

Accelerated Article

Zinc, Copper, and Iron Metabolism During Porcine Fetal Development*

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ABSTRACT

Zinc, copper, and iron levels in maternal and fetal pig tissues and fluids were measured starting on d 30 of gestation and continuing to term (d 114) at 10-d intervals. Fetal hematocrit increased from a low of 19% on d 30 to 32% by d 50, after which it remained above 30% to term. Amniotic fluid zinc, copper, and iron all reached maximal levels by d 60 of gestation. Maternal serum zinc levels fluctuated little during gestation, but fetal serum zinc concentration was significantly elevated above maternal levels during the second trimester. Fetal serum copper levels were significantly lower than maternal values throughout gestation and this was also the case for ceruloplasmin oxidase activity. Maternal serum iron reached its lowest level by d 80 of gestation when rate of transfer of iron to the developing fetuses was high. Fetal serum iron declined throughout gestation, reaching its lowest level on d 100. In general, fetal liver concentrations of zinc, copper, and iron were higher than the corresponding maternal values throughout gestation. Distinct increases were noted for fetal hepatic zinc and copper concentrations during the second trimester of pregnancy and these were accompanied by increases in cytosolic and metallothionein-bound zinc and copper levels. Maternal hepatic iron declined during the second trimester, reaching its lowest point on d 80, indicative of the shunting of maternal iron reserves to fetal tissues. Fetal kidney metal levels did not demonstrate any distinctive developmental patterns with respect to zinc, copper, or iron concentrations, but a general accumulation of each metal was observed as gestation progressed. The results of this study highlight some of the distinct changes occurring in the metabolism of zinc, copper, and iron in both maternal and fetal tissues and fluids during gestation in the pig.

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Index Entries: Trace elements; development; porcine; zinc; copper; iron; metallothionein; fetus; placenta; gestation.

INTRODUCTION

The importance of maternal trace element nutrition to the development of the mammalian fetus *in utero* is well recognized (1–3). Normal fetal development is dependent on an adequate and regulated supply of trace elements, including zinc, copper, and iron. Deficiencies of these essential nutrients can have profound effects on fetal and postnatal development that, if severe enough, can result in prenatal or neonatal death, congenital and behavioral abnormalities, reduced birth weight, and impaired neurologic, cardiovascular, pulmonary, and immune function (4–5). Therefore, it is important to investigate trace element metabolism and its regulation in both maternal and fetal tissues in order to understand the mechanisms governing the transfer of zinc, copper and iron from the mother to the fetus.

Placental regulation of nutrient flux is a crucial aspect of prenatal development and, as such, is central to understanding the mechanisms involved in maternal–fetal transfer of trace elements. The pig has an epitheliochorial type of placenta in which the fetal membranes do not invade or erode the endometrial lining of the uterus (6). Because several cell layers separate maternal blood from the absorptive surface of the fetal chorion, the transfer of nutrients, including trace elements, from the mother to the fetus is accomplished by secretion from the uterine endometrial epithelium followed by uptake into the fetal compartment (7). This secretory route for nutrient transfer is a particularly important one, especially during the early stages of fetal development in the pig. Because zinc, copper, and iron would not be soluble in the ionic state under physiological conditions, transport proteins or other metal-chelating substances presumably mediate this transfer process. Albumin and transferrin, which have been shown to be taken up by the yolk sac placenta of the fetal rat, could play a role in trace element transport from maternal to fetal circulation (8). Using *in situ* perfusion of the guinea pig placenta, Patterson et al. (9) demonstrated that the availability of binding ligands such as albumin and the amino acids cysteine and histidine in fetal circulation can influence placental zinc transfer. Douglas et al. (10) investigated the role of α_2 -macroglobulin and albumin in maternal–fetal zinc transfer using human placental syncytiotrophoblasts in culture and reported specific binding and uptake of ^{65}Zn -labeled α_2 -macroglobulin at the cell surface. Lee et al. (11) reported that copper from the mother that is destined to be deposited in the rat fetus is delivered primarily by ceruloplasmin and that the exchange occurs at the placenta via a receptor-mediated event.

In the pig, transplacental iron transport, at least in part, involves the synthesis and secretion of the iron-containing basic glycoprotein uteroferrin by the glandular epithelial cells of the uterus in response to progesterone stimulation (12). The iron-bearing uteroferrin is absorbed by the chorionic epithelial cells in the areolae regions of the placenta and is subsequently released into fetal circulation (13). Once in fetal circulation, uteroferrin is rapidly cleared by reticuloendothelial cells lining the sinusoids of the liver and by the spleen via receptor-mediated endocytosis or it is deposited as a temporary store into allantoic fluid via filtration through the fetal kidneys (14). Uteroferrin is degraded intracellularly in lysosomes and the iron released is initially incorporated into the liver and the spleen, then into red cell hemoglobin, and, ultimately, into all tissues of the fetus (12). The mechanisms involved in the transplacental transport of zinc and copper in the pig have not been elucidated. However, it is of interest to note that some preparations of uteroferrin, as originally isolated, did not contain the full complement (i.e., two atoms per molecule) of iron (15). This, coupled with the fact that uteroferrin is capable of binding other metals including copper and zinc at one of the iron-binding sites (16), could indicate a more general role for uteroferrin in trace element transport across the placenta of the pig.

In order to more fully understand the transfer of trace elements from mother to fetus, data are needed concerning the trace element levels in maternal and fetal tissues and fluids collected at specific times during gestation. Although some data exist for a number of species, including rats (17), mice (18), rabbits (19), sheep (20), cattle (21), and humans (1,22), relatively less is known about pigs. The purpose of this study was to collect and analyze maternal and fetal tissues and fluids at discrete (10-d) intervals beginning on day 30 and continuing throughout the gestation period of the pig (approximately 114 d to term) in order to compare the levels of zinc, copper, and iron as a function of stage of fetal development.

MATERIALS AND METHODS

Animals and Tissue Collection

Thirty first-parity Duroc gilts were bred to Duroc boars on the first 2 d of the estrous cycle. If signs of estrus were not evident after 25 d, the gilts were considered to be pregnant and the first day of estrus was designated as day 0. On days 30, 40, 50, 60, 70, 80, 90, 100, and 110–114 (term) of gestation, three gilts were anesthetized with an intravenous (iv) dose of sodium pentobarbital, and anesthesia was maintained by further iv administration of this drug. Prior to anesthesia, a 10-mL sample of whole blood was collected from each gilt. The uterus was exteri-

orized after a mid-ventral laparotomy and dissected to carefully expose each conceptus (fetus, placental membranes, and fetal fluids). After rupturing the allantoic sac, a 10-mL syringe fitted with a 20-gauge needle was used to collect a sample of amniotic fluid by penetrating the amniotic sac, taking care to avoid contamination from blood or allantoic fluid. Following rupture of the amniotic sac, a sample of fetal arterial blood was obtained from umbilical vessels and a small aliquot was collected into a microcapillary tube for hematocrit determination. The umbilical cord was then clamped off and the fetus severed from this connection at a point close to the abdomen. After recording fetal body weight, the liver and kidneys were removed, rinsed in 0.9% saline, blotted on a clean paper towel and their weights recorded. The gilt was sacrificed by exsanguination following surgery, at which time the liver was removed, weighed, and a sample taken for analysis. Fetal hematocrit values were determined after centrifugation of the capillary tubes and expressed as percentage of packed red cells per volume of whole blood. Maternal and fetal serum was harvested following centrifugation of blood allowed to clot for 4 h at room temperature. Tissue, serum, and fluid samples were stored at -20°C prior to analysis. All animal handling and surgical procedures were conducted in accordance with institutional animal care and use guidelines.

Analytical Procedures

Maternal and fetal serum (diluted 1:5 with deionized water) and amniotic fluid (undiluted) were analyzed directly for zinc, copper, and iron concentration using flame atomic absorption spectrophotometry (AAS). Tissue samples were weighed into clean porcelain crucibles, dried at 110°C overnight, and ashed in a muffle furnace at 450°C for 24 h. The remaining ash residue was dissolved in 3N HCl, diluted to an appropriate volume and the zinc, copper, and iron concentrations determined using AAS. Ceruloplasmin was determined in samples of maternal and fetal serum using the procedure of Rice (23) measuring the oxidation of *p*-phenylenediamine. A unit of oxidase activity was defined as the change in absorbance at 540 nm/min/L of serum.

Column Chromatography

Samples of maternal and fetal liver were homogenized in four volumes of 0.25M sucrose, 10 mM Tris-HCl, pH 8.6 containing 4 mM phenylmethylsulfonyl fluoride (PMSF). Homogenates were centrifuged at 100,000g for 90 min at 4°C to obtain cytosol extracts. Four milliliters of the cytosol was applied to a column (1.6×80 cm) of Sephadex G-75 and eluted with 10 mM Tris-HCl, pH 8.6. Five-milliliter fractions were collected and analyzed for zinc and copper using AAS. The amounts of zinc

and copper bound to metallothionein (MT) were determined as that portion of cytosol metal eluting with peak fractions at v_e/v_o of 1.8–2.2. The amounts of zinc and copper in this peak were summed and expressed as microgram per gram of fresh liver weight.

Statistical Analyses

Data were analyzed by analysis of variance, and the significance of the maternal–fetal difference was tested using the Student's t-test (SAS, SAS Institute, Cary, NC, USA).

RESULTS

Fetal body weight increased 50-fold during the second trimester of gestation (d 40–80) and 3.6-fold during the third trimester (d 80 to term; see Fig. 1). Growth of the liver and the kidneys differed from that of body weight in that fetal liver weight increased 12-fold during the second trimester and kidney weight (pair) increased 4-fold. As a result, the size of fetal liver and kidneys relative to that of the body declined during the second trimester (Fig. 1, inset). During the third trimester, the increase in fetal body weight (3.6-fold) was similar to the increases in liver (5-fold) and kidney (3-fold) weights such that the relative sizes of these two organs declined to a lesser extent or even increased slightly (liver) compared to the second trimester. An overall average of 10.5 ± 0.5 fetuses per gilt was observed in this study (data not shown).

Fetal hematocrit (packed cell volume) increased from 19% on d 30 to 32% by d 50 of gestation (Fig. 2). Fetal hematocrit values remained above 30% throughout the remainder of gestation.

The concentrations of zinc, copper, and iron in amniotic fluid all reached a maximum level on d 60 of gestation (Fig. 3). At any point during gestation, the concentration of iron in amniotic fluid was greater than that of zinc, which exceeded that of copper.

Maternal serum zinc levels fluctuated little during gestation (Fig. 4). Fetal serum zinc levels were significantly ($p < 0.05$) higher than the corresponding maternal values during the second trimester of gestation. Fetal serum zinc levels also declined during this time such that by d 100 of gestation, there was no significant ($p > 0.05$) difference between maternal and fetal values. Copper levels in maternal serum fluctuated more than zinc, reaching a maximum on d 70 and a minimum on d 80. There was a small increase in maternal serum copper levels during the third trimester. At all times sampled, maternal serum copper levels were significantly ($p < 0.05$) higher than fetal levels. Fetal serum copper declined during gestation, with the most dramatic reduction occurring between d 70 and 80 of gestation. The lowest level of fetal serum copper occurred

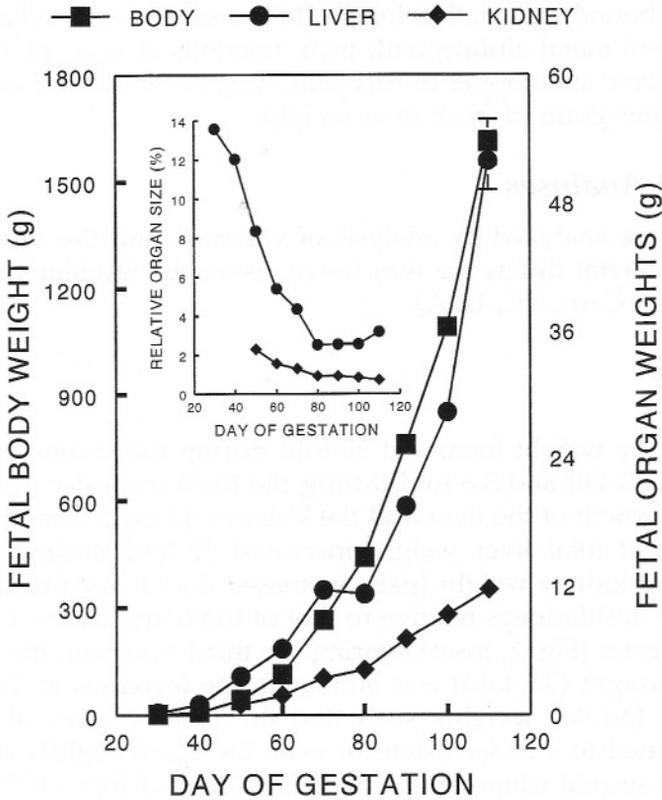


Fig. 1. Fetal body and organ (liver and kidney) weights as a function of gestational age. Inset: Relative organ size (organ weight/body weight $\times 100$) as a function of gestational age. Values represent mean \pm SEM (because of their small size, most of SEM values are not visible).

on d 110 of gestation, just prior to parturition. Maternal serum iron concentration declined during the second trimester, reaching the lowest level on d 80 of gestation. During the third trimester, the maternal serum iron level increased to term. In contrast, the fetal serum iron concentration declined from d 50 of gestation through d 100, with a slight increase by d 110. Between d 90 and 110 of gestation, fetal serum iron was significantly ($p < 0.05$) lower than the corresponding maternal level. In the case of fetal serum metals, the declines in zinc, copper, and iron concentrations were four-fold, three-fold, and four-fold, respectively, between d 50 and 110 of gestation.

The oxidase activity of the copper-containing α_2 -globulin ceruloplasmin (i.e., oxidation of *p*-phenylenediamine, PPD) for maternal and fetal

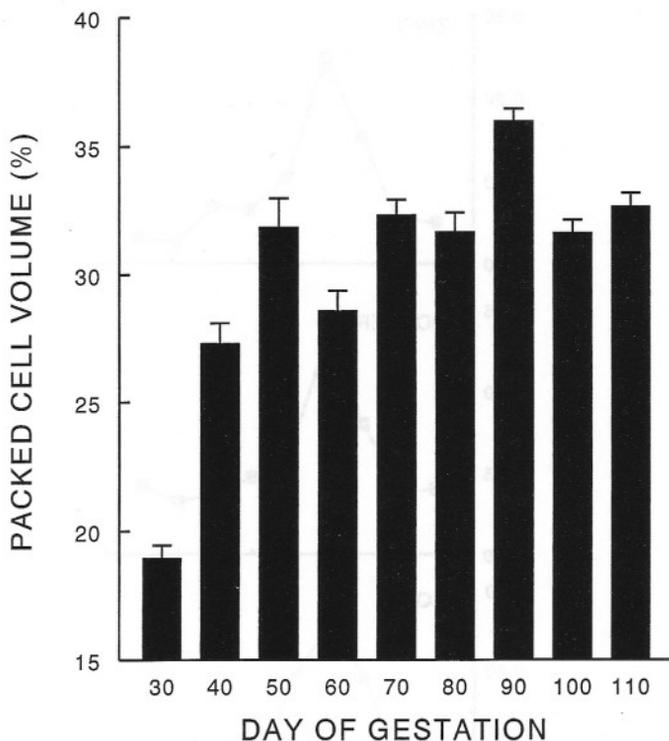


Fig. 2. Fetal hematocrit (% red cell volume) as a function of gestational age. Values represent mean \pm SEM.

serum samples is depicted in Fig. 5. Maternal serum ceruloplasmin activity levels were significantly ($p < 0.05$) higher (approximately 40-fold) than the corresponding fetal levels at all times during gestation. In general, for both maternal and fetal serum samples, the trends in ceruloplasmin oxidase activity paralleled those of copper concentration.

Table 1 summarizes the zinc, copper, and iron concentrations and total organ contents for maternal and fetal liver. In general, fetal hepatic concentrations of zinc, copper, and iron were significantly ($p < 0.05$) higher than those of maternal liver throughout gestation, whereas the total liver content of each metal was much higher in maternal as compared to fetal liver because of the large difference in liver size. Some important trends were apparent in both fetal and maternal liver with respect to metal levels. For maternal liver, the most apparent trend observed was a decline in the concentration and total content of iron beginning at the end of the first trimester and continuing throughout the second trimester of pregnancy. During the third trimester, maternal

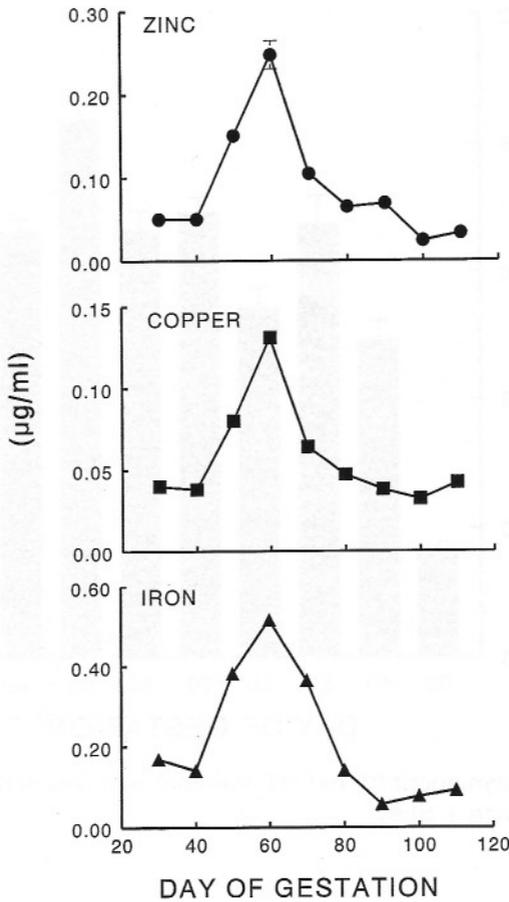


Fig. 3. Zinc, copper, and iron concentrations in amniotic fluid as a function of day of gestation. Values represent mean \pm SEM (because of their small size, most of SEM values are not visible).

hepatic iron concentration and content increased to term. Fetal liver zinc concentration increased to a peak at the end of the second trimester. Copper concentration in fetal liver reached a maximum level early in the second trimester and then declined toward term. Fetal liver iron concentration was highest by the end of the second trimester. Total liver contents of zinc and iron increased commensurate with the increase in fetal liver mass; that is, zinc and iron contents increased 11- and 14-fold, respectively, during the second trimester and 3-fold during the third trimester. In contrast, copper content of fetal liver increased only five-fold during the second trimester and two-fold during the third.

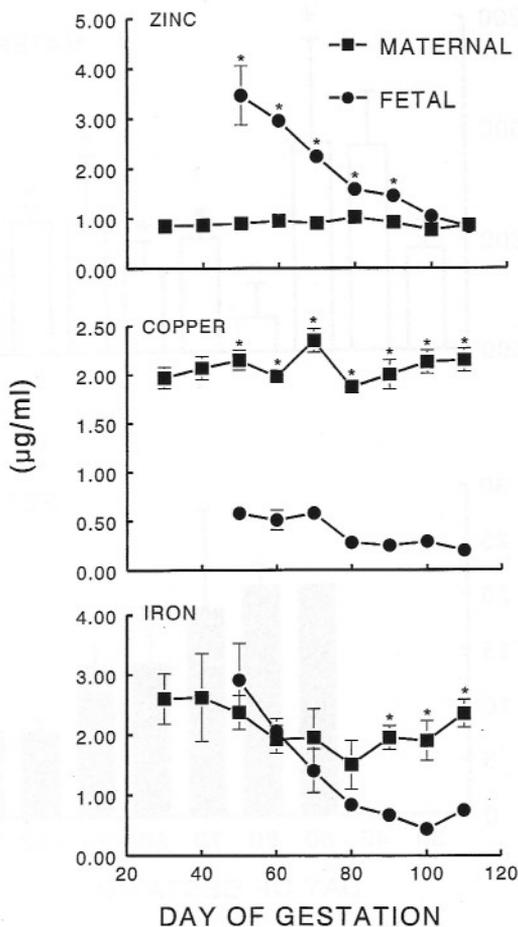


Fig. 4. Zinc, copper, and iron concentrations in maternal and fetal serum as a function of day of gestation. Values represent mean \pm SEM (because of their small size, many of SEM values are not visible). The asterisk denotes a significant ($p < 0.05$) difference for the maternal-fetal comparison on a given day of gestation.

The zinc and copper concentrations of fetal and maternal hepatic cytosol and those of cytosolic metallothionein (MT) are summarized in Table 2. In general, the zinc and copper concentrations of hepatic cytosol and MT followed the trends observed for maternal and fetal hepatic tissue metal levels. The percentage of cytosolic zinc bound specifically to the MT protein ranged from 29% to 50% in maternal liver, whereas 67–81% of the cytosolic zinc in fetal liver was associated

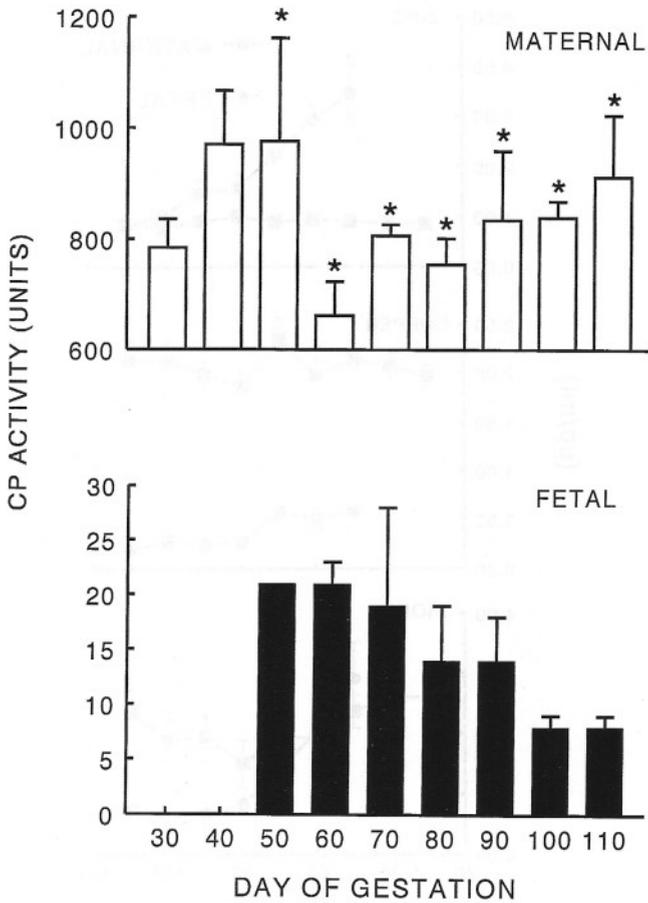


Fig. 5. Maternal and fetal serum ceruloplasmin (CP) *p*-phenylenediamine oxidase activity as a function of day of gestation. Values represent mean \pm SEM. The fetal value for d 50 represents a pooled sample caused by insufficient serum available for individual determinations. The asterisk denotes a significant ($p < 0.05$) difference for the maternal-fetal comparison on a given day of gestation.

with MT. This higher percentage in fetal liver reflects the higher zinc concentration of fetal liver as compared to maternal liver. The range of cytosolic copper associated with MT was similar for maternal (70–80%) as compared to fetal liver (63–83%). The Zn/Cu ratio of MT from fetal liver was significantly ($p < 0.05$) higher than that of maternal liver during the second and third trimesters, reflecting the relatively zinc-rich nature of fetal liver as compared to that of the mother during this time.

Table 1
Zinc, Copper, and Iron in Maternal and Fetal Liver¹

DAY	ZINC		COPPER		IRON	
	($\mu\text{g/g}$) ²	(mg) ³	($\mu\text{g/g}$)	(mg)	($\mu\text{g/g}$)	(mg)
Maternal Liver						
30	178±16*	102±14*	66±15*	38±9*	433±60*	249±47*
40	200±25*	116±8*	85±18*	50±10*	306±68*	177±35*
50	157±7*	92±20*	101±30*	58±15*	267±44*	156±26*
60	235±46*	135±16*	94±20*	54±7*	219±58*	134±43*
70	208±30*	121±9*	102±25*	59±10*	147±47*	90±34*
80	264±59*	108±14*	162±110*	63±40*	192±42*	78±10*
90	195±29*	112±15*	120±18*	69±9*	149±52*	88±33*
100	194±26*	127±12*	128±35*	89±30*	294±154*	212±127*
110	208±28*	147±16*	86±17*	63±15*	245±79*	182±63*
Fetal Liver						
30	600±18	0.02±0.01	358±36	0.01±0.001	763±21	0.03±0.001
40	653±36	0.12±0.01	871±28	0.16±0.005	663±19	0.12±0.006
50	740±54	0.56±0.07	672±25	0.50±0.03	650±35	0.48±0.05
60	865±123	1.04±0.14	450±5	0.55±0.01	687±44	0.84±0.06
70	1148±33	2.78±0.15	423±12	1.01±0.04	573±48	1.41±0.14
80	592±11	1.34±0.06	347±16	0.77±0.01	808±95	1.72±0.13
90	586±44	2.25±0.19	282±9	1.07±0.03	538±27	2.03±0.09
100	532±55	3.29±0.45	187±11	1.06±0.04	556±39	3.14±0.16
110	333±18	4.00±0.20	109±5	1.32±0.06	474±27	5.65±0.26

¹Each value represents the mean ± SEM.

²Metal concentrations are expressed as μg metal/g dried liver weight.

³Total liver metal content.

*Designates a significant ($p < 0.05$) difference for maternal versus fetal mean comparison.

The zinc, copper, and iron contents of fetal kidney tissue are summarized in Table 3. The concentrations of zinc and copper were remarkably constant throughout development, with a small increase occurring during the latter part of the third trimester. Renal iron concentration showed a biphasic response with peak levels on d 70 and 110 of gestation. The total content of zinc, copper, and iron in fetal kidney tissue increased 13-, 17-, and 20-fold, respectively, between d 50 and 110 of gestation. Like the liver, there was a reduced rate of accumulation of all three metals between d 70 and 80 of gestation, which correlated with a slowing in the rate of kidney growth. The increase in total zinc content paralleled the increase in kidney mass (Fig. 1), whereas the overall increases in total copper and iron contents exceeded the increase in kidney mass.

Table 2
Zinc and Copper Contents of Maternal and Fetal Liver Cytosol
and Metallothionein¹

DAY	CYTOSOL		METALLOTHIONEIN		
	Zinc ($\mu\text{g/g}$) ²	Copper ($\mu\text{g/g}$) ²	Zinc ($\mu\text{g/g}$) ²	Copper ($\mu\text{g/g}$) ²	Zinc/Copper
Maternal Liver					
30	48±5*	13±2*	23±4*	10±2*	2.3±0.1
40	46±4*	15±2*	23±5*	12±1*	2.0±0.4*
50	36±2*	20±4*	14±1*	16±3*	1.2±0.2*
60	49±8*	19±2*	22±6*	15±2	1.3±0.2*
70	49±4*	19±4	20±3*	15±3	1.6±0.4*
80	67±11*	42±17	30±8*	32±14	1.6±0.5*
90	42±1*	20±1*	12±1*	14±0.4*	0.7±0.1*
100	46±4	25±4*	16±2*	18±3*	1.1±0.3*
110	50±7	14±2*	20±4*	11±2*	2.1±0.5*
Fetal Liver					
30	114±8	46±0.3	77±3	38±1	2.0±0.1
40	113±16	86±6	59±10	67±4	0.9±0.1
50	107±10	42±4	79±11	35±2	2.3±0.4
60	94±1	26±2	64±3	19±2	3.4±0.3
70	220±14	19±1	164±13	13±1	13.0±0.8
80	102±2	18±1	75±4	14±1	5.6±0.2
90	110±15	14±1	77±11	11±1	7.4±1.5
100	88±20	10±1	65±16	8±1	8.8±2.6
110	70±6	8±1	53±5	6±1	8.9±1.0

¹Each value represents the mean \pm SEM.

²Metal concentrations are expressed as μg metal/g fresh liver weight.

*Designates a significant ($p < 0.05$) difference for maternal versus fetal mean comparison.

Table 3
Zinc, Copper, and Iron Contents of Fetal Kidneys¹

DAY	ZINC		COPPER		IRON	
	($\mu\text{g/g}$) ²	(μg) ³	($\mu\text{g/g}$)	(μg)	($\mu\text{g/g}$)	(μg)
50	81±0.9	11±0.5	17±0.7	2±0.1	147±5	20±1
60	80±0.5	18±0.3	18±0.5	4±0.2	200±8	46±2
70	82±0.9	38±3	17±0.4	8±0.5	213±20	102±14
80	78±0.8	39±6	15±1.0	7±0.8	174±10	86±13
90	79±1.0	71±2	17±1.0	15±0.8	157±6	140±7
100	90±0.9	115±7	18±1.0	24±3.0	185±15	236±24
110	89±0.7	143±8	21±0.7	34±2.0	243±14	400±40

¹Each value represents the mean \pm SEM.

²Metal concentrations are expressed as μg metal/g dried kidney weight.

³Total kidney metal content (for pair of kidneys).

DISCUSSION

The results of this study demonstrate distinct differences in the levels of zinc, copper, and iron between maternal and fetal tissues and fluids throughout gestation in pigs. Not only were significant differences apparent between maternal and fetal tissues and fluids, but within both groups, the stage of gestation had a major influence on trace element levels as well. In mammalian species, accumulation of fetal liver zinc, copper, and iron stores generally occurs during the latter part of gestation (11). In fetal liver, a significant proportion of the accumulated zinc and copper is stored bound to cytosolic metallothionein, whereas nonheme iron is sequestered by ferritin. Presumably, this accumulation of trace elements by fetal liver assures a readily available store for use during the early postnatal period when milk, which is generally low in trace element content, is the primary food source (11). Pigs are born with low iron status indicative of insufficient iron stores to meet the needs of rapid growth during the early neonatal period (24). An intramuscular injection of iron has become a routine management practice performed within the first week postpartum to augment neonatal iron stores, whereas supplemental iron administered to the dam prior to birth has proven to be ineffective in raising neonatal iron status (24). Pigs are also born with low levels of circulating copper and ceruloplasmin and elevated hepatic copper levels, a situation that was observed throughout fetal development in this study. However, this trend quickly reverses during the first 2 wk postpartum as plasma copper and ceruloplasmin levels increase toward adult levels and hepatic copper concentration decreases (25,26). Thus, it is likely that events occurring during the latter stages of development *in utero* set the stage for specific responses in trace element metabolism that occur during the early neonatal period.

Fetal accretion of essential trace elements results from their mobilization and transfer from maternal sources to fetal tissue stores. This process does not involve passive diffusion across the placental barrier because significant gradients exist for maternal-fetal plasma concentrations of zinc, copper, and iron during much of gestation in the pig. Such gradients have been observed in other species, including rodents (27). In the pig, which has an epitheliolchorial type of placentation, mechanisms for nutrient transfer, in part, involve the induction and synthesis of the transport proteins by uterine endometrial glands and their subsequent uptake and accumulation by fetal tissues and fluids. Perhaps one of the best characterized mechanism in pigs involves the transfer of iron to the developing fetus via the synthesis and secretion of uteroferrin (12). This type of transfer mechanism constitutes what has been referred to as an embryotrophic route (i.e., nutrients bound to transporting proteins released from the uterine endometrial glands and taken up into fetal circulation). A similar embryotrophic mechanism might be envisioned for the transfer of copper involving uterine synthesis and secretion of ceru-

loplasmin. In fact, ceruloplasmin has been detected in the uterus, placenta, and amniotic fluid (11). Interestingly, in pigs, maternal serum ceruloplasmin activity is the highest at the point in gestation when fetal hepatic copper concentration is maximal (approx d 40–50 of gestation). However, its role, if any, in shuttling copper between mother and fetus remains speculative. Although unique changes in the levels of fetal hepatic zinc, copper, and iron occur during the development of the pig, it cannot be ruled out that a common or shared mechanism exists for the transplacental transfer of trace elements. Metal-transporting proteins such as albumin, α_2 -macroglobulin, and even uteroferrin, which is known to bind zinc and copper in addition to iron (16), might fill this role. The role of such proteins in metal transfer might also involve alternative routes of transfer, including transcytotic exchange mechanisms such as those reported for the placental transfer of iron via transferrin in other mammalian species (28). Indeed, there is some doubt as to whether uteroferrin can account for all of the iron transferred to the pig fetus during its development *in utero* (7,12). Because there is close contiguity between maternal and fetal blood supplies over much of the placental surface in the pig (7), this would permit the interaction or passage of large protein molecules carrying metals and provide for an alternate route of trace element transfer.

The accumulation of zinc, copper, and iron in fetal liver at concentrations exceeding those of maternal liver most likely reflects the net effect of a number of distinct mechanisms. Trace element supplies coming from the dam can originate from dietary intake, which increases significantly during pregnancy, or from mobilization of deposits in such tissues as the liver in response to fetal demands for these nutrients. The decline in maternal hepatic iron concentration during the second trimester coincided with a decrease in maternal and an increase in fetal hepatic ferritin-iron concentration (29), emphasizing the potential importance of this iron storage protein in both the release and the accumulation of iron stores during gestation in pigs.

The ability of fetal liver to take up and store trace elements from fetal circulation is a complex process that depends on the combined effects of transport into (influx), binding to intracellular storage proteins, and transport out (efflux) of fetal hepatocytes. The role of metal-transporting proteins such as transferrin, ceruloplasmin, albumin, and others in facilitating the uptake of zinc, copper, and iron by different cells in maternal and fetal tissues is well recognized. Recently, membrane-bound, metal-transporting proteins, studied in animal and cell culture models, have attracted greater attention from researchers as an important component of cellular metal homeostasis. Two membrane-bound, copper-transport proteins (ATP7A and ATP7B) have been identified and characterized as P-type ATPases (30). Similarly, other proteins have been reported to mediate transmembrane zinc and iron transport (31). One type of membrane transport protein (proton coupled) has also been iden-

tified in rat duodenal tissue (DCT-1), which is capable of transporting several different divalent cations, including zinc, copper, and iron (32). The combined transporting action (both into and out of cells) by membrane proteins such as these, in part, would determine the concentration of a particular metal within the fetal hepatocyte. Very little is known about the developmental regulation of membrane-bound metal-transport proteins. However, it was observed that the pig is born lacking the ability to transport copper out of the liver and into bile, a process which develops rapidly during the first week postpartum (33). Biliary copper excretion has been attributed to the copper-transporting protein product of the Wilson's disease gene (ATP7B), a membrane-bound P-type ATPase (34). Taken together, these data suggest that the expression of the gene for this membrane copper-transport protein exhibits some degree of developmental control. In support of this concept, Harris et al. (30) noted that human choriocarcinoma placental (BeWo) cells lacked a membrane-bound copper transporter, preventing them from releasing absorbed copper back into the medium unless they were first cultured on porous filters, which allowed them to form apical and basolateral surfaces. This suggests that development of active copper transport mediated by membrane metal transporters may depend on the degree of cellular differentiation and/or maturation (30).

Active synthesis of intracellular hepatic metal-sequestering proteins such as metallothionein and ferritin during fetal development may occur in response to the intracellular metal levels determined by the actions of the membrane metal transporters. However, because gene expression of such metal sequestering proteins is also regulated by nonmetal inducers such as hormones and cytokines, it is also possible that the synthesis of these proteins could lead to enhanced intracellular metal accumulation by acting as a metal sinks. The highest levels of hepatic zinc and copper in porcine fetal liver correspond with peak levels of metallothionein-bound zinc and copper (this study) and hepatic iron with that of ferritin (29). Thus, it is not known whether the elevated level of fetal hepatic metallothionein observed in this study is the result of increased uptake of zinc and/or copper by the liver or is itself the mechanism for increased hepatic metal accretion. Clearly, the balance of cellular influx, efflux and intracellular sequestration significantly influences trace element accretion by fetal liver.

Partitioning and repartitioning among different fetal tissues and fluids can influence hepatic trace element levels. In the fetal pig, the liver is an active site of plasma protein synthesis (35) and this may constitute one mechanism for the export of hepatic metals as they are bound to transporting proteins such as α -fetoprotein, albumin, ceruloplasmin, transferrin, and perhaps others. In the case of iron, allantoic fluid serves as a dynamic reserve that can deliver iron (via a uteroferrin-transferrin transfer) to the reticuloendothelial cells of the liver and spleen for its subsequent incorporation into erythrocyte hemoglobin (36). Throughout

much of gestation, the fetal liver is a major site of erythropoiesis (12) and this activity represents a major demand for iron. The extent to which fetal hepatic reserves might be utilized for or affected by hemoglobin biosynthesis during erythropoiesis is not known.

The role of trace elements contained in amniotic fluid is not clear. Undoubtedly, these metals are complexed by specific proteins and the presence of plasma metal-transporting proteins such as transferrin, α -fetoprotein, and albumin in porcine amniotic fluid has been reported (36). Because amniotic fluid is swallowed by the fetus, it has been suggested that this fluid may be an important source of certain trace elements such as copper (bound to ceruloplasmin) for fetal nutrition (37). In the case of the pig, trace element levels in amniotic fluid are quite low and probably do not constitute a major source of these nutrients for the fetus. However, this does not completely rule out the possibility of some localized effects of the trace-element-bearing proteins on the growth and development of the fetal gut, for example, or some protective role as antimicrobial or antioxidant agents. It has been definitively shown that uteroferrin does not accumulate in amniotic fluid and, therefore, cannot account for changes in iron levels in this fetal fluid compartment (36). The unique pattern for changes in amniotic fluid zinc, copper, and iron levels observed in this study most likely reflects changes in the fluid volume of the amniotic sac, which, in the pig, is known to reach its peak at about d 60 of gestation (38).

In conclusion, this study demonstrated some of the changes in zinc, copper, and iron levels in maternal and fetal pig tissues and fluids occurring during gestation. This information is useful in determining specific developmental periods of heightened demand for particular trace elements, such as occurs for iron at the end of the second trimester. The results of this study also offer new insight into potential mechanisms involved in the maternal-fetal transfer of trace elements as mediated by the placenta in gestating pigs. Moreover, such information highlights the potential importance of intrauterine trace element metabolism on the preparation of the pig fetus for its subsequent growth and development as a neonate postpartum.

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