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5 Metabolism in normal pregnancy

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Introduction

During pregnancy, the mother adapts her metabolism to ensure the continuous supply of nutrients to the fetus in order to sustain its exponential growth. Among those nutrients crossing the placenta, glucose is quantitatively the most important, followed by amino acids. Although lipids cross the placenta in much lower proportion than the other nutrients, maternal lipid metabolism is consistently and intensely affected during pregnancy in order to satisfy maternal and fetal needs. Fetal growth and development also depend on other essential nutrients, like vitamins. The metabolism of certain vitamins is therefore affected during pregnancy to ensure their proper availability to the fetus. The purpose of this chapter is to review the main changes in carbohydrate, amino acids, lipid and vitamin metabolism that take place throughout pregnancy under normal conditions

Carbohydrate metabolism

Glucose is the primary energy source of fetoplacental tissues. During early pregnancy, basal plasma glucose and insulin levels and hepatic gluconeogenesis are unchanged.[1] However, during late pregnancy, the mother develops hypoglycemia, which is specially manifest under fasting conditions, when the rate of gluconeogenesis from different substrates is enhanced.[2,3] The use of different substrates for such increased gluconeogenesis is variable: the conversion of glycerol to glucose rather than other more classical gluconeogenetic substrates like pyruvate or alanine is specially intense.[4] The development of maternal hypoglycemia despite the enhanced gluconeogenesis and the reduced consumption of glucose by maternal tissues, due to her insulin-resistant condition, is the result of the high rate of placental transfer of glucose, which is greater than that of any other substrate (Figure 5.1).[5] This preponderance of placental transfer of glucose over other metabolites has been demonstrated in different species. It is carried out by facilitated diffusion according to concentration-dependent kinetics and thanks to the presence of a high number of glucose transporters, particularly GLUT1.[6] The fetus does not synthesize glucose but uses it as its main oxidative substrate. This causes fetal glycemia to be normally lower than that of its mother, allowing a positive maternal–fetal glucose gradient, which facilitates its placental transfer.

Protein and amino acid metabolism

The accretion of protein is essential for fetal growth and must be sustained by the active transfer of amino acids from maternal circulation. There is no evidence that pregnant women store protein during early pregnancy, when fetal needs are scarce. Therefore, the increased requirements of late pregnancy must be met by metabolic adjustments that enhance both dietary protein utilization and nitrogen retention in

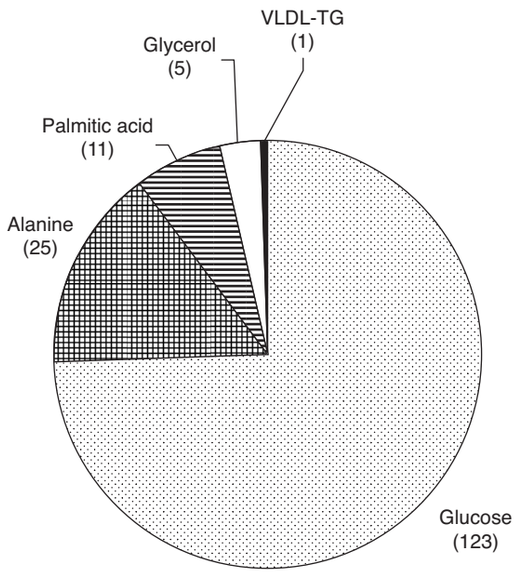

Figure 5.1 *In situ* placental transfer of D-glucose, L-alanine, palmitic acid, glycerol and VLDL-triacylglycerols (VLDL-TG) in 20-day pregnant rats. Placental transfer was measured by the infusion of ^{14}C-labeled substrates through the left uterine artery for 20 min and making the proper correction of data for specific activity dilution and uterine blood flow. Data are expressed as percentual value of all the studied substrates, numbers between breakers indicate the mean absolute value of the transfer for each substrate, expressed as nmol/min/g fetal body weight.

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order to satisfy fetal demands. Protein metabolism changes gradually throughout gestation, so that nitrogen conservation for fetal growth achieves full potential during the last quarter of pregnancy.[7] Nitrogen balance studies showed that the rate of maternal nitrogen retention between 20 and 40 weeks of gestation was greater than the predicted need,[8] leading to the proposal that the mother gains additional protein in her own tissues. The increased nitrogen retention in late pregnancy is due to a reduction in urinary nitrogen excretion as a consequence of decreased urea synthesis.[7] In late pregnancy, nitrogen balance is improved, allowing a more efficient use of dietary proteins.[9]

Although these alterations in protein metabolism during late pregnancy favor nitrogen conservation, pregnancy is associated with hypoaminoacidemia, which is specially evident during fasting, is present at early gestation, and persists throughout pregnancy.[10,11] Since insulin infusion in non-pregnant adults decreases both plasma amino acid levels and protein breakdown, it is proposed that the decrease in plasma amino acid levels found during normal pregnancy is not associated with the pregnancy insulin resistant condition. Thus, maternal hypoaminoacidemia reflects enhanced placental amino acid uptake. Additionally, maternal oxidation of branched-chain amino acids decreases in late pregnancy, increasing their availability for transfer to the fetus.[12]

Contrary to glucose, the concentration of most amino acids in fetal plasma is higher than that found in the mother, because placental transfer of amino acids is carried out by an active process, using selective transporters and metabolic energy.[13] This capacity to concentrate amino acids in the fetal side against the gradient versus maternal levels is clearly seen in the fed and 24 h fasted rat. As shown in Figure 5.2, under fed conditions, maternal plasma total amino acid levels are similar in 20 day pregnant rats and sex- and age-matched virgin animals, whereas the levels in fetal plasma are already higher than in the mother. However, after fasting, the decline of plasma amino acids in the late pregnant rat is greater than that seen in virgin animals, whereas fetal plasma total amino acid concentration remains the same as when fed. Thus, under fasting, the fetal/maternal total amino acid ratio becomes even higher than when fed, showing the efficiency of the placenta in transfering amino acids against the gradient. A multiplicity of factors affects the overall placental amino acid delivery rates, including the activity and location of the amino acid transporter systems, changes in placental surface area, uteroplacental blood flow and maternal concentrations of amino acids,[13] all of which change as gestation advances and are dependent on maternal health conditions.[14]

Lipid metabolism

Accumulation of fat depots in maternal tissues and maternal hyperlipidemia are characteristic features during normal pregnancy. Besides, although lipids cross the placenta with difficulty, essential fatty acids (EFA) and long-chain polyunsaturated fatty acids (LCPUFA) are needed for fetal growth and development and must arrive from maternal circulation. Thus, throughout pregnancy there are major changes in lipid metabolism.

Adipose tissue metabolism

Fat accumulation takes place during the first two-thirds of gestation[15,16] and represents most of the increase in maternal structures that take place during pregnancy.[17] It is the result of both hyperphagia and enhanced lipid synthesis, and is driven by the enhanced adipose tissue insulin responsiveness that occurs during early pregnancy.[18]

Increments of maternal fat depots stop during the third trimester of gestation as a consequence of two changes: (1) a decrease in lipoprotein lipase (LPL) activity,[19] which mainly corresponds to that present in adipose tissue[20] and causes a decline in the hydrolysis and tissue uptake of triacylglycerols

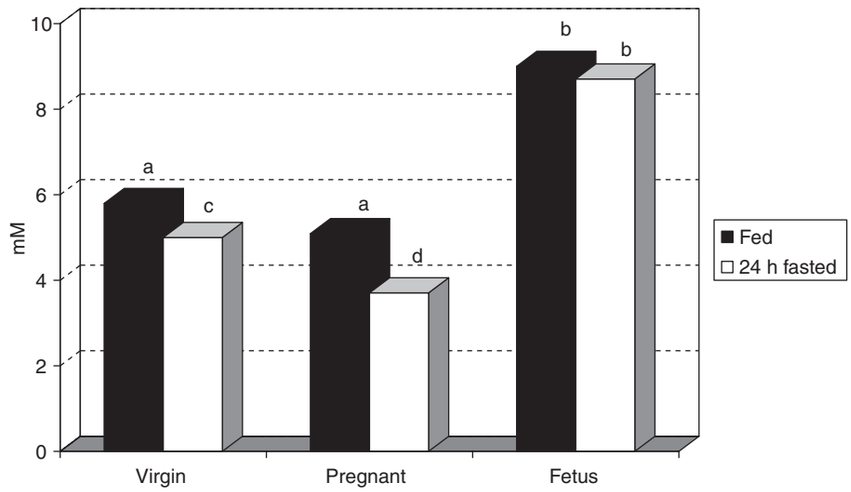

Figure 5.2 Plasma concentration of total amino acids in fed and 24 h fasted virgin and 20-day pregnant rats and their fetuses. Letters above each bar correspond to the statistical comparison between the groups: different letters indicate statistical differences ($P < 0.05$).

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circulating in triacylglycerol-rich lipoproteins (chylomicrons and very low density lipoproteins, VLDL); and (2) an increased adipose tissue lipolytic activity, which is specially manifest under fasting conditions.[21,22]

The placental transfer of the products of adipose tissue lipolysis released into the circulation, non-esterified fatty acids (NEFA) and glycerol, is quantitatively low,[23] and therefore their main destiny is maternal liver. In liver, NEFA are converted into acyl-CoA, and glycerol into glycerol-3-phosphate, which are partially re-esterified for the synthesis of triacylglycerols. These are released back into the circulation in the form of VLDL, as maternal liver production is enhanced. In addition, whereas glycerol is also used as a preferential substrate for gluconeogenesis, NEFA are used for β-oxidation, leading to energy production and ketone body synthesis. These pathways are markedly increased under fasting conditions in late pregnancy.[3,24] Ketone bodies easily cross the placenta.[25] Although not synthesized by the fetus, in fetal circulation, they reach the same concentration as in the mother.[26] Different to what occurs in adults, ketone bodies can be used by the fetus not only as energetic fuels but as substrates for brain lipids.[27,28]

Thus, as shown in Figure 5.3, both the mother and the fetus benefit from the enhanced adipose tissue lipolytic activity during late pregnancy, and very especially during the fasting periods. The preferential conversion of glycerol to glucose allows the preservation of other gluconeogenic substrates like alanine and other amino acids for their transfer to the fetus. The active production of ketone bodies from fatty acids by fasting maternal liver, besides their transfer to the fetus, allows their use by certain maternal tissues such as skeletal muscle as alternative fuels. This production also saves glucose for its use by maternal tissues like the nervous system, which depends on glucose as well as for its placental transfer.

Although pregnancy hormones may contribute to some of these changes, it is thought that the insulin-resistant condition of late pregnancy is the main factor contributing to the increased adipose tissue lipolytic activity and hepatic VLDL production, as well as the increased gluconeogenesis and ketogenesis under fasting conditions.

Hyperlipidemia

Hyperlipidemia normally develops during the last third of gestation and mainly corresponds to increases in triacylglycerols, with smaller rises in phospholipids and cholesterol.[17,19] Besides an increase in VLDL levels as a result of their enhanced liver production and decreased removal from circulation as a consequence of reduced adipose tissue LPL activity, the increase in plasma triacylglycerols corresponds to their proportional enrichment in both LDL and HDL,[19] lipoproteins that are normally poor in triacylglycerols. Such changes in the maternal lipoprotein profile and composition are the result of the simultaneous action of several factors, which are schematically summarized in Figure 5.4: (1) enhanced arrival of the adipose tissue lipolytic products, NEFA and glycerol, to the liver, which facilitates the hepatic synthesis of triacylglycerols and their subsequent release into the circulation as VLDL; (2) decreased removal of VLDL from circulation as a consequence of the reduced adipose tissue LPL activity; (3) increase in cholesteryl ester transfer protein (CETP) activity that takes place at mid-gestation,[29] facilitating the exchange of cholesterol by triacylglycerols from LDL and HDL with VLDL; and (4) intense decrease in hepatic lipase (HL)[19] which decreases the conversion of buoyant HDL_{2b} triacylglycerol-rich particles, into small triacylglycerol-poor and cholesterol-rich particles (HDL_3), allowing the accumulation of the former.[19]

Besides the insulin-resistant condition, which enhances adipose tissue lipolytic activity and decreases its LPL activity,[30] the increase in plasma estrogen concentrations during gestation also contributes to maternal hypertriglyceridemia,

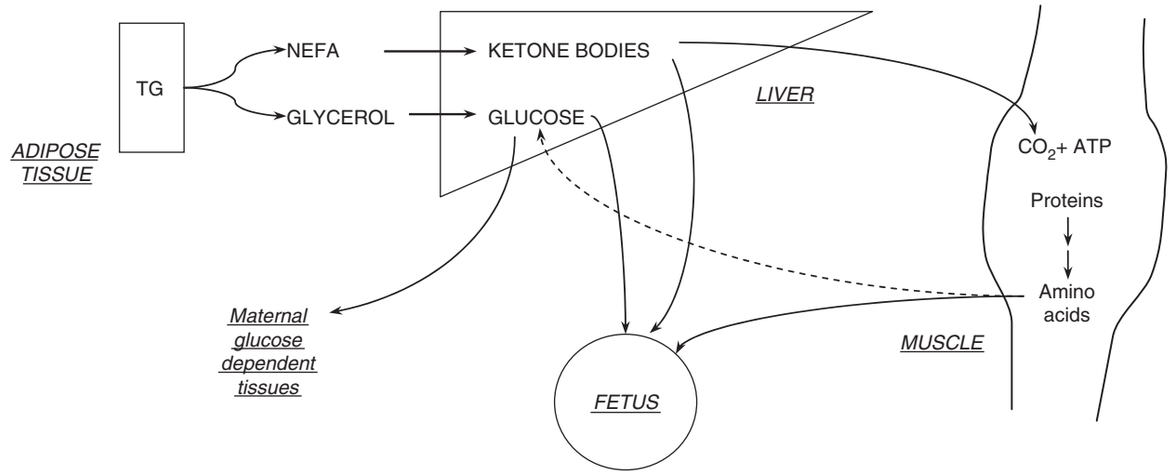

Figure 5.3 Schematic representation of maternal response to starvation during late pregnancy. Enhanced adipose tissue lipolysis increases the availability of glycerol in the liver, where it is used as preferential substrate for gluconeogenesis, and of non-esterified fatty acids (NEFA) for ketogenesis. Throughout this mechanism, the mother, besides producing glucose for the fetus and her own needs, preserves other gluconeogenic substrates, such as amino acids (mainly, alanine), and ensures their availability to the fetus.

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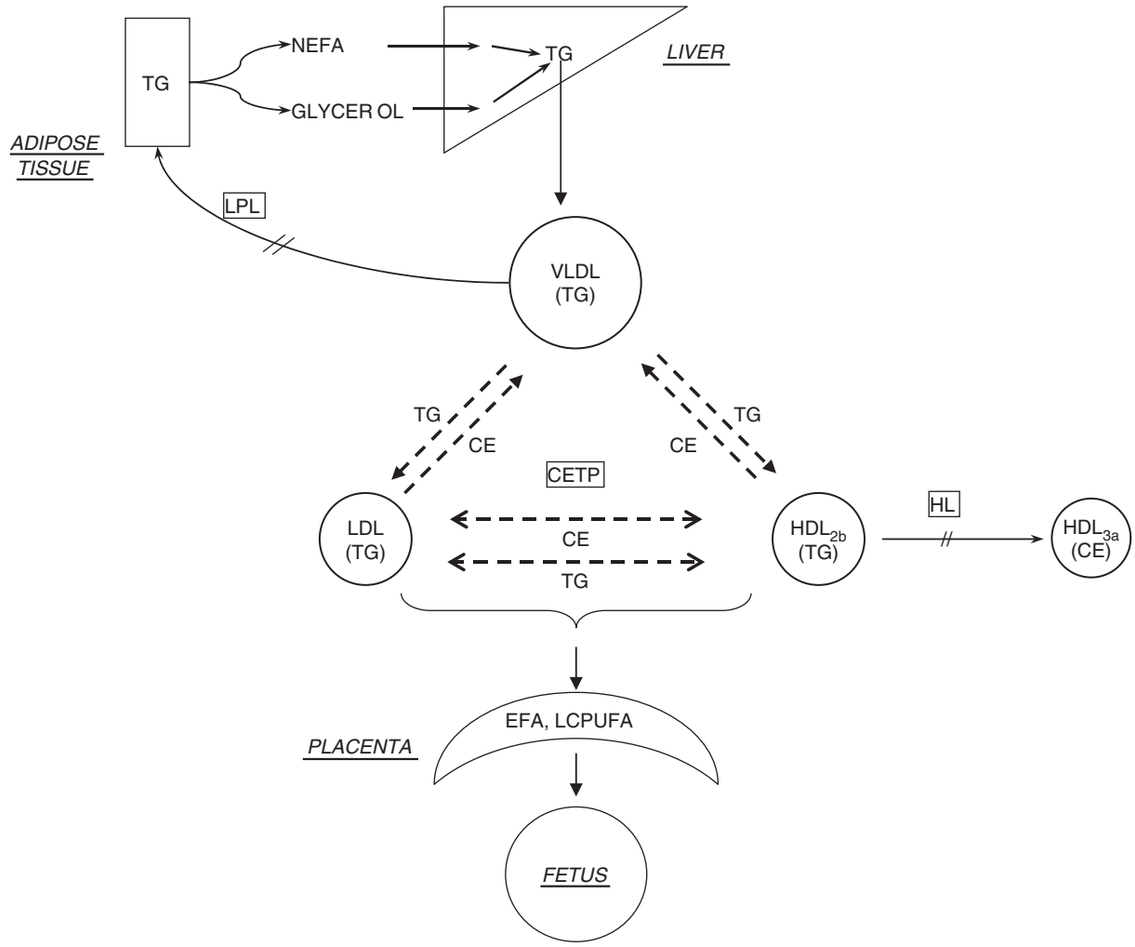

Figure 5.4 Schematic representation of the relationship of adipose tissue lipolytic activity with lipoprotein metabolism during late pregnancy, and its role as a source of essential- (EFA) and long-chain-fatty acids (LCPUFA) for the fetus. CE = cholesterol esters; CETP= cholesterol ester transfer protein; HDL = high density lipoproteins; LDL = low density lipoproteins; VLDL = very low density lipoproteins; LPL = lipoprotein lipase; HL = hepatic lipase.

since it enhances hepatic VLDL production[31] and decreases HL expression and activity.[32]

Benefits of maternal hypertriglyceridemia to the fetus and newborn

Although maternal triacylglycerols do not directly cross the placenta,[23] we think that there are several ways by which the fetus and newborn may benefit from maternal hypertriglyceridemia, as follows.

Use of triacylglycerols as metabolic fuels

Although adult liver does not normally express LPL activity, studies in the rat have shown that under fasting conditions during late pregnancy, there is an increment in liver LPL activity.[33] This liver LPL seems to be the result of the wash-out of LPL from extra-hepatic tissues carried out by the remnants of the triacylglycerol-rich lipoproteins. In this way, under fasting conditions, the maternal liver switches from an exporter organ to an importer of plasma triacylglycerols, which may be used as substrates for ketogenesis. This allows the exaggerated increase in plasma ketone bodies, which, as commented above, save glucose in maternal tissues as well as cross the placental barrier and are directly metabolized by the fetus.

Placental transfer of polyunsaturated fatty acids (PUFA)

Essential fatty acids (EFA) and LCPUFA from either maternal diet or endogenous interconversion are mainly transported in their esterified form in maternal plasma lipoproteins rather than as NEFA.[34] The placenta expresses receptors for all the major plasma lipoproteins. It has different lipolytic activities, including LPL, phospholipase A_2 and an intracellular lipase and it also expresses fatty acid-binding proteins. (for a review see Herrera et al.[35]) Thus, esterified PUFA in maternal plasma lipoproteins are taken up either intact through the placenta receptors or only their constituent fatty acids after hydrolysis. Within the placenta, fatty acids are re-esterified to be latterly hydrolyzed and, in their free form, finally diffused to fetal plasma. This process, together with the direct transfer of NEFA

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and the intrinsic placental fatty acid metabolism, determines the actual rate of the selective placental fatty acid transfer, which is essential for fetal development.

Contribution to milk synthesis in preparation for lactation

Around parturition, there is a rapid increase in mammary gland LPL activity,[36] which, together with the low LPL activity in adipose tissue,[20] drive circulating triacylglycerols to the mammary gland. Through this mechanism there is a rapid disappearance of maternal hypertriglyceridemia,[19] and EFA and LCPUFA from maternal circulation are taken up by mammary gland for milk synthesis to become available to the suckling newborn, contributing to its normal development.

Vitamin metabolism in pregnancy

Adequate maternal micronutrient and vitamin status is especially critical during pregnancy and lactation. Several micronutrient deficiencies (like iron, iodine, zinc) are well established as contributors to abnormal prenatal development and/or pregnancy outcome. But less well-recognized for their importance are deficiencies of vitamins. Evidence is accumulating that maternal antioxidant status is important to prevent abnormal pregnancy outcomes. In lactation, the maternal status of several of these vitamins affects their concentration in breast milk. The main cause of multiple vitamin deficiencies is a poor quality diet, even though gene polymorphism can also impair vitamin absorption or alter their metabolism, and cause vitamin deficiency. In some diets high in unrefined grains and legumes, the amount of nutrients consumed may be adequate, but dietary constituents, such as phytanes and polyphenols, can also limit their absorption.

We summarize here the main changes in the metabolism of the vitamins during pregnancy which have the highest implications in fetal growth and development.

Hydrophilic vitamins

Folic acid

Folates act in different one-carbon transfer reactions, including purine and thymidylate biosynthesis, amino acid metabolism and formate oxidation. Purine and thymidylate biosynthesis is a fundamental requisite event underlying DNA and RNA synthesis. Thus, it is obvious that these folate-dependent reactions are essential for fetal growth and development and for maternal well-being.

Pregnancy is associated with an increased folate demand and, in some cases, leads to overt folate deficiency. The increase in folate requirement during pregnancy is due to the growth of the fetus and uteroplacental organs. Circulating folate concentrations decline in pregnant women who are not supplemented with folic acid.[37] Possible causes for the declines in blood folate include increased folate demand for the fetus, increased folate catabolism, increased folate clearance and excretion, decreased folate absorption, hormonal influence on folate metabolism as a physiological response to pregnancy and low folate intake[38,39] Whatever the reasons for the decline, it is essential that plasma folate be kept above a critical level (>7.0 nmol/L) because plasma maternal folate is the main determinant of transplacental folate delivery to the fetus. There is a strong positive association between maternal plasma, cord plasma and placental folate concentrations, suggesting that transplacental folate delivery depends on maternal plasma folate concentrations. In placental perfusion studies, it has been found that 5-methyltetrahydrofolate (the main form of folate in plasma) is extensively and rapidly bound in the placenta but transferred to the fetus in low amounts at a slow rate.[40] The placental folate receptor (FR) favors the binding of 5-methyltetrahydrofolate and can transfer folate against a concentration gradient; hence, the fetal perfusate is about 3-fold that of the maternal perfusate. The transfer of 5-methyltetrahydrofolate from the maternal to the fetal perfusate is not saturable in a range well above typical physiologic concentrations.[41] The placenta is rich in FR and is one of the tissues that express the α-isoform of FR (FR-α) in abundance. FR-α is a membrane-bound glycosylphosphatidylinositol-linked glycoprotein and the primary form of FR in the epithelial cells. The importance of FR-α in placental folate transfer is inferred from the fact that an FR-α knockout mouse is embryo-lethal.[42] Maternal folate status should be kept adequate to maintain plasma folate above a certain concentration for placental transfer.

Studies conducted in recent years led to recognition that supplementing with folic acid reduced the prevalence of folate deficiency in pregnancy and prevented pregnancy-related disorders. Data from these studies suggest that 200–300 μg folic acid per day is needed in addition to dietary folate to maintain normal folate status and to prevent folate deficiency during this time.[43,44]

Vitamin C

In addition to the prevention of scurvy, vitamin C has numerous other functions and is a co-factor for several enzyme systems. For humans, vitamin C is an essential vitamin, with an important antioxidant function. As antioxidant defense systems are important to protect tissues and cells from damage caused by oxidative stress, an imbalance between increased oxidative stress and decreased antioxidant defenses impairs fetal growth.[45] Thus, pregnant women utilize a defense mechanism, composed of antioxidant enzymes and nutrients including vitamin C, against oxidative stress and free-radical damage. It is believed that ascorbic acid, through conversion to dehydroascorbic acid, crosses the placenta to enter fetal circulation. Once dehydroascorbic acid is present in the fetal circulation, it is reduced back into ascorbic acid and is maintained in high concentrations on the fetal side in the placenta.[46] Maternal serum vitamin C levels during the second trimester of gestation are correlated with birthweight and length in full-term babies.[47]

Lipophilic vitamins

Because lipophilic vitamins are fat soluble, they share several common mechanisms with other lipidic substances concerning

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their metabolism and transfer to the offspring. Although lipophilic vitamins are essential during intrauterine and early postnatal life, little is known about their placental transfer during pregnancy and mammary gland uptake during lactation.

Vitamin D

Vitamin D metabolites have numerous potential physiological and pharmacological actions, but their principal physiological function is maintaining serum calcium and phosphorus concentrations in a range that supports cellular processes, neuromuscular function and bone mineralization. In humans, vitamin D (cholecalciferol or vitamin D_3) can be synthesized endogenously from 7-dehydrocholesterol in the epidermis of the skin after exposure to ultraviolet B radiation, or can come from dietary sources. Vitamin D_3 is then transported to the liver and hydroxylated to the inactive but biologically abundant 25-hydroxyvitamin D (25-OH D), which is the major circulating form of vitamin D. The active metabolite of vitamin D_3 is 1,25-dihydroxyvitamin D (1,25-$(OH)_2$ D), which is formed after further hydroxylation in the kidney. This active metabolite of vitamin D increases the efficiency of intestinal calcium absorption, decreases renal calcium excretion and, in conjunction with the parathyroid hormone (PTH), mobilizes calcium from bone.

Significant changes in maternal vitamin D and calcium metabolism occur during pregnancy to provide the calcium needed for fetal bone mineral accretion. Fetal $1,25(OH)_2D_3$ levels are low, whereas maternal levels are strikingly elevated during pregnancy before rapidly returning to normal after parturition.[48] This increase in maternal $1,25(OH)_2D_3$ levels appears to be caused by increased production rather than decreased clearance, but the precise source of the increased $1,25(OH)_2D_3$ synthesis has yet to be fully defined. There is evidence of 1α-hydroxylase activity in the placenta and deciduas, suggesting that these tissues might also contribute to $1,25(OH)_2D_3$ levels.[49] The placenta is a possible site of $1,25(OH)_2D_3$ production, independent of the maternal and fetal kidneys. The presence of a specific vitamin D receptor in placenta and deciduas has also been well documented, underlining the potential for autocrine or paracrine effects of $1,25(OH)_2D_3$ within these tissues.[50] The precise function of the $1,25(OH)_2D_3$ produced by placenta has yet to be fully defined. $1,25(OH)_2D_3$ passes through the placenta barrier bidirectionally to sustain the active transport of calcium across the placenta during late gestation, although current data suggest that the production of $1,25(OH)_2D_3$ by placenta may be less crucial to the maintenance of maternal and fetal calcium homeostasis than originally thought.[51] The fetus has developed several ways to either induce tolerance or escape from the maternal immune system, and it has been proposed that placental produced $1,25(OH)_2D_3$, acting in concert with other mechanisms, may play a key role in maintaining pregnancy by suppression of the maternal immune system.[52]

Approximately 25–30 g of calcium are transferred to the fetal skeleton by the end of pregnancy, most during the last trimester. It has been estimated that the fetus accumulates up to 250 mg/dL calcium during the third trimester. The three possible calcium sources that may supply the mother with the necessary calcium to support fetal growth include increased intestinal absorption from the diet, increased renal conservation, and increased bone mobilization.[53]

To date, there is no evidence to indicate a beneficial effect of vitamin D intake during pregnancy above amounts routinely required to prevent vitamin D deficiency among non-pregnant women.

Vitamin A

Vitamin A exists in several forms in animal tissues: retinol, retinal, retinoic acid and retinyl esters, mainly as retinyl palmitate. All forms of vitamin A are hydrophobic compounds that are highly unstable in the presence of O_2. A diet deficient in either retinol or in the provitamin A carotenoids that can be metabolized to retinol results in impaired growth, night blindness and ultimately, xerophthalmia and blindness. We now know that there are two metabolites of vitamin A, retinoic acid and retinal, which are responsible for growth and development by regulating gene expression, whereas retinal and its isomers are responsible for the visual function of vitamin A. The potential adverse effect of poor vitamin A status on pregnancy outcomes was demonstrated in an intervention study in a region of Nepal with endemic vitamin A deficiency: supplementation of these women with the recommended daily intake of vitamin A reduced maternal mortality by 40%, and supplementation with β-carotene reduced mortality by 49%. The apparent cause of the reduced mortality risk was a decreased susceptibility to infection.[54] Another advantage of vitamin A supplementation of pregnant women is that it can increase hemoglobin concentrations.[55]

During pregnancy, maternal plasma retinol concentrations fall as gestation advances (Figure 5.5),[56] and this effect reflects the increasing demands of the rapidly growing maternal and fetus tissues. Fetal retinol supply is essential, as retinoids are involved in growth and cellular differentiation of the fetus. Even though retinol is the only form of vitamin A that supports reproduction in full, all-*trans* retinoic acid appears to be the most important form for proper embryonic development. Vitamin A plays an essential role in the development of organs such as the lungs, heart, and skeleton; retinoic acid also enables the setting up of the vascular and nervous system, and is involved as a morphogenic agent during embryonic development.[57,58] Both cytoplasmatic and nuclear classes of retinoid binding proteins (CRBP, CRABP and RAR, RXR) are expressed early in development and are proposed to control the concentration of retinoic acid and the transcription activity of retinoid responsive genes, respectively. RAR regulate many developmental control genes, including homeobox genes and growth factor genes. Multiple fetal anomalies occur in vitamin A-deficient, as well as in RAR-deficient knockout mice, but an excess of vitamin A also induces the same type of abnormality: the importance of the abnormality depends on the period of gestation and the duration of the excessive or deficient supply.

The transfer of vitamin A from mother to fetus is carefully regulated in such a way that it allows vitamin A levels in the fetus to remain unaffected by alterations in maternal vitamin A status, except in conditions of deficiency or excess.[59] The placenta's vitamin A content increases in the last trimester of

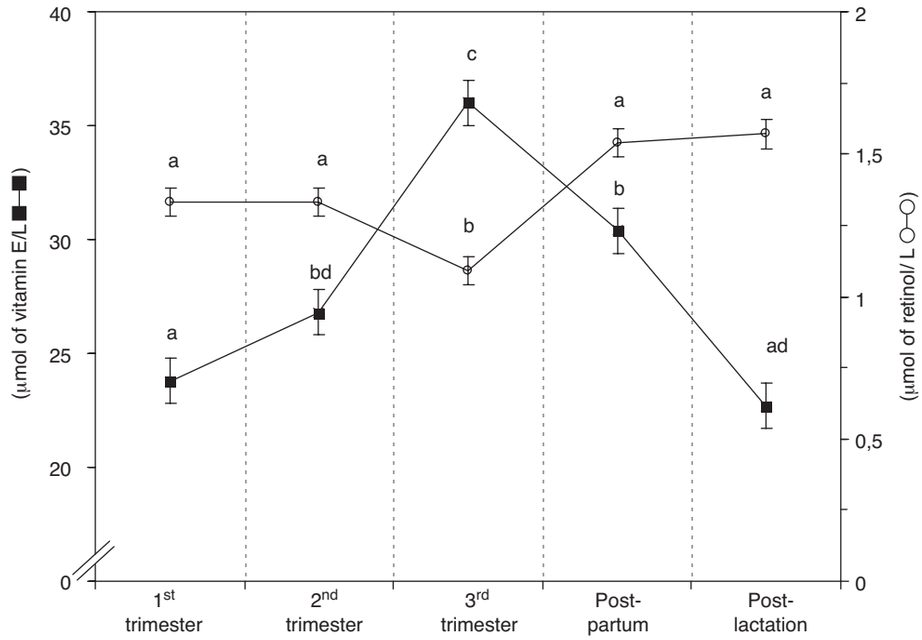

Figure 5.5 Plasma levels of vitamin E (α- and γ-tocopherol) and vitamin A (retinol) at different trimesters of pregnancy, 6–8 days postpartum and at postlactation in healthy women. Data are expressed as means ± SEM. Statistical comparisons are shown by the letters above the points. Different letters for the corresponding vitamin between the groups indicate statistical significance ($P < 0.05$).

pregnancy thanks to the supply of vitamin A from maternal stores (i.e. liver).[60] The amount of retinol provided to the fetus usually remains constant until maternal stores are almost totally depleted. Perfusion studies show that retinol is taken up and concentrated in the placenta, but the exact mechanism of transfer remains unknown. Although the retinol binding protein (RBP) seems to be involved,[61] it might be dispensable for retinol transfer,[62] because homozygous RBP-null mutant mice are viable and fertile. Studies in rats showed that in early gestation, maternal RBP is transported across the placenta and delivers retinol, whereas in late gestation, a different mechanism appears to be operating because fetal liver is capable of synthesizing RBP.[63] *In vivo* studies show that maternal RBP does not cross the placental membrane barrier in the last trimester of gestation and cannot enter fetal circulation.[64] In humans, serum apo-RBP (retinol-free) concentration appears to be elevated during pregnancy, suggesting that pregnancy may alter the affinity of RBP for retinol or induce the binding of the vitamin to other uncharacterized proteins.[65] Other forms of vitamin A, such as retinyl esters and retinoic acid, can also be taken up at the placental barrier.

Although under normal conditions there are no significant correlations between maternal and cord plasma concentrations of retinol or carotenoids, some authors report a weak but statistically significant correlation when the concentration of retinol in cord and maternal plasma are low.[66] Published studies in humans show that maternal subclinical vitamin A deficiency is related to neonatal subclinical vitamin A deficiency and to low birth weight,[66,67] and a high percentage of preterm neonates have marginal values of vitamin A (<0.35μmol/L).

The situation with vitamin A in early lactation is peculiar. Because of the limited transplacental transfer, infant liver stores of vitamin A at birth are small even in well-nourished populations, so newborns are greatly dependent on dietary intake of this vitamin to establish proper tissue stores, maintain rapid growth, and develop their immune system. Colostrum contains higher vitamin A concentration than milk, and has an important role to play in providing initial protection against vitamin A deficiency to the newborn.[68] The timing of colostrum ingestion seems to play a role in the efficiency of intestinal vitamin absorption: thus colostrum feeding on the day of birth is important for the establishment of absorptive mechanism allowing intestinal transport of fat-soluble vitamins. Further, breast milk is a good source of vitamin A and clinical vitamin A deficiency is rare in breast-fed infants during their first year of life.

Debate surrounds the use of retinol supplements during pregnancy. The use of retinol supplements in well-nourished mothers does not affect fetus concentrations. High doses of retinol are teratogenic, and in some countries pregnant women are advised to avoid retinol-containing supplements.[69] However, this advice may lead to vitamin A deficiency.[70] Serum retinol is a relatively insensitive indicator of body vitamin A status: only 1% of the body's reserves circulate in the plasma, and homeostatic mechanisms control concentrations via retinol binding protein concentrations.

Vitamin E

Dietary vitamin E is present as tocopherol, mainly α- and γ-tocopherol, and tocopheryl esters. As for retinyl esters,

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tocopheryl esters are hydrolyzed into tocopherol within the intestinal lumen by pancreatic esterase as well as by intestinal enzymes. The uptake of tocopherol by enterocytes appears to occur by passive diffusion, and the efficiency of the absorption is largely dependent on the quantity and type of fat present in the diet, even though β- and δ-tocopherol are poorly absorbed. Tocopherol is not re-esterified during the absorption process, which does not require any cellular transfer protein, and within the enterocyte, is incorporated to the chylomicrons and transported from the intestine to the lymphatic pathway to reach the bloodstream.

Plasma levels of vitamin E significantly increased from the first trimester of gestation and reached a maximum in the third trimester of gestation.[56,71,72] Different to vitamin A, there is no specific protein carrier protein in the serum to transport vitamin E, which circulates in its alcohol form in serum lipoproteins. Thus, changes in plasma α-tocopherol levels during pregnancy parallel maternal hyperlipidemia (see above), and are also accompanied by the increase of lipid peroxides. However, γ-tocopherol reaches a maximum concentration in maternal plasma at mid-gestation. The reason for this different concentration pattern between α- and γ-tocopherol during pregnancy is unknown, but could be related to differences in their tissue uptake and intracellular metabolism.

α-Tocopherol concentration in the plasma of human fetuses is lower than in their mothers, but rises towards the end of pregnancy. Since α-tocopherol is carried in plasma associated to the different lipoproteins, its uptake and handling by the placenta is similar to that of the other lipoprotein lipophilic components (see above in this chapter). Besides, the placenta expresses α-tocopherol transfer protein (α-TTP) and similar to the role of this protein in liver, it may actively contribute to the specific transfer of α-tocopherol to the fetus.[73,74]

Despite the existence of these processes, efforts to investigate the actual kinetics of the transfer of vitamin E by isolated human placental systems have found that although it is specific for natural RRR-α-tocopherol rather than any other form of vitamin E, its rate is very low, only 10% of passively transferred L-glucose. This justifies the consistent finding of much lower α-tocopherol levels in fetal plasma and red blood cells than in maternal ones, indicating an insufficient vitamin E supply for the fetus throughout gestation.

During lactation, vitamin E intake through milk is the way of supplying the newborn with an essential defense against oxygen toxicity and of stimulating the development of its immune system. A good supply of vitamin E to the offspring is therefore particularly critical in this period. The increase in vitamin E content in body tissues of the offspring following birth is attributed to the ingestion of colostrums and milk, emphasizing the limited placental vitamin E transfer and the importance of milk consumption. Colostrum contains higher vitamin E concentration than milk,[75] which may imply an active uptake by the mammary gland in compensation for the limited placental transport. A decline in maternal circulating vitamin E concentration is noticed at the end of gestation or in early lactation; this decrease may be the consequence of a considerable amount of α-tocopherol present in colostrum. The mechanism of transfer from blood into milk is not completely understood. Perhaps the transfer of vitamin E into milk occurs through a protein-mediated transport: the presence of an α-TTP like mechanism in the mammary gland cannot be excluded, nor can the presence of an SR-BI receptor in the mammary gland, which could be involved in the uptake of α-tocopherol from HDL. Further, the high concentration of vitamin E found in colostrum compared with mature milk might be due to an increase in activity of the mammary LDL receptors and thus, to an important uptake of LDL by the mammary gland around parturition. LPL also seems to influence in modulating the mammary gland uptake of α-tocopherol.[76] Contrary to the placental transfer, which remains low even with increased maternal serum levels, the transfer through colostrum and milk can be increased via higher vitamin E ingestion by the mother.

It is important to note that tocopherol is able to affect the metabolism of vitamin A in several tissues and may play a role in tissue retinol homeostasis. It has been shown to modulate the levels of retinol and total vitamin A in tissues such as the liver, kidney and intestine. *In vitro*, tocopherol exerts an inhibiting or stimulating action (depending on the tissue) on retinyl palmitate hydrolysis.

Summary

Maternal metabolic adaptations during pregnancy are mainly directed to maintaining a continuous availability of substrates to warrant fetal growth. Glucose, used as a primary energy source of fetoplacental tissues, is quantitatively the most important substrate crossing the placenta. During late pregnancy the mother develops hypoglycemia as a result of the high rate of placental transfer, despite of enhanced gluconeogenesis and reduced consumption of glucose. Amino acids cross the placenta against the gradient thanks to an active process. Fetal growth is sustained by the transfer of amino acids from maternal circulation. Protein metabolism changes gradually throughout gestation, and although during late pregnancy there is increased nitrogen retention, the mother develops hypoaminoacidemia, which is specially evident during fasting. Fat depot accumulation and maternal hyperlipidemia are characteristic features of pregnancy. Maternal adipose tissue lipolytic activity is increased and the main destination of released non-esterified fatty acids (NEFA) and glycerol is the liver, where they are used for the synthesis of triacylglycerols. Alternatively, in the case of glycerol, it is also used as a gluconeogenetic substrate and, in the case of NEFA, oxidized for ketogenesis. Major changes also occur in vitamin metabolism. Vitamin A and E are the most affected. Maternal plasma retinol falls as gestation advances, whereas vitamin E levels increase parallel to the increase in plasma lipids. Transplacental transfer of these vitamins is limited, but both the fetus and the newborn need them. They are taken up by mammary gland and their high content in colostrum seems to play an important role in the extrauterine adaptations of the suckling newborn.

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REFERENCES

1. Catalano PM, Tyzbir ED, Wolfe RR, et al. Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. Am J Obstet Gynecol 1992; 167: 913–9.
2. Kalhan S, Rossi K, Gruca L, Burkett E, O'Brien A. Glucose turnover and gluconeogenesis in human pregnancy. J Clin Invest 1997; 100: 1775–81.
3. Herrera E, Knopp RH, Freinkel N. Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis and nitrogen metabolism during gestation in the fed and fasted rat. J Clin Invest 1969; 48: 2260–72.
4. Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. Eur J Clin Nutr 2000; 54(suppl. 1): S47–S51.
5. Herrera E, Amusquivar E. Lipid metabolism in the fetus and the newborn. Diabetes Metab Res Rev 2000; 16: 202–10.
6. Jansson T, Wennergren M, Illsley NP. Glucose transporter protein expression in human placenta throughout gestation and in intrauterine growth retardation. J Clin Endocrinol Metab 1993; 77: 1554–62.
7. King JC. Physiology of pregnancy and nutrient metabolism. Am J Clin Nutr 2001; 71(suppl.): 1218S–25S.
8. Calloway DH. Nitrogen balance during pregnancy. In: Winick M, ed. Nutrition and Fetal Development. Philadelphia: Wiley & Sons; 1974, pp. 79–94.
9. Mojtahedi M, de Groot LCPGM, Boekholt HA, van Raaij JMA. Nitrogen balance of healthy Dutch women before and during pregnancy. Am J Clin Nutr 2002; 75: 1078–83.
10. Metzger BE, Unger RH, Freinkel N. Carbohydrate metabolism in pregnancy.XIV. Relationships between circulating glucagon, insulin, glucose and amino acids in response to a "mixed meal" in late pregnancy. Metabolism 1977; 26: 151–6.
11. Schoengold DM, DeFiore RH, Parlett RC. Free amino acids in plasma throughout pregnancy. Am J Obstet Gynecol 1978; 131: 490–9.
12. Fitch WL, King JC. Plasma amino acid, glucose, and insulin responses to moderate-protein and high-protein test meals in pregnant, nonpregnant, and gestational diabetic women. Am J Clin Nutr 1987; 46: 243–9.
13. Regnault TRH, De Vrijer B, Battaglia FC. Transport and metabolism of amino acids in placenta. Endocrine 2002; 19: 23–41.
14. Herrera E. Metabolic changes in diabetic pregnancy. In: Djelmis J, Desoye G, Ivanisevic M, eds. Diabetology of Pregnancy. Basel: Karger; 2005: 34–45.
15. Villar J, Cogswell M, Kestler E, et al. Effect of fat and fat-free mass deposition during pregnancy on birth weight. Am J Obstet Gynecol 1992; 167: 1344–52.
16. Lopez Luna P, Maier I, Herrera E. Carcass and tissue fat content in the pregnant rat. Biol Neonate 1991; 60: 29–38.
17. Herrera E, Lasunción MA, Gomez Coronado D, et al. Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triacylglycerides in pregnancy. Am J Obstet Gynecol 1988; 158: 1575–83.
18. Ramos MP, Crespo-Solans MD, Del Campo S, Cacho J, Herrera E. Fat accumulation in the rat during early pregnancy is modulated by enhanced insulin responsiveness. Am J Physiol Endocrinol Metab 2003; 285: E318–28.
19. Álvarez JJ, Montelongo A, Iglesias A, Lasunción MA, Herrera E. Longitudinal study of lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. J Lipid Res 1996; 37: 299–308.
20. Martín-Hidalgo A, Holm C, Belfrage P, Scott J, Herrera E. Lipoprotein lipase and hormone sensitive lipase activity and mRNA in rat adipose tissue during pregnancy. Am J Physiol 1994; 266: E930–5.
21. Williams C, Coltart TM. Adipose tissue metabolism in pregnancy: the lipolytic effect of human placental lactogen. Br J Obstet Gynaecol 1978; 85: 43–6.
22. Knopp RH, Herrera E, Freinkel N. Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. J Clin Invest 1970; 49: 1438–46.
23. Herrera E, Lasunción MA. Maternal-fetal transfer of lipid metabolites. In: Polin RA, Fox WW, Abman SH, eds. Fetal and Neonatal Physiology. Philadelphia: Saunders; 2004, pp. 375–88.
24. Zorzano A, Lasunción MA, Herrera E. Role of the availability of substrates on hepatic and renal gluconeogenesis in the fasted late pregnant rat. Metabolism 1986; 35: 297–303.
25. Alonso de la Torre SR, Serrano MA, Medina JM. Carrier-mediated β-D-hydroxybutyrate transport in brush-border membrane vesicles from rat placenta. Pediatr Res 1992; 32: 317–23.
26. Herrera E, Gomez Coronado D, Lasunción MA. Lipid metabolism in pregnancy. Biol Neonate 1987; 51: 70–7.
27. Page MA, Williamson DH. Enzymes of ketone-body utilisation in human brain. Lancet 1971; 2: 66–9.
28. Shambaugh GE, Koehler RR, Yokoo H. Fetal fuels III: ketone utilization by fetal hepatocyte. Am J Physiol 1978; 235: E330–7.
29. Iglesias A, Montelongo A, Herrera E, Lasunción MA. Changes in cholesteryl ester transfer protein activity during normal gestation and postpartum. Clin Biochem 1994; 27: 63–8.
30. Ramos P, Herrera E. Reversion of insulin resistance in the rat during late pregnancy by 72-h glucose infusion. Am J Physiol Endocrinol Metab 1995; 269: E858–63.
31. Knopp RH, Bonet B, Lasunción MA, Montelongo A, Herrera E. Lipoprotein metabolism in pregnancy. In: Herrera E, Knopp RH, eds. Perinatal Biochemistry. Boca Raton: CRC Press; 2006, pp. 19–51.
32. Brinton EA. Oral estrogen replacement therapy in postmenopausal women selectively raises levels and production rates of lipoprotein A-I and lowers hepatic lipase activity without lowering the fractional catabolic rate. Arterioscler Thromb Vasc Biol 1996; 16: 431–40.
33. López-Luna P, Olea J, Herrera E. Effect of starvation on lipoprotein lipase activity in different tissues during gestation in the rat. Biochim Biophys Acta 1994; 1215: 275–9.
34. Herrera E. Lipid metabolism in pregnancy and its consequences in the fetus and newborn. Endocrine 2002; 19: 43–55.
35. Herrera E, Amusquivar E, López-Soldado I, Ortega H. Maternal lipid metabolism and placental lipid transfer. Horm Res 2006; 65 (suppl. 3): 58–63.
36. Ramirez I, Llobera M, Herrera E. Circulating triacylglycerols, lipoproteins, and tissue lipoprotein lipase activities in rat mothers and offspring during the perinatal period: effect of postmaturity. Metabolism 1983; 32: 333–41.
37. Cikot RJ, Steegers-Theunissen RP, Thomas CM, et al. Longitudinal vitamin and homocysteine levels in normal pregnancy. Br J Nutr 2001; 85: 49–58.
38. Caudill MA, Gregory JF, Hutson AD, Bailey LB. Folate catabolism in pregnant and nonpregnant women with controlled folate intakes. J Nutr 1998; 128: 204–8.
39. Higgins JR, Quinlivan EP, McPartlin J, et al. The relationship between increased folate catabolism and the increased requirement for folate in pregnancy. Br J Obstet Gynaecol 2000; 107: 1149–54.
40. Henderson GI, Perez T, Schenker S, Mackins J, Antony AC. Maternal-to-fetal transfer of 5-methyltetrahydrofolate by the perfused human placental cotyledon: evidence for a concentrative role by placental folate receptors in fetal folate delivery. J Lab Clin Med 1995; 126: 184–203.
41. Bisseling TM, Steegers EA, van den Heuvel JJ, et al. Placental folate transport and binding are not impaired in pregnancies complicated by fetal growth restriction. Placenta 2004; 25: 588–93.
42. Piedrahita JA, Oetama B, Bennett GD, et al. Mice lacking the folic acid-binding protein Folbp1 are defective in early embryonic development. Nat Genet 1999; 23: 228–32.
43. Rayburn WF, Stanley JR, Garrett ME. Periconceptional folate intake and neural tube defects. J Am Coll Nutr 1996; 15: 121–5.
44. Swain RA, St Clair L. The role of folic acid in deficiency states and prevention of disease. J Fam Pract 1997; 44: 138–44.
45. Myatt L, Eis AL, Brockman DE, et al. Differential localization of superoxide dismutase isoforms in placental villous tissue of normotensive, pre-eclamptic, and intrauterine growth-restricted pregnancies. J Histochem Cytochem 1997; 45: 1433–8.

46. Guajardo L, Beharry KD, Modanlou HD, Aranda JV. Ascorbic acid concentrations in umbilical cord veins and arteries of preterm and term newborns. Biol Neonate 1995; 68: 1–9.
47. Lee BE, Hong YC, Lee KH, et al. Influence of maternal serum levels of vitamins C and E during the second trimester on birth weight and length. Eur J Clin Nutr 2004; 58: 1365–71.
48. Kovacs CS, Kronenberg HM. Maternal-fetal calcium and bone metabolism during pregnancy, puerperium, and lactation. Endocrine Rev 1997; 18: 832–72.
49. Gray TK, Lowe W, Lester GE. Vitamin D and pregnancy: the maternal-fetal metabolism of vitamin D. Endocr Rev 1981; 2: 264–74.
50. Henry HL, Norman AW. Vitamin D: metabolism and biological actions. Annu Rev Nutr 1984; 4: 493–520.
51. Kovacs CS, Woodland ML, Fudge NJ, Friel JK. The vitamin D receptor is not required for fetal mineral homeostasis or for the regulation of placental calcium transfer in mice. Am J Physiol Endocrinol Metab 2005; 289: E133–44.
52. Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. J Soc Gynecol Invest 2004; 11: 263–71.
53. Ritchie LD, Fung EB, Halloran BP, et al. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 1998; 67: 693–6.
54. West Jr. KP, Katz J, Khatry SK, et al. Double blind, cluster randomised trial of low dose supplementation with vitamin A or beta carotene on mortality related to pregnancy in Nepal. The NNIPS-2 Study Group. BMJ 1999; 318: 570–5.
55. Tanumihardjo SA, Permaesih D, Muhilal ??. Vitamin A status and hemoglobin concentrations are improved in Indonesian children with vitamin A and deworming interventions. Eur J Clin Nutr 2004; 58: 1223–30.
56. Herrera E, Ortega H, Alvino G, et al. Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. Eur J Clin Nutr 2004; 58: 1231–8.
57. Zachman RD. Role of vitamin A in lung development. J Nutr 1995; 125(6, suppl.): 1634S–8S.
58. Rohwedel J, Guan K, Wobus AM. Induction of cellular differentiation by retinoic acid in vitro. Cells Tissues Organs 1999; 165: 190–202.
59. Underwood BA. Maternal vitamin A status and its importance in infancy and early childhood. Am J Clin Nutr 1994; 59(2, suppl.): S517–24.
60. Ismadi SD, Olson JA. Dynamics of the fetal distribution and transfer of Vitamin A between rat fetuses and their mother. Int J Vit Nutr Res 1982; 52: 112–9.
61. Sivaprasadarao A, Boudjelal M, Findlay JB. Solubilization and purification of the retinol-binding protein receptor from human placental membranes. Biochem J 1994; 302(pt 1): 245–51.
62. Dancis J, Levitz M, Katz J, et al. Transfer and metabolism of retinol by the perfused human placenta. Pediatr Res 1992; 32: 195–9.
63. Takahashi YI, Smith JE, Goodman DS. Vitamin A and retinol-binding protein metabolism during fetal development in the rat. Am J Physiol 1977; 233: E263–72.
64. Quadro L, Hamberger L, Gottesman ME, et al. Transplacental delivery of retinoid: the role of retinol-binding protein and lipoprotein retinyl ester. Am J Physiol Endocrinol Metab 2004; 286: E844–51.
65. Sapin V, Alexandre MC, Chaib S, et al. Effect of vitamin A status at the end of term pregnancy on the saturation of retinol binding protein with retinol. Am J Clin Nutr 2000; 71: 537–43.
66. Gazala E, Sarov B, Hershkovitz E, et al. Retinol concentration in maternal and cord serum: its relation to birth weight in healthy mother-infant pairs. Early Hum Dev 2003; 71: 19–28.
67. Ghebremeskel K, Burns L, Burden TJ, et al. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. Early Hum Dev 1994; 39: 177–88.
68. Schweigert FJ, Bathe K, Chen F, Buscher U, Dudenhausen JW. Effect of the stage of lactation in humans on carotenoid levels in milk, blood plasma and plasma lipoprotein fractions. Eur J Nutr 2004; 43: 39–44.
69. Keusch GT. Vitamin A supplements: too good not to be true. N Engl J Med 1990; 323: 985–6.
70. Sanders TA. Vitamin A and pregnancy. Lancet 1990; 336: 1375.
71. González-Corbella MJ, Lopez-Sabater MC, Castellote-Bargallo AI, Campoy-Folgoso C, Rivero-Urgell M. Influence of caesarean delivery and maternal factors on fat-soluble vitamins in blood from cord and neonates. Early Hum Dev 1998; 53: S121–34.
72. Vriese SR, Dhont M, Christophe AB. Oxidative stability of low density lipoproteins and vitamin E levels increase in maternal blood during normal pregnancy. Lipids 2001; 36: 361–6.
73. Jishage K, Arita M, Igarashi K, et al. Alpha-tocopherol transfer protein is important for the normal development of placental labyrinthine trophoblasts in mice. J Biol Chem 2001; 276: 1669–72.
74. de Kaempf-Rotzoll ??, Horiguchi M, Hashiguchi K, et al. Human Placental trophoblast cells express alpha-tocopherol transfer protein. Placenta 2003; 24: 439–44.
75. Barbas C, Herrera E. Lipid composition and vitamin E content in human colostrum and mature milk. J Physiol Biochem 1998; 54: 167–73.
76. Martinez S, Barbas C, Herrera E. Uptake of alpha-tocopherol by the mammary gland but not by white adipose tissue is dependent on lipoprotein lipase activity around parturition and during lactation in the rat. Metabolism 2002; 51: 1444–51.