The Neonatal Piglet as a Model for Human Neonatal Carnitine Metabolism

JANET K. BALTZELL,* FULLER W. BAZER,† STANLEY G. MIGUEL‡ AND PEGGY R. BORUM*

*Department of Food Science and Human Nutrition and †Department of Animal Science, University of Florida, Gainesville, FL 32611 and ‡Mead Johnson Nutritional Division, Evansville, IN 47721

ABSTRACT Investigations concerning carnitine metabolism and possible requirements for exogenous carnitine in human preterm neonates are limited by ethical considerations. The neonatal piglet is a potential animal model for these investigations. Tissue carnitine concentrations were determined in fetuses from cross-bred domestic gilts at stages of gestation corresponding to those of neonates found in neonatal intensive care units. Fetal piglet plasma and red blood cell carnitine levels decreased from approximately 90 d to term. Skeletal muscle carnitine increased from 60 d to term. Temporal changes in fetal carnitine concentrations in plasma, red blood cells and skeletal muscle throughout gestation are similar to the pattern reported by our laboratory for the human neonate. Cardiac muscle carnitine increased earlier than skeletal muscle but also continued to increase to term. Carnitine concentrations in fetal liver, kidney and intestine were maximal at 90 d and decreased until term. Similarities in physiology, metabolism and profiles of tissue carnitine concentration between the newborn piglet and the human neonate indicate that the neonatal piglet is an appropriate animal model for investigations concerning neonatal carnitine metabolism. J. Nutr. 117: 754–757, 1987.

INDEXING KEY WORDS:
• carnitine • neonate • piglet

Carnitine is a critically important nutrient for the human neonate. The roles of carnitine in facilitating the transport of fatty acids into the mitochondrial matrix for oxidation, in the initiation of ketogenesis and in the maintenance of thermogenesis are well documented [1]. The roles of carnitine in facilitating the maintenance of blood glucose concentrations and the control of blood ammonia concentrations require additional investigations but have far-reaching physiological importance [1]. The critical need for carnitine coupled with reduced stores of carnitine and reduced biosynthetic capability in the neonate compared with the adult have caused nutritionists to consider the possibility that carnitine may be an essential nutrient for the human neonate [2]. Most neonates receive exogenous carnitine from breast milk, milk-based formulas or carnitine-supplemented soy-based formulas. However, neonates requiring total parenteral nutrition as a result of prematurity and its accompanying metabolic consequences receive no exogenous carnitine. Neonates born at < 32 wk gestation have the smallest carnitine stores [3]. They are often in a very fragile metabolic state and may actually have the greatest need of any neonate for exogenous carnitine. At present our knowledge of the metabolic handling and compartmentalization of exogenous carnitine by neonates of all gestational ages is severely limited.

Ethical considerations prohibit many investigations in the neonate that would provide data concerning metabolic compartmentalization of exogenous carnitine. An appropriate animal model is thus essential for these investigations. Data are presented that indicate the neonatal piglet as an appropriate animal model.
MATERIALS AND METHODS

Animals. Piglets were taken on d 60, 79, 85, 90, 97, 103, 110, and 112 of gestation by Caesarean section from domestic cross-bred (Yorkshire × Hampshire × Duroc) gilts. The gilts were fed a diet prepared with no carnitine-containing ingredients. The gilts were anesthetized initially with one g thiamylal sodium (5% solution) injected via an ear vein. Anesthesia was maintained by halothane. A midventral laparotomy was performed to expose the uterus. The fetuses were removed from the uterus, assigned an identification number and transported to the laboratory for tissue sampling. Maternal blood was collected from the uterine vein before removal of the uterus. Fetal blood was collected from the umbilical vein as the fetuses were removed from the uterus.

Tissue sampling. Fetuses were covered with ice until dissection and tissue collection were completed. Heart, lungs, liver, intestine, kidneys and skeletal muscle (gastrocnemius region) samples were removed from fetuses and placed in individual plastic weighing boats on ice. Heart ventricles were trimmed of excess tissue, cut open and swirled gently in chilled saline (0.9% NaCl) to remove blood. Lungs, kidneys and skeletal muscle were trimmed of excess tissue and rinsed in saline. Intestinal tissue was cut lengthwise and rinsed with saline to remove the intestinal contents. Whole livers were perfused with saline (via the intact vasculature) to clear any blood. All tissue samples were blotted to remove excess moisture, placed in prelabeled plastic vials and stored at -80°C. Fetal and maternal blood were perfused with saline (via the intact vasculature) and frozen.

Assay procedures. A Ten Broeck homogenizer (purchased from Fisher Scientific, Springfield, NJ) was used to prepare tissue homogenates of 250 [liver and lung] or 150 mg [all other tissues] in 3 mL of distilled water. The carnitine concentrations of the tissue homogenates, plasma and red blood cells were measured by a modification of the method of Cederblad and Lindstedt (4) as described previously (5). Protein concentration was determined by the microbiuret method of Itzhaki and Gill (6). The method of Lilienthal et al. (7) was used for the solubilization of noncollagen protein. Hemoglobin concentration of the red blood cells was measured using a Fisher Diagnostic Kit (Fisher Diagnostics, Orangeburg, NY) based on a modified Drabkin method (8).

Statistical analysis of data. Statistical analyses of data were performed using the RS/1 Integrated Data Analysis System for the Digital Professional 350 Computer (Digital Equipment Corporation, Maynard, MA). Sample populations were subjected to the Wilk-Shapiro test of normality. Normal populations were tested for equal or unequal variances with an F-test for variance ratio. A t-test for either equal or unequal variances was then performed. In the case of nonnormal populations the Ansari-Bradley test for equal dispersions was used, and then the Mann-Whitney test for unpaired samples with equal dispersions was applied (9).

RESULTS

Fetal piglets' plasma carnitine levels decreased from 97 to 112 d gestation (Fig. 1, top graph). The piglets' red blood cell total carnitine concentrations decreased from 90 to 112 d gestation (Fig. 1, bottom graph). The ratio of maternal to fetal plasma carnitine concentrations increased steadily from 0.2 at 60 d to 3.7 at 112 d. The ratio of maternal to fetal red blood cell carnitine did not follow the same pattern, with values remaining at < 0.5 until after d 103 and then increasing to a mean of 0.69 in one litter and 1.56 in a second litter at d 112.

The piglets' skeletal muscle total carnitine levels increased with gestational age (Fig. 2, top graph). Although cardiac muscle carnitine concentrations (Fig. 2, bottom graph) also increased between 60 and 112 d gestation, the levels reached peak concentrations earlier than in the skeletal muscle.

Total carnitine concentrations in the fetal liver (Fig. 3, top graph), kidney (Fig. 3, middle graph) and intestine (Fig. 3, bottom graph) reached a peak at 90 d gestation.
FIGURE 2 Skeletal muscle total carnitine concentrations (top graph) at various days of gestation in the piglet. Values at 60 d gestation were lower than values at 79, 85, 90, 97, 103, 110 and 112 d gestation (P < 0.01). Cardiac muscle total carnitine concentrations (bottom graph) at various days of gestation. Value at 60 d gestation was lower than value at 112 d gestation. Bars represent means ±SD. Numbers in parentheses represent the number of piglets in each sample population.

DISCUSSION

Many investigations necessary to determine nutritional requirements of the human neonate (including the possible requirement for exogenous carnitine) involve techniques too invasive to be performed in the neonate. An animal model is required for these investigations. This animal model must possess certain characteristics in common with the neonate to permit appropriate extrapolation of experimental results. Table 1 lists characteristics important in investigations of all types of nutrients, as well as characteristics specifically relevant to the investigation of possible carnitine requirements in the neonate.

Maturity at birth. A significant problem with many neonatal animal models, such as rodents, is that the degree of maturity at birth is much less for the animal model than for the neonate. The degree of maturity of piglets and of neonates is more comparable.

Anatomy and physiology. For the animal model to be appropriate, the anatomy and physiology of the animal model and the neonate must be comparable. Neonatal piglets and human neonates are comparable both anatomically and physiologically for most processes (10–12). Additionally, unlike many other animal models, the litter size provides a statistically significant number of animals, which helps to decrease variables in experimental design. Twenty years ago, Glauser (10) pointed out the possible importance of the piglet as an animal model when she stated, “The similarities between human infants and piglets is noted and the possibility of using piglets as the prototype for infants in pediatric research is advocated.”

Susceptibility to hypothermia and hypoglycemia. In 1959 McCance and Widdowson (13) reported apparent similarities between the response of newborn piglets

| TABLE 1 |
| Characteristics that must be common to the human neonate and the animal model |

| Characteristics for investigations of all nutrients |
| Maturity at birth |
| Anatomy and physiology |

Specific characteristics for carnitine investigations

Susceptibility to hypothermia and hypoglycemia

Adaptation at birth from carbohydrate as the sole energy source to use of lipid as an important energy source

Profile of tissue carnitine concentrations during gestation

Survival and growth of colostrum deprived newborn animals maintained with nutritional support techniques used in neonatal intensive care units
and newborn infants to a cold environment. Hypothermia and hypoglycemia are problems for both. Because carnitine deficiency is associated with both hypoglycemia and hypothermia [1], the common problems of hypoglycemia and hypothermia may be of significance in studying the possible nutritional requirement of carnitine.

**Adaptation at birth from use of carbohydrate as the sole energy source to use of lipid as an important energy source.** The fetal piglet and the fetal human both utilize carbohydrate as the sole energy source. The metabolic adaptations required by both the neonatal piglet and the human neonate to utilize lipid as an energy source include the development of the physiological processes involving carnitine.

**Profile of tissue carnitine concentration during gestation.** If the neonatal piglet (both preterm and term) is a valid animal model for the neonate, the profile of tissue carnitine concentrations during gestation must be similar. The patterns observed for plasma and red blood cell carnitine concentrations in piglets are similar to those in neonates [14]. Both species show decreases from around 90 d (corresponding to 30 wk human gestation) to term. Similar patterns are observed for carnitine in fetal red blood cell, plasma, liver, kidney and intestine. One possible explanation of the low maternal-to-fetal plasma carnitine ratio in early gestation is that it may reflect maternal-to-fetal transfer of carnitine. The increase in this ratio in late gestation could reflect a decrease in maternal-to-fetal transfer. The decreased carnitine levels in the liver, intestine and kidney detected in this study support this hypothesis.

Similarly the increasing skeletal muscle carnitine concentrations from 60 ( < 26 wk human gestation) to 112 d (38 wk human gestation) observed in this study are similar to those noted in a study of humans [3]. Cardiac muscle carnitine concentration also increases but appears to reach a maximal concentration earlier. This may reflect a greater or earlier need for carnitine in the fetal heart. It is possible that by 90 d (30 wk human) gestation the fetus has obtained a critical minimal level of carnitine in the muscle "stores." The 90-d gestational age corresponds to a human gestational age above which neonatal survival increases greatly.

**Survival and growth of colostrum-deprived newborn animals maintained with nutritional support techniques used in neonatal intensive care units.** There are reports indicating that colostrum-deprived neonatal piglets can be reared by artificial means [11, 15–17]. Our experience to date confirms that the animals do not have to be pathogen free but that the same strict adherence to cleanliness and constant care observed in a neonatal intensive care unit is also required in a piglet neonatal intensive care unit. In experiments in progress, nutritional support regimens commonly used in neonatal intensive care units in maintaining colostrum-deprived neonatal piglets are being tested. These nutritional support techniques include formula feeding and total parenteral nutrition. Elucidation of the nutritional requirements of the neonatal piglet should provide valuable information concerning nutritional requirements of the neonate. The same data also provide valuable information for improvement of piglet husbandry and may be useful in reducing piglet mortality.

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