of which were distributed in uterine positions 1–4. Fetal weight was reduced in the ligated horn, whether analyzed as individual fetal weights (3.02 ± 0.10 g, $n = 43$) or as litter mean (3.03 ± 0.14 g, $n = 15$) compared to fetuses in the control horn (4.32 ± 0.09 for individual fetuses, $n = 58$; $p < 0.0001$; 4.41 ± 0.17 for litter means, $n = 15$, $p < 0.0001$, respectively).

As initially reported by Wigglesworth (12), the fetuses farthest from the ligation exhibited the least degree of growth retardation, and when analyzed by analysis of variance, position had a significant ($p < 0.03$) influence on fetal weight. Analysis of variance also showed that fetal weight, serum and liver Sm-C/IGF I, and serum glucose concentrations were reduced in the fetuses of the uterine artery ligated horn ($p < 0.0001$). Although this analysis did not indicate that uterine position had a significant influence on any variable other than weight, we chose to further analyze the data by comparing fetuses at each uterine position of ligated and nonligated horns. The strong relationship of fetal size and uterine position makes this approach intuitively appealing, and this more conservative test of significance is less likely to yield a type I error (false-positive). At each position, fetal weight, serum glucose concentration, liver and serum Sm-C/IGF I concentrations were significantly reduced in the IUGR fetuses, compared to their position-matched appropriately grown control counterparts (Table 1).

Because insufficient quantities of sera necessitated pooling of serum from fetuses in the same horn to perform insulin determinations, comparison of insulin concentrations was performed by t test on the means of each horn. Insulin concentrations were significantly lower in the IUGR fetuses (64 ± 12 μU/ml) than in control fetuses (131 ± 18 μU/ml, $p < 0.01$). Lung Sm-C/IGF I concentrations did not differ between fetuses from control and ligated horns, 0.28 ± 0.03 and 0.24 ± 0.03 U/gm, respectively.

Fetal weight correlated positively with serum glucose concentrations ($r = 0.703$; $n = 97$; $p < 0.001$), liver Sm-C/IGF I concentration ($r = 0.682$; $n = 101$; $p < 0.001$; Fig. 1), and serum Sm-C/IGF I ($r = 0.452$; $n = 101$; $p < 0.001$) when all fetuses were considered together. Stepwise linear regression demonstrated that these three factors in combination correlated very highly with fetal weight ($r = 0.836$). Significant correlations were not found for mean horn insulin concentrations ($r = 0.378$; $n = 22$) or for mean horn lung Sm-C/IGF I concentrations ($r = 0.256$; $n = 30$) and fetal weight.

Other correlations of possible biologic relevance include a significant relationship between serum glucose and liver Sm-C/

Table 1. *Fetal wt, serum glucose, liver, and serum Sm-C/IGF I concentrations (mean ± SEM) at each uterine position in control and IUGR fetuses**

	Uterine position			
	1 (n)	2 (n)	3 (n)	4 (n)
Wt† (g)				
Control	4.41 ± 0.19 (13)	4.44 ± 0.18 (15)	4.37 ± 0.18 (14)	3.46 ± 0.45 (10)
IUGR	3.15 ± 0.17 (15)	2.95 ± 0.24 (10)	2.84 ± 0.17 (8)	2.62 ± 0.10 (5)
Serum glucose (mg/dl)				
Control	66 ± 3 (13)	64 ± 7 (14)	71 ± 9 (13)	67 ± 8 (10)
IUGR	41 ± 3 (15)	30 ± 5 (9)	34 ± 5 (8)	37 ± 8 (5)
Liver Sm-C/IGF I (U/g)				
Control	0.29 ± 0.03 (13)	0.30 ± 0.03 (15)	0.32 ± 0.03 (14)	0.22 ± 0.02 (10)
IUGR	0.20 ± 0.02 (15)	0.18 ± 0.02 (10)	0.15 ± 0.02 (8)	0.12 ± 0.03 (5)
Serum Sm-C/IGF I (U/ml)				
Control	1.53 ± 0.05 (13)	1.53 ± 0.08 (15)	1.49 ± 0.11 (14)	1.34 ± 0.07 (10)
IUGR	1.08 ± 0.10 (15)	0.95 ± 0.10 (10)	0.89 ± 0.16 (8)	0.79 ± 0.26 (5)

* All comparisons between IUGR and control are different at $p < 0.05$.

† After exsanguination.

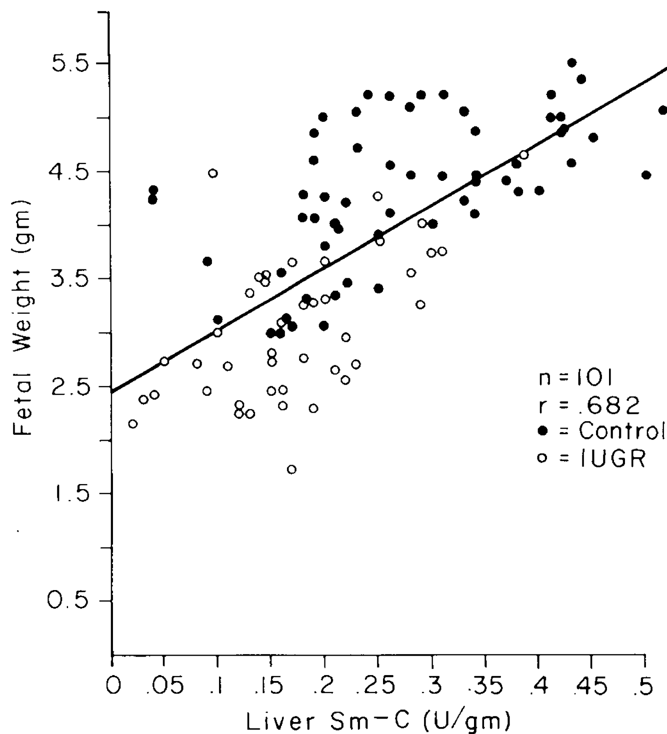

Fig. 1. Liver Sm-C/IGF I concentration in IUGR (○) and control (●) fetal rats compared to fetal weight ($r = 0.682$; $n = 101$; $p < 0.001$). Mean (± SEM) liver Sm-C/IGF I concentrations are 0.17 ± 0.01 U/g ($n = 43$) for IUGR fetuses and 0.27 ± 0.02 ($n = 58$) for control fetuses ($p < 0.001$). Serum Sm-C/IGF I in the same fetuses are 1.01 ± 0.06 U/ml and 1.45 ± 0.04, respectively ($p < 0.001$).

IGF I concentrations ($r = 0.308$; $n = 101$; $p < 0.002$). In addition, serum Sm-C/IGF I correlated with liver Sm-C/IGF I ($r = 0.361$; $n = 101$; $p < 0.001$). We also attempted to correlate litter mean insulin concentrations with litter mean serum and with liver Sm-C/IGF I concentrations. Although there is a significant relationship between insulin and serum Sm-C/IGF I ($r = 0.499$; $n = 22$; $p < 0.02$), there is none between insulin and liver Sm-C/IGF I ($r = 0.400$; $n = 22$).

DISCUSSION

Our findings that both liver and serum Sm-C/IGF I concentrations are reduced in IUGR fetuses and that both measures correlate with fetal weight suggest a mediating role for Sm-C/IGF I in the control of fetal growth. These results are consistent with those of DePrins *et al.* (21) who found decreased serum concentrations of somatomedin bioactivity in fetal rats made growth retarded by uterine artery ligation. They also reported a significant correlation between the bioactive somatomedin concentrations and fetal body size. Unlike the original report of Wigglesworth (12) and this study, DePrins *et al.* (21) did not find that fetal size was influenced by the proximity of the conceptus to the site of uterine artery ligation. Subtle differences in techniques may explain this discrepancy.

The RIA used in this study employs an antibody directed against human Sm-C/IGF I and has been validated for the estimation of this peptide in rats (17, 18). Rat homologues of human somatomedins (Sm-C/IGF I and IGF II) have been purified. A peptide purified from adult rat serum bears marked homology to Sm-C/IGF I (22) and has been reported to be 30–40% as potent as Sm-C/IGF I in competing for binding to this antibody (23); MSA, the rat homologue of IGF II (24), cross-reacts minimally with our antibody (1.2% for MSA III-2 and 0.025% for MSA II-1) (25). When this RIA is used to measure specimens from rats, it is likely to underestimate rat Sm-C/IGF I by 2.5 to 3.3-fold and to detect MSA only when it is present in very high concentrations. In the fetal rat, however, serum Sm-C/IGF I concentrations are low compared to those of MSA (23, 26), and, although unlikely to be a significant factor, it remains possible that the immunoactivity reported in this study in part reflects cross-activity with MSA. Precise determination of the quantities of Sm-C/IGF I and MSA in fetal rat sera awaits simultaneous studies of the same sera with antibodies directed against both peptides and/or use of antibodies with no cross-reactivity.

Although not directly addressed in the study, it is reasonable to suspect that fetal nutritional deficiency occurring from uterine artery ligation (13) leads to the decrease in Sm-C/IGF I concentrations and, in turn, results in a decrease in fetal growth. Serum Sm-C/IGF I in the postnatal rat and in man has been shown to be highly dependent on nutritional status (1, 6–10). In the postweanling rat, we have shown that both energy and protein intake determined serum Sm-C/IGF I and growth (11). In man, serum Sm-C/IGF I is reduced dramatically by fasting and returns to normal with refeeding (6). The rate of return of serum Sm-C/IGF I concentrations from low basal fasting levels to normal is dependent on the quality of the diet which is refed (7–9).

Inadequate nutrient transfer to the fetus may account, in large part, for the growth failure resulting from uterine artery ligation. In the Wigglesworth (12) model, the reduction in uteroplacental blood flow is associated with a decrease in placental transfer of maternally administered analogues of glucose and amino acids (13). In spontaneous growth retardation occurring in fetal guinea pigs, Saintonge and Rosso (27) also demonstrated an association between reduced placental blood flow and a reduction in the transfer of both amino acid and glucose analogues. Studies utilizing *in situ* perfusion of the guinea pig placenta substantiate the conclusion that the transfer of these analogues to the fetus is dependent on placental blood flow (28, 29). Placental hypoxia, which probably occurs in uteroplacental insufficiency, per se may result in decreased glucose and amino acid transport (30, 31). Fetal oxygen uptake is also uterine blood flow dependent under conditions of severe uterine blood flow restriction (32). Thus, fetal substrate deprivation is a major component of the pathophysiology induced by uterine artery ligation.

The strongest correlation in this study is that of glucose concentrations and fetal size. This is not surprising given that glucose has long been implicated as the major fetal metabolic fuel, both for maintenance of fetal oxidation and formation of new tissue (33). States of pathologic fetal growth, either overgrowth or undergrowth, have a direct positive relationship with fetal glucose uptake (33–35). Fetal glucose uptake accounts for a large fraction of the total oxygen consumption and serves as a substrate source for fetal glycogen and lipid synthesis (36, 37). Maternal hyperglycemia results in augmentation of both of these biosynthetic functions, even in cases of flow restricted intrauterine growth retardation (38, 39). Our finding of a correlation between glucose and Sm-C/IGF I concentrations in liver and serum suggests a role for nutrient availability (specifically of glucose) in the control of fetal Sm-C/IGF I production and/or release.

Fetal hypoinsulinemia has been suggested as an etiology of fetal growth retardation (40). Analysis of our data has failed to establish a relationship between fetal weight and insulin concentrations. In addition, investigations by others have not led to conclusive evidence that insulin is the primary controller of fetal anabolism (4,41). It may be that insulin in the fetus serves "to maintain a favorable homeostatic environment" (4), or alternately insulin may stimulate fetal somatomedin production. Both fetal rabbits and pigs respond to hyperinsulinemia with elevation of somatomedin bioactivity (42–44). However, our observations of an association between insulin and serum Sm-C/IGF I, but not with liver Sm-C/IGF I, do not support a strong direct role for insulin in the control of Sm-C/IGF I synthesis. We speculate that insulin does not directly influence Sm-C/IGF I, but that substrate (possibly glucose) concentrations influence both insulin and somatomedin homeostasis.

The somatomedins are synthesized in many fetal tissues (45) and are thought to act in an autocrine and/or paracrine fashion, *i.e.* they exert their biologic activity on their cells of origin or on cells near their sites of synthesis (18). Because the liver is considered to be a major site of somatomedin synthesis (1, 2), it is not surprising that liver Sm-C/IGF I concentrations correlate better with fetal weight than do serum Sm-C/IGF I concentrations. The significant correlations of liver Sm-C/IGF I with serum Sm-C/IGF I concentrations support the hypothesis that the fetal liver is a major source of somatomedin activity in the circulation. Because of its location as the first organ to be exposed to changes in nutrient flux (whether they be from the placenta prenatally or from the gut postnatally), the liver is likely to be profoundly influenced by nutritional status. Our finding that there is a reduction in liver, but not in lung, of Sm-C/IGF I concentrations associated with uterine artery ligation is consistent with this hypothesis. This is also consistent with our previous findings that there is a greater reduction of liver Sm-C/IGF I than of lung or kidney Sm-C/IGF I in calorically deprived adult rats (46). That lung Sm-C/IGF I concentrations are not reduced in IUGR fetuses and do not correlate with fetal weight suggest that in this organ Sm-C/IGF I is regulated differently (by a different mechanism or by the same mechanism but to a much lesser degree) than liver Sm-C/IGF I.

Evidence for an important role for Sm-C/IGF I in fetal development comes from multiple sources (4, 5). Sm-C/IGF I is a potent mitogen for a variety of cultured fetal cells (4, 5) including those derived from rodents (47, 48). Fetal tissues (49, 50), including those derived from the rat (51), possess type I insulin-like growth factor receptors, *i.e.* plasma membrane receptors which have a higher affinity for Sm-C/IGF I than for IGF II/MSA (52). Despite its relatively low circulating concentrations, plasma Sm-C/IGF I concentrations in cord blood correlate well with birth size (4, 5). Sm-C/IGF I is capable of potent anabolic actions other than its mitogenic effect. It can stimulate amino acid uptake in cultured fibroblasts, including those derived from the fetus and neonate (53). Sm-C/IGF I is more potent than insulin in the stimulation of glycogen synthesis in cultured fetal rat hepatocytes (54), suggesting a specific role for Sm-C/IGF I in the rat fetus. In this regard, MSA appears to have less than 5% of the potency of Sm-C/IGF I (55). Although it has been suggested that MSA (rat IGF II) is the relevant somatomedin for the rat fetus (56), it is likely that Sm-C/IGF I also has an important role.

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