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Neonatal liver physiology

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ABSTRACT

In the neonate, the liver is relatively immature and undergoes several changes in its functional capacity during the early postnatal period. The essential liver functions can be classified into three categories: metabolism, detoxification, and bile synthesis. In general, the immature liver function has limited consequences on the healthy term neonate. However, preterm neonates are particularly susceptible to the effects of the immature liver function placing them at risk of hypoglycemia, hyperbilirubinemia, cholestasis, bleeding, and impaired drug metabolism. An appreciation of the dynamic changes in liver function during the neonatal period is essential for successful management of neonates who require medical and surgical interventions. This review will focus on the neonatal liver function as well as the changes that the liver undergoes as it matures.

Introduction

The liver is the largest solid organ and is responsible for several critical functions including the metabolism of dietary compounds, regulation of blood glucose levels, production of clotting factors and serum proteins, bile synthesis, and biotransformation of xenobiotics and endogenous by-products of metabolism.[1] During gestation, the fetus is able to rely on the metabolic activity of the maternal liver while the fetal liver develops the capacity to perform these functions. In the neonate, the liver is relatively immature and undergoes several changes in its functional capacity during the early postnatal period. In general, the immature liver function has limited consequences on the healthy term neonate. However, preterm neonates are particularly susceptible to the effects of the immature liver function placing them at risk of hypoglycemia, hyperbilirubinemia, cholestasis, bleeding, and impaired drug metabolism. An appreciation of the dynamic changes in liver function during the neonatal period is essential for successful management of neonates who require medical and surgical interventions. This review will focus on the neonatal liver function as well as the changes that the liver undergoes as it matures.

Liver embryology

Liver organogenesis begins during the fourth week of embryogenesis with the development of an outpouching from the ventral foregut endoderm referred to as the hepatic diverticulum or liver bud. The cranial portion of the hepatic diverticulum develops into the liver and intrahepatic biliary tree while the caudal portion develops into the gallbladder and extraheptic biliary tree. The primitive endodermal cells of this bud, also referred to as hepatoblasts, are bipotential cells with the ability to differentiate into hepatocytes and biliary epithelial cells (e.g., cholangiocytes).[2] The cardiac mesoderm and septum transversum are in close contact with hepatoblasts[3,4] and secrete growth factors necessary for induction of hepatoblasts and differentiation and maturation of hepatocytes.[5,6] Rapidly growing hepatoblasts migrate into the mesenchyme of the septum transversum eventually forming long cords of hepatocytes called hepatic plates. Hepatic plates are separated by mesenchymal cells that form the specialized porous endothelium of the sinusoids. The intrahepatic bile ducts are formed by the periportal hepatoblasts, which through interactions with neighboring cells and matrix proteins form a continuous single-layered ring around the portal mesenchyme and give rise to the bile ducts.[7–9]

Fetal liver circulation

The fetal liver receives highly oxygenated blood via the umbilical vein which travels along the falciform ligament and connects to the left branch of the portal vein via the portal sinus.[10,11] The umbilical vein supplies oxygen and nutrients to the fetal liver, supplying approximately 75% of the total blood to the liver.[12] The umbilical vein continues past the portal vein branches as the ductus venosus and delivers highly oxygenated blood to the inferior vena cava. The oxygenated blood is eventually directed across the patent foramen ovale to the left side of the heart.[10,13]

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Thus, the ductus venosus acts as a shunt supplying oxygenated blood to the growing fetus. The degree of shunting through the ductus venosus is highest during early pregnancy when approximately 30% of the blood supplied by the umbilical vein is shunted through the ductus venosus. By 30 weeks of gestation, this value is reduced to 20%, resulting in approximately 80% of umbilical blood perfusing the liver. The ductus venosus is under adrenergic tonic control and vasodilates in the presence of nitric oxide and prostaglandins. During fetal hypoxemia, extensive dilation of the ductus venosus results in increased shunting to supply oxygenated blood to the heart and brain.[14] The degree of shunting through the ductus venosus is also influenced by the hematocrit levels, umbilical venous pressure, and neural and endocrine regulation resulting in a dynamic variation in the degree of shunting. Closure of the ductus venosus occurs within the first 2 weeks of life in the majority of neonates. However, there is a significant delay in ductus venosus closure in preterm neonates and neonates that received antenatal corticosteroids.[15,16]

Functional organization of the liver

The functional unit of the liver consists of the hepatic acinus which is developed by the third month of gestation.[17] Each acinus includes a central hepatic vein connected to four to six portal triads via hepatic plates. Blood from the portal vein and hepatic artery enters the sinusoids which are located between hepatic plates before emptying into the central hepatic veins. Hepatocytes located within these functional units perform specific functions depending on their location within the acinus. Hepatocytes with similar functions are divided into three zones within the acinus. Hepatocytes closest to the portal triad are located in zone 1 and are exposed to blood with highest oxygen and nutrient concentration. As a result, these cells perform the majority of the liver's metabolic functions including glycogen synthesis, gluconeogenesis, protein synthesis, and lipid metabolism.[18–22] The hepatocytes closest to the central veins are located in zone 3 and are specialized for biotransformation reactions, glycolysis, and urea synthesis. Hepatocytes in zone 2 are located between zones 1 and 3 and their function is dependent on their relative proximity to zone 1 or 3.

Plasma protein synthesis

Plasma protein synthesis and homeostasis is a vital function of the liver. The substrates necessary for plasma protein synthesis are provided by amino acids transported via the portal vein across the basolateral membrane of sinusoidal hepatocytes. The main serum protein produced by the fetal liver is alpha-fetoprotein which reaches its maximal concentration by the end of the first trimester. Albumin synthesis begins at approximately the 16th week of gestation and reaches adult levels by the end of gestation. All of the coagulation proteins are synthesized in the liver with the exception of factor VIII. The serum concentrations of coagulation proteins are low in newborn infants and reach adult levels within the first few days following birth.[23]

Glucose and fatty acid metabolism

During gestation, the fetus receives a continuous supply of nutrients from the maternal circulation. The fetal glucose concentration is slightly lower, but in equilibrium with maternal glucose concentration and is the principle source of energy for the fetus under physiologic conditions. The fetal liver contains the necessary enzymes for gluconeogenesis and glycogen synthesis by the eighth week of gestation. Gluconeogenesis does not occur *in utero* under physiologic conditions; however, glycogen synthesis begins early in pregnancy and glycogen deposition increases from 3.4 mg/g of liver tissue at 8 weeks gestational age to 50 mg/g of liver tissue by term.[24]

Newborn infants have labile blood glucose levels as they transition from receiving nutrients from the maternal circulation to metabolizing hepatic stores and enteral feeds. At birth, the glucose concentration in umbilical venous blood is 80–90% of the maternal venous glucose concentration.[25] Blood glucose concentration in the neonate falls rapidly until 1 h of age after which glucose concentrations rise and stabilize by 3 h of age regardless of any enteral intake by the neonate.[26,27] During this same period, plasma insulin levels fall and glucagon levels markedly increase.[28,29] This resultant decrease in the insulin/glucagon ratio promotes mobilization of the limited hepatic glycogen stores which get reduced to 10% of their initial levels within 12 h of birth.[30] Following the depletion of hepatic glycogen stores, maintenance of normoglycemia in the neonate is dependent on metabolism of milk lactose provided enterally or through gluconeogenesis.

Extremely low blood glucose concentrations are uncommon in full-term breast-fed infants with modern feeding practices. The rate of glucose production in the neonate during the first few days following birth is estimated to be between 4 and 6 mg/kg/min.[31,32] Glycogenolysis accounts for approximately one-third of this glucose production and gluconeogenesis supplies a significant proportion of the remainder. Fetal gluconeogenesis is limited secondary to low activity of phosphoenolpyruvate carboxykinase; however, following birth, the activity of this rate limiting enzyme markedly increases and results in the activation of gluconeogenesis as early as 2 h after birth.[24] In term neonates, hepatic glucose-6-phosphatase activity, the final enzymatic step in glycogenolysis and gluconeogenesis, is significantly reduced in the fetus, increasing to 10% of adult levels by term and reaching adult levels by 3 days of age.[33]

Fatty acid metabolism provides another significant energy source for the neonate during the early postnatal period. Maternal plasma triglycerides undergo lipolysis in the placenta to allow for transport of fatty acids to the fetus. However, fetal fatty acid oxidation is low allowing for shuttling of fatty acids to adipose tissue for triglyceride synthesis.[34] Shortly after birth, the rate of lipolysis is increased and appears to be modulated by catecholamine release. During suckling, lypolysis is triggered due to enhanced sensitivity to lipolytic hormones (e.g., thyrotropin)[35] and decreased plasma insulin/glucagon ratio.[36,37] Elevated lipolysis provides non-esterified fatty acids for oxidation and ketone body synthesis. Within the first day of life, hepatic ketogenesis is markedly elevated in term infants[38] and remains elevated during the first 3 days of life.[39] These findings suggest that ketone bodies may provide up to 25% of the neonate's basal energy requirements during the first few days of life.[24] Additionally, elevated hepatic ketogenesis has been shown to continue through the suckling period possibly due to a direct ketogenic effect of breast milk.[39]

Preterm neonates have lower hepatic glycogen reserves[40] as well as decreased activity of gluconeogenic enzymes[34,41] which result in lower postnatal glucose concentrations. During the first few hours following birth, there is a significantly greater decrease in glucose concentrations in preterm neonates compared to term neonates. This is due to lower hepatic glucose-6-phosphatase activity in preterm neonates. As a result, preterm neonates have a very limited response to exogenous glucagon administration which has been shown to stimulate hepatic glucose-6-phosphatase expression in animal studies.[42,43] This illustrates the limited gluconeogenic capacity in premature neonates secondary to immaturity of responsible enzymatic pathways. In addition, serum ketone body levels are significantly lower in preterm

neonates indicating an inability to produce an appropriate ketogenic response to falling blood glucose levels in the early postnatal period. This immature hepatic ketogenic response to hypoglycemia in preterm infants may continue through the first 8 weeks of postnatal life.[25,44] Therefore, maintenance of glucose levels in preterm neonates is complicated by reduced glycogen stores, immaturity of the gluconoegenic pathway, and an inadequate ketogenic response to hypoglycemia.

Bilirubin metabolism

Bilirubin is a by-product of hemoglobin catabolism from senescent red blood cells in the neonate. The heme moiety is metabolized to bilirubin within the reticuloendothelial system and transported to the liver while bound to albumin. Unconjugated bilirubin is highly lipophilic and water insoluble thus limiting its ability to be readily eliminated from the body. Conjugation of bilirubin with glucuronide is catalyzed by the hepatic enzyme uridine diphosphate glucuronyl transferase (UDPGT). Expression of hepatic UDPGT is approximately 1% of adult values between 30 and 40 weeks of gestation and increases significantly to adult levels during the first few weeks of life.[45] Conjugation of bilirubin increases its aqueous solubility and increases its transport into the canaliculus via the multispecific organic anion transport system. Conjugation of bilirubin also attenuates its toxic detergent effect on bile ducts.[23] Ultimately, conjugated bilirubin is excreted into bile and transported via the biliary tract to the small intestines where it is either excreted in stool or deconjugated by bacterial or enteric β-glucuronidase and reabsorbed into blood as part of the enterohepatic circulation.[46,47]

A rise in unconjugated bilirubin during the first 2 weeks of life is very common and is usually a self-limiting process in the neonate. This physiologic jaundice results from increased bilirubin production, deficient conjugation, and/or increased reabsorption of unconjugated bilirubin via the enterohepatic circulation. During the first few days after birth, the neonate is not receiving full feeds and has not developed a normal stooling pattern. In addition, the hepatic uptake, conjugation, and biliary excretion of bilirubin are not functioning at their eventual full capacity.[48]

Several factors are responsible for the increased severity and longer duration of hyperbilirubinemia observed in preterm neonates compared to term neonates.[48] Preterm neonates experience higher rates of bilirubin production due to the higher proportion of senescent red blood cells. The preterm neonatal liver also has a reduced ability to uptake bilirubin due to deficient organic anion transport proteins. Additionally, preterm neonates have reduced hepatic UDPGT activity resulting in decreased conjugation of bilirubin. Furthermore, feeding difficulties and increased conversion of conjugated bilirubin to its unconjugated form by enteric β-glucuronidase increases enterohepatic absorption of bilirubin. These factors result in an early onset, longer duration, and increased severity of hyperbilirubinemia in preterm infants.

Bile synthesis

Primary bile acids cholic acid and chenodeoxycholic acid are synthesized from cholesterol in the liver and are first detected in the fetus by the 14th week of gestation. Examination of bile present in meconium has revealed several distinguishing features between bile acids produced by fetal and adult livers. Fetal bile acids demonstrate an increased ratio of chenodeoxycholic acid to cholic acid which is in contrast to adults in whom there is a predominance of cholic acid. Another difference is the predominance of taurine conjugation in the fetus in contrast to glycine-conjugated bile acids in adults. Finally, fetal bile acids have additional hydroxylations at carbons 1, 2, and 4 or 6 of the sterol nucleus.[49–51] These findings demonstrate unique differences in the bile acid synthetic pathway between fetal and adult livers. The physiologic significance of these differences is not known but has been hypothesized to serve a protective role against hepatotoxic products produced by the fetal liver, such as lithocholate. In both preterm and term neonates, the bile acid pool is reduced; however, term neonates experience a significant expansion of the bile acid pool size at the end of gestation.[52,53] By 7 weeks of age, the bile acid pool size in infants is comparable to adults when corrected for body surface area.[54]

The molecular mechanisms responsible for generating bile flow and bile acid uptake are not fully developed in the neonate. Animal studies have been used to investigate the development of these processes in fetal and neonatal livers. Through these studies the developmental course of the enterohepatic circulation during fetal and early postnatal life has been demonstrated and provides some insight into the predisposition of neonates to cholestatic liver disease. These studies have demonstrated decreased bile flow and bile acid secretion in the developing liver.[55] During late gestation, there is increased activity of enzymes involved in bile acid synthesis resulting in an increase in bile acid pool size before birth.[56] At birth, bile acid transport across the basolateral and canalicular membranes of hepatocytes increases, resulting in a shift of bile acid pool from the liver to intestine.[52] Additionally, the sodium–bile acid co-transport activity of the terminal ileum is fully developed by the time of weaning.[57] The final step in maturation of the enterohepatic circulation occurs when the hepatocyte sodium–bile acid co-transporter reaches its full potential, resulting in a decrease in serum bile acid concentration.[56] These findings demonstrate the development and maturation of the enterohepatic circulation in animal models; however, these findings have not been confirmed in human neonates. Shortly after birth, the serum bile acid levels in the neonate follow a characteristic developmental pattern. During the first week of life, the serum primary bile acids reach a significantly higher concentration than healthy older children and adults.[58] Additionally, serum bile acid levels do not decrease to levels comparable to those in adults until 6 months of age.[58] The high serum bile acid levels observed in infants during this time are the result of impaired hepatic uptake resulting in a physiologic cholestasis of infancy.[59,60] The mechanisms for impaired hepatic uptake have been investigated in animal studies and revealed attenuated uptake of bile acids across the basolateral membrane of fetal and neonatal hepatocytes. This impaired hepatic bile acid uptake is correlated with decreased expression of the sodium–bile acid co-transporter mRNA, decreased sodium–bile acid co-transporter protein levels, and attenuated bile acid transport.[61] Maturation of bile acid metabolism and the enterohepatic circulation occurs by the end of the first year of life.[60,62]

Maturation of the neonatal gallbladder is necessary for efficient bile flow into the duodenum. Term neonates have more effective gallbladder function compared to preterm neonates demonstrated by larger fasting gallbladder volume and more effective gallbladder contractions.[63] The duodenal bile acid concentration in neonates following enteral administration of milk or magnesium sulfate is reduced compared to older children.[64] Additionally, in term neonates, bile secretion appears to be unresponsive to postprandial stimulation.[60] These findings indicate that the neonatal gallbladder function is not fully developed at birth.

Biotransformation

The liver plays a critical role in modulating the absorption, excretion, and metabolism of xenobiotics and endogenous by-products of metabolism. During the neonatal period, the main

limiting factor affecting hepatic clearance of these products is either an immature drug metabolism pathway or hepatic transport mechanisms.[65] The role of membrane transporters in hepatic clearance of xenobiotics is poorly understood; however, limited investigation has demonstrated that the hepatic excretory function is immature in neonates.[66]

Hepatic biotransformation is generally divided into phase I and phase II reactions. Phase I or activation reactions involve oxidation, reduction, and hydrolytic reactions. The principal enzymes involved in these activation reactions belong to the cytochrome P450 family. Phase II or detoxification reactions involve the conjugation of a water-soluble endogenous molecule (e.g., glucuronic acid, sulfate, glycine, glutathione, and acetate) in order to catalyze the metabolism of endogenous compounds or xenobiotics. Many of the enzymes involved in phase I and II reactions are differentially expressed during the perinatal period and subject to developmental alterations.

The neonatal liver undergoes a rapid maturation process during the first year of life which affects its many biotransformation functions. The cytochrome P450 enzymes are members of a superfamily that catalyze the oxidative metabolism of lipophilic substrates. The cytochrome P450 families 1, 2, and 3 play an important role in drug metabolism in the liver and are expressed early in fetal life.[65] The protein concentrations of cytochrome P450 enzymes remain relatively stable throughout gestation and are at 30% of adult concentrations at birth.[67,68] These findings are not surprising given their role in modulating levels of endogenous substrates involved in fetal homeostasis, growth, and differentiation.[69] Individual cytochrome P450 enzymes demonstrate significant differences in expression and activity in the fetus and neonate compared to adults, resulting in vastly different metabolic profiles[65] between neonates and adults. During the postnatal period, total hepatic cytochrome P450 expression increases, reaching adult levels by 1 year of age.[67] These changes in cytochrome P450 expression during the first year of life result in decreased half-lives and increased clearance of certain drugs.[70,71]

Phase II reactions increase the water solubility of xenobiotics or endogenous compounds for renal or biliary excretion through conjugation reactions. Important conjugation reactions in the infant include glucuronidation, sulfation, acetylation, glutathione conjugation, methylation, and amino acid conjugation.[65] In general, the developmental pattern and degree of fetal and neonatal expression of phase II enzymes is enzyme specific.[72] Neonates have limited enzymatic glucuronidation capacity as demonstrated by their limited ability to conjugate bilirubin during early postnatal life resulting in unconjugated hyperbilirubinemia. These observations are consistent with the finding of low expression levels of hepatic UDPGT enzymes during fetal and early postnatal development. UDPGT expression increases after birth, reaching about 25% of adult levels by 3 months of age.[73] For example, both term and preterm infants demonstrate reduced and variable morphine clearances secondary to immature glucuronidation pathways.[74] Another important group of phase II enzymes are the sulfotransferases that catalyze sulfate conjugation of xenobiotics and endogenous products. Investigation of hepatic sulfotransferase activity in the fetus and neonates revealed high activity of this enzymatic pathway suggesting that it may have an essential role in homeostasis and detoxification in the fetus and neonate.[65]

The hepatic drug metabolism pathways in the neonate are in general immature, resulting in prolonged drug elimination and increased plasma half-lives. However, the extent of compromised drug elimination in the neonate depends on the maturity of the metabolic pathway for a particular substrate and alternative routes of elimination. It should be noted that significant interindividual variability in maturation of these metabolic pathways does exist.

Summary

The liver is undergoing dynamic changes during the neonatal period as it continues to mature. Immediately after birth, it has a vital role in glucose and fatty acid metabolism to ensure a continuous supply of energy as the neonate adapts to enteral feeding. It is responsible for the synthesis of plasma proteins and coagulation factors. In addition, it is responsible for bilirubin metabolism and bile synthesis for the elimination of metabolic wastes and absorption of essential nutrients as well as metabolism and clearance of xenobiotics. The maturation of the liver in term neonates is an expected natural process. However, due to the delay in liver maturation of preterm neonates, they have a significantly lower capacity to respond to the various environmental and physiologic demands. An appreciation of neonatal liver function is essential to the management of neonates undergoing medical and surgical interventions.

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