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Serum Insulin, Glucose, and Free Fatty Acids in the Cow and Fetus during Gestation[1] (38449)

J. S. GRIGSBY, W. D. OXENDER, H. D. HAFS, D. G. BRITT, AND R. A. MERKEL

Departments of Food Science and Human Nutrition, Dairy Science and Animal Husbandry, Michigan State University, East Lansing, Michigan 48824

Insulin is found in ovine fetal pancreatic islets by the end of the first trimester of gestation (1, 2) and in fetal plasma between Day 42 and term (1). In rabbits, the ratio of maternal to fetal plasma insulin concentration decreases (3) during gestation due to a decrease in maternal insulin concentration. Serum insulin also decreases in cows during gestation (4). The mechanism of insulin release during fetal life is not well understood. Exogenous glucose or fructose increases insulin concentration in serum of the fetus (5, 6) with glucose being the more potent stimulant. Short-chain fatty acids infused into mature sheep also increase plasma insulin (7), but to our knowledge the relationships between endogenous free fatty acids (FFA), glucose and insulin during gestation in cattle have not been investigated.

The objective of this experiment was to examine these relationships as part of a study of endocrine control of growth, development, and carcass composition in cattle.

Materials and Methods. Pregnant primaparous Holstein heifers were 18–26 mo old when laparotomy was performed on Day 90, 180, or 260 of gestation (8). Heifers were fasted 24 hr before surgery. During surgery, blood samples were collected from the uterine and umbilical blood vessels or from the fetal heart in the case of Day 90 fetuses (9).

Serum insulin was measured using a double antibody radioimmunoassay (10). Bovine insulin standards (Lot No. 795372; 24.2 units/mg) were graciously provided by Dr. Mary Root, Eli Lilly and Co. (Indianapolis, IN) and ^{125}I-insulin was purchased from Amersham Searle (Arlington Heights, IL). Antibodies against guinea pig gamma globulin (SAGPGG) were described previously (11, 12). Lyophilized guinea pig serum containing antibodies to bovine insulin (GPABI) (Miles Laboratories, Kankakee, IL) was reconstituted with 1 ml of deionized water, diluted to 1:400 in 0.05 *M* EDTA–phosphate-buffered saline (pH 7.0) and frozen in 10-ml aliquots at −30°. The bovine insulin standards used contained from 0.04 to 5 ng per tube. A dilution of GPABI of 1:105,000 provided approximately 35% binding of ^{125}I-insulin and was used in all assays. The sensitivity of the assay was optimal under our assay conditions between 0.08 and 1.0 ng per tube (Fig. 1). Dilution curves between 100 and 300 μl of both bovine and ovine sera were parallel to the standard insulin curve between 0.08 and 1.0 ng per tube (Fig. 1). The slopes of the three curves were -50.152 ± 0.137 ($r = 0.99$), -50.078 ± 0.423 ($r = 0.99$), and -51.368 ± 0.476 ($r = 0.99$) for the insulin standard, bovine sera dilution, and ovine sera dilution (between 100 and 300 μl) curves, respectively, and they were not significantly different from each other. All samples were assayed at two concentrations (between 100 and 300 μl) to determine parallelism with the standard curve (13).

Bovine prolactin, growth hormone, luteinizing hormone, and ovine follicle stimulating hormone in amounts up to 500 ng per tube did not cross react with the GPABI antibody. The recovery of bovine standard insulin added to bovine sera ranged from 96 to 102% (Fig. 2). The mean insulin concentration (± standard error) of normal bovine sera used in all assays was 38 ± 1 μunits/ml ($n = 20$).

Serum FFA were determined using the procedure of Ko and Royer (14) and glucose by the Glucostat assay (Worthington Biochemical Corporation, Freehold, NJ).

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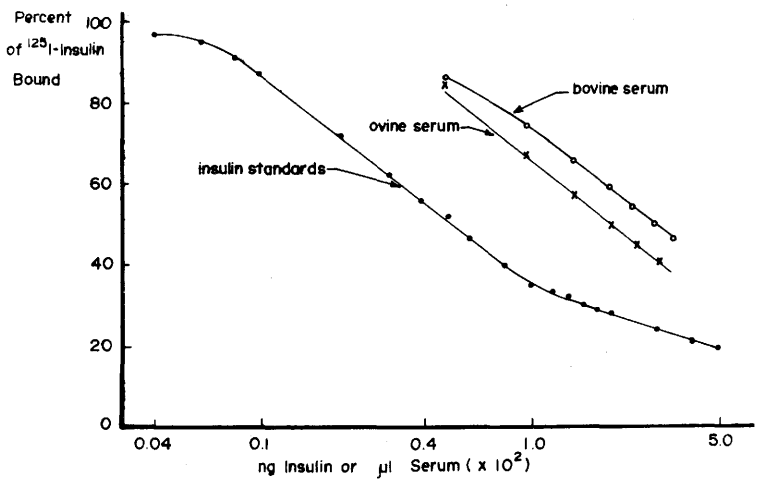

FIG. 1. Assay curves obtained with insulin standards and with ovine and bovine sera.

The data were statistically analyzed by analysis of variance. Fetal data were analyzed in two steps: (a) The data from the umbilical artery and vein were compared and, when there were no differences, (b) data from the umbilical artery and vein were combined for a test of fetal sex and age differences. Similarly, the maternal data were analyzed in two steps: (a) The data from the jugular vein, uterine artery, and vein were compared and, when there were no differences in uterine artery and vein data, then (b) data from the uterine artery and vein were combined for a test of fetal sex and stage of gestation differences. Finally the data (combined artery and vein) for the fetus and dam were tested to determine fetal and maternal differences (9). The statistical analyses of the data did not include a test for individual differences within a single cell of the analysis of variance (*i.e.*, between males and females at 90 days); therefore, interpretation of these data are limited to the type of statistical analysis described above.

Results. Insulin. Insulin concentrations in sera from the umbilical vein and artery were not significantly different; therefore they were averaged (Table I). Fetal average serum insulin more than doubled ($P < 0.05$) from Day 90 to 260 of gestation. The gestation average serum insulin concentration of male fetuses was higher ($P < 0.05$) than that of female fetuses. Although fetal serum insulin was only slightly greater in males than in females on Day 180, by Day 260 of

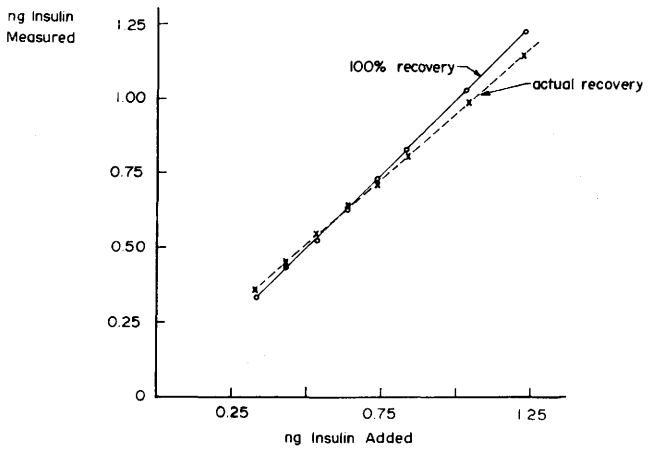

FIG. 2. Recovery of bovine insulin added to 150 µl of bovine serum.

TABLE I. Insulin Concentration in the Bovine Fetus and Dam.[a]

Source of blood	Days of gestation			
	90	180	260	Gestation average[b]
Male fetus	8.5 ± .05 (3)	14.5 ± 2.0 (7)	33.2 ± 8.7 (6)	21.6 ± 3.1 (16)
Female fetus	9.6 ± 1.1 (3)	11.5 ± 1.3 (6)	21.6 ± 3.0 (5)	15.6 ± 1.4 (14)
Fetal average[c]	9.1 ± 0.6 (6)	13.1 ± 1.3 (13)	27.9 ± 5.1 (11)	18.9 ± 1.9 (30) (overall mean)
Dam (male fetus)	40.0 ± 3.6 (5)	34.9 ± 8.9 (7)	18.3 ± 3.0 (6)	30.8 ± 3.0 (18)
Dam (female fetus)	43.5 ± 15.7 (8)	32.8 ± 5.8 (6)	33.0 ± 13.9 (5)	37.4 ± 5.3 (19)
Dam average	42.1 ± 9.5 (13)	33.9 ± 5.3 (13)	25.0 ± 6.5 (11)	34.1 ± 3.1 (37) (overall mean)

[a] Values are means ± SE in μunits/ml, (n) = number of animals; samples from 90 day fetuses taken by cardiac puncture; 180 and 260 day values are means for umbilical artery and vein; dam values are the means for uterine artery and vein.

[b] Gestation average for males was higher ($P < 0.05$) than females and fetal average was lower ($P < 0.05$) than the dam average.

[c] Average fetal insulin increased ($P < 0.05$) at each trimester of gestation.

gestation in males insulin was 50% greater than in females.

Uterine arterial and venous concentrations of insulin were similar at all stages of gestation, so these data were combined (Table I). Average insulin concentrations of dams decreased during gestation but this decrease was not statistically significant. The decrease during gestation of serum insulin from dams carrying male fetuses was twice that from dams carrying female fetuses; however, the AOV used did not permit us to test the significance of this difference. The gestation average insulin concentration was not statistically different between dams with male or female fetuses.

The overall mean serum insulin of pregnant heifers during gestation was nearly twice that of the fetus ($P < 0.01$). This difference was greatest on Day 90 of gestation but by Day 260 average serum insulin concentrations in the dam and fetus were similar. This decrease in the dam-to-fetal insulin ratio was mainly due to an increase in fetal serum insulin and to a gradual, although nonsignificant, decrease in maternal serum insulin.

Free fatty acids. Serum FFA concentrations are given in Table II. Fetal average FFA concentrations decreased ($P < 0.05$) significantly from 455 ± 34 on Day 90 to 308 ± 19 μequiv/liter on Day 260 of gestation. The decrease was 45% greater in females than in male fetuses, but this difference was not tested (AOV). In contrast to the decrease in fetal serum FFA concentration during gestation, the average FFA concentration in serum from the uterine vessels increased 64% ($P < 0.05$) as gestation progressed from Day 90 to 260.

Gestation average FFA for male and female fetuses did not differ significantly, but dams carrying male fetuses had greater ($P < 0.05$) FFA concentrations than dams with female fetuses (Table II). The significant sex effect on maternal FFA concentration was due to the difference at 260 days, since at 90 days FFA in dams with male and female fetuses were similar.

Glucose. Average fetal glucose concentrations (Table III) decreased from 53 ± 6 at 180 days to 29 ± 6 mg/100 ml at 260 days ($P < 0.05$). Gestation average serum glucose concentrations were not significantly different between male and female fetuses. Likewise, sex of the fetus did not significantly influence gestation average maternal glucose concentrations. Glucose decreased (dam average) slightly from 90 to 260 days gestation but this decrease was not significant. The overall mean of glucose in the dams was 40% greater than that of the fetuses ($P < 0.05$).

Discussion. The average insulin concentrations in the serum of the bovine fetuses increased twofold between Day 180 and 260 of gestation in a manner similar to that reported for fetal rats (15) but in contrast to the stable concentrations observed in fetal rabbits (3). Pancreatic insulin (16) in human fetuses also increases from Day 50 to 110 of gestation which is similar to the increase in serum insulin observed for fetuses in this study. It has been demonstrated (17, 18) that fetal lambs can secrete increased quantities of insulin when glucose or valeric acid is infused.

TABLE II. Serum Free Fatty Acid (FFA) Concentration in the Bovine Fetus and Dam.[a]

Source of blood	Days of gestation			
	90	180	260	Gestation average[b]
Male fetus	443 ± 71 (5)	345 ± 35 (7)	332 ± 28 (6)	356 ± 21 (18)
Female fetus	479 ± 34 (8)	349 ± 27 (6)	279 ± 19 (5)	360 ± 21 (19)
Fetal average[c]	455 ± 34 (13)	347 ± 22 (13)	308 ± 19 (11)	358 ± 15 (37) (overall mean)
Dam (male fetus)	954 ± 121 (5)	1209 ± 141 (7)	1740 ± 230 (6)	1218 ± 85 (18)
Dam (female fetus)	952 ± 122 (8)	1039 ± 157 (6)	1343 ± 257 (5)	1083 ± 78 (19)
Dam average[c]	953 ± 85 (13)	1130 ± 103 (13)	1560 ± 174 (11)	1196 ± 63 (37) (overall mean)

[a] Values are means ± SE in μequiv/liter, (n) = number of animals; samples from 90-day fetuses taken by cardiac puncture; 180- and 260-day samples are mean for umbilical artery and vein. Dam values are mean for uterine artery and vein.

[b] Fetal gestation average FFA was lower ($P < 0.01$) than gestation average for dam; gestation average for dams with male fetuses was higher ($P < 0.05$) than dams with females.

[c] Average fetal FFA decreased ($P < 0.05$) each trimester of gestation; dam average FFA increased ($P < 0.05$) the last trimester of gestation.

However, it was not within the scope of this experiment to demonstrate whether or not the increase in fetal serum insulin observed was the result of a response to one or more endogenous blood metabolites.

Fetal growth is greater in late gestation and Trenkle (19) has shown that the serum insulin level is positively correlated with growth in cattle. Therefore, elevated fetal serum insulin concentrations during late gestation (Table I) may cause increased fetal growth. The insulin secretory mechanism, like many other functions, develops in the fetus according to a recognizable time schedule and when insulin becomes available, some of the insulin-dependent metabolic pathways, such as glucose utilization (6), are stimulated. Development of the insulin-dependent metabolic pathways in the bovine fetus could account for the decreased concentrations of glucose (Table II) and FFA (Table III) that we observed in the fetal sera on Day 260 of gestation.

The decrease in serum insulin with advancing gestation in these nonlactating heifers is comparable to that observed by Koprowski and Tucker (4) in lactating pregnant cows. The significant decrease in serum insulin during gestation is the opposite of what happens for growth hormone (GH) in these animals, in whom GH increases during gestation (8). An inverse relationship between changing serum insulin and GH concentrations in pregnant, lactating cattle

TABLE III. Serum Glucose Concentration in the Bovine Fetus and Dam.[a]

Source of blood	Days of gestation			
	90	180	260	Gestation average[b]
Male fetus	—	60 ± 11 (5)	32 ± 9 (5)	46 ± 8 (10)
Female fetus	—	51 ± 7 (5)	26 ± 8 (5)	38 ± 6 (10)
Fetal average[c]	—	53 ± 6 (10)	29 ± 6 (10)	42 ± 5 (20) (overall mean)
Dam (male fetus)	78 ± 4 (5)	73 ± 7 (5)	67 ± 7 (5)	73 ± 4 (15)
Dam (female fetus)	74 ± 5 (5)	65 ± 5 (5)	58 ± 5 (5)	66 ± 3 (15)
Dam average	76 ± 3 (10)	69 ± 4 (10)	63 ± 4 (10)	69 ± 2 (30) (overall mean)

[a] Values are means ± SE in mg/100 ml; (n) = number of animals; 180- and 260- day fetal values are for umbilical vein samples; dam values are for jugular vein samples..

[b] The gestation average fetal glucose was lower ($P < 0.05$) than the gestation average for the dams.

[c] Fetal average serum glucose decreased ($P < 0.05$) from 180 to 260 days of gestation.

(4) and feedlot cattle (10) has been reported previously.

In contrast to insulin, average FFA increased significantly in maternal sera during gestation, while average maternal glucose remained constant. Thus, the increased energy demands of gestation appear to be supplied by increased serum FFA.

It was unexpected to find that on Day 260 of gestation the concentrations of serum insulin, glucose, and FFA were greater in males than in female fetuses, although not all the differences were statistically significant. Perhaps these differences contribute to the more rapid growth rate of the males during late gestation and subsequently to the heavier birth weight of males. This could be in addition to the effect of the significantly greater androgen concentrations in these male fetuses compared to the females (20).

Summary. Insulin, free fatty acids (FFA), and glucose were measured in blood sera from 37 primiparous Holstein heifers and their fetuses on Day 90, 180, or 260 of gestation. Blood was collected from the uterine artery and the uterine vein of the heifers, by cardiac puncture in the 90-day fetuses and from an umbilical artery and vein in 180- and 260-day fetuses.

The average serum insulin concentration was higher ($P < 0.05$) in male than in female fetuses although the average of the two increased ($P < 0.05$) during gestation. The overall mean of fetal insulin was only 55% of that of the dam ($P < 0.05$) and the difference was greatest at 90 days gestation.

Fetal FFA decreased ($P < 0.05$) during gestation, but maternal FFA increased ($P < 0.05$) as pregnancy progressed. Maternal FFA were higher ($P < 0.01$) than fetal FFA and the heifers carrying male fetuses had higher ($P < 0.05$) average FFA concentrations than heifers with male fetuses.

Average fetal glucose decreased ($P < 0.05$) from Day 180 to 260 of gestation and the overall mean of fetal glucose was lower ($P < 0.05$) than maternal glucose.

We conclude that, during gestation, fetal insulin increased while FFA and glucose decreased. In contrast, maternal insulin decreased and FFA increased but glucose remained constant.

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