Fuel selection: the maternal adaptation to fetal nutrient demand

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The necessity to accommodate the developmental and fuel requirements of the fetus confers upon the pregnant mother a considerable nutritional burden. Pregnancy is thus a state in which the maternal response to feeding and fasting is substantially modified to ensure a constant supply of glucose for the developing fetus, while maintaining the glucose and energy requirements of the mother. One of the major adaptations to pregnancy involves a decreased threshold and an increased response of the pancreatic β cell to glucose [1]. This adaptation, through facilitating insulin hyperssecretion during meals [2], effectively compensates for maternal insulin resistance at the level of glucose uptake by skeletal muscle [3–5]. It also permits storage of excess dietary glucose as glycogen in maternal muscle, thereby dampening excursions in glycaemia which might be considered to be detrimental to the fetus. The recent observations that the placenta can secrete leptin [6,7] and leptin levels are raised in pregnant women [6,8] have raised the intriguing possibility that leptin may play an important role in nutritional signalling from the mother to the fetus in pregnancy. During the progression from the fed to the fasted state, metabolic adaptations to pregnancy include post-absorptive hypoglycaemia [9–12] and resistance of lipolysis to the action of insulin in vivo at low concentrations [5]. This results in an altered balance between glucose and lipid-derived fuels which impinges on maternal fuel selection.

Further impetus to gaining an understanding of maternal fuel homeostasis during pregnancy has been provided by the hypothesis that environmental factors operating in fetal life are important in the development of several adult onset diseases, including hypertension, insulin resistance, Type 2 diabetes and Syndrome X (insulin resistance syndrome, the cluster of pathological states including insulin resistance, hypertension and hypertriglyceridaemia) [13–16]. This suggestion is based largely on epidemiological associations between low birthweight and weight at age 1 (implying early growth retardation) and the subsequent development of hypertension [17,18] and Type 2 diabetes [19,20], decreasing birthweight and increased incidence of Syndrome X [21], and impaired fetal development (thinness at birth) and insulin resistance (as assessed by an insulin tolerance test)/Type 2 diabetes [22].

In this review, we discuss the normal homeostatic adaptations to pregnancy within the context of fetal growth, with emphasis on insulin secretion and action and how fetal growth and development may be impaired by inappropriate nutrition. Consideration is given mainly to mechanisms sparing maternal glucose utilization, rather than glucose production.

Glucose utilization and sparing by maternal muscle during pregnancy

As the major site of insulin-stimulated glucose disposal, the skeletal muscle mass collectively plays a key role in the utilization of blood-borne glucose [23,24] and therefore plays a central role in the control of whole-body glucose regulation. Glucose is used as an energy substrate and, during insulin stimulation — when muscle glucose uptake via the insulin-regulatable glucose transporter GLUT4 is increased — as a precursor for stored glycogen [25]. The importance of the muscle mass for the rapid adjustment of glucose metabolism to the fluctuations in glycaemia and insulin concentrations caused by changes in carbohydrate supply is exemplified by the degree of variation in skeletal-muscle glucose utilization and glycogen storage observed during intermittent meal ingestion [26]. The skeletal muscle mass is not homogeneous with respect to glucose requirement and insulin action. In the post-absorptive state, rates of glucose utilization by the fast-twitch skeletal muscles are low [27,28], unless they are exercising [29]. Nevertheless, fast-twitch muscles are able to increase their rates of glucose transport/phosphorylation several fold under hyperinsulinaemic conditions. The predominant fate of glucose in these skeletal muscles during insulin stimulation is storage as...
glycogen [28,30,31]. The slow-twitch oxidative skeletal muscles, which maintain posture, are continually under tension, even in the absence of exercise. These skeletal muscles have a high capacity for fatty acid oxidation and are less reliant on glucose and/or glycogen for energy than the fast-twitch muscles but, under hyperinsulinaemic conditions, still require glucose to contribute to ATP synthesis, as well as to act as a precursor for glycogen synthesis [27,28,30,31]. Because of their energy requirements, the slow-twitch skeletal muscles continue to exhibit relatively high rates of glucose transport/phosphorylation in the post-absorptive states and, as a consequence, the response of slow-twitch muscle to hyperinsulinaemia is usually less marked than that of fast-twitch muscles [27,30]. Nevertheless, euglycaemic–hyperinsulinaemic clamp studies have demonstrated that the more oxidative, slow-twitch muscle shows a higher degree of insulin sensitivity than fast-twitch muscle in terms of stimulation of glucose transport/phosphorylation [28,31].

During late pregnancy, maternal peripheral insulin resistance is thought to contribute to glucose ‘sparing’ for feto-placental use in the fed state [5]. A clear effect of pregnancy to diminish skeletal muscle glucose utilization can be identified, the magnitude of which is greatest in late gestation [32]. Differences in the time-courses and magnitude of the response to pregnancy are observed between individual muscles. In the rat at day 20 of pregnancy (term is approx. 23 days), glucose utilization is suppressed by approx. 80% in fast-twitch muscles and approx. 60% in slow-twitch muscles and, in general, effects of pregnancy are observed earlier and are of greater magnitude in fast-twitch than in slow-twitch muscles [32] i.e. those muscles with lower intrinsic insulin sensitivity [31]. We have suggested that the suppression of glucose utilization by the slow-twitch skeletal muscles — which in the non-pregnant state are more insulin sensitive and have much higher rates of glucose utilization than fast-twitch muscles [31] — is a result of modification of their substrate selection towards the increased use of lipid fuels [9,32].

**Fuel homeostasis in the absorptive phase during pregnancy**

Elevated rates of lipid-fuel oxidation are generally associated with peripheral insulin resistance [33–38], and thus the increased use of lipids in late pregnancy participates in attenuating the response of maternal slow-twitch muscle glucose utilization to small increments in insulin. As a consequence, a greater proportion of available glucose is spared for fetal use. However, when higher insulin levels are achieved (e.g. by insulin infusion under the conditions of the euglycaemic–hyperinsulinaemic clamp), glucose utilization (transport/phosphorylation) by skeletal muscles of late-pregnant rats is increased over and above that observed postprandially [9]. This contrasts with the non-pregnant state, where an acute elevation in insulin to high physiological levels does not increase skeletal-muscle glucose uptake/phosphorylation rate over and above those normally observed during free feeding [9]. It can therefore be concluded that the ‘insulin resistance’ of late pregnancy [3] renders glucose utilization by maternal muscle much more dependent on the extent to which nutrient ingestion enhances insulin secretion (Figure 1). Importantly, within the framework of insulin-dependent (Type 1) diabetes mellitus, the enhanced response of skeletal muscle to insulin at high concentrations has clear implications for the control of glycaemia in pregnancy after insulin injection, implicating inappropriately high insulin-dependent glucose utilization by skeletal muscle as a factor contributing towards the increased frequency and severity of hypoglycaemic episodes found in pregnant, intensively treated Type 1 diabetic patients [39,40].

**Mechanisms underlying the gluco-regulatory response to fasting during pregnancy**

Glucose is required by several maternal tissues (e.g. red blood cells, nervous tissue) and by the fetus during starvation; however, the major fuel reserve is storage triacylglycerol in maternal adipose tissue. Adipose tissue lipolysis provides glycerol, which serves as a substrate for hepatic gluconeogenesis, and non-esterified fatty acids (NEFA). Fatty acids cannot give rise to glucose, but can be used as oxidative substrate by some tissues (e.g. oxidative muscle), thereby reducing their requirement for glucose. The suppression of skeletal-muscle pyruvate oxidation via the pyruvate dehydrogenase complex (PDHC) [41] facilitates the release and/or sparing of lactate (and related 3-C compounds) derived from circulating glucose or muscle glycogen, and also precludes the use of circulating lactate as an oxidative substrate. As a consequence, lactate is
Figure 1
Maternal glucose homeostasis during pregnancy

Enhanced nutrient-stimulated insulin secretion during meals counters maternal muscle insulin resistance, facilitating glucose clearance and storage.

Postabsorptive

MATERNAL MUSCLE
INSULIN RESISTANCE
GLUCOSE
PLACENTA & FETUS

Absorptive

MATERNAL MUSCLE
INSULIN HYPERSECRETION
GLUCOSE
PLACENTA & FETUS

Directed towards hepatic gluconeogenesis and, potentially, towards the fetus.

Although maternal peripheral insulin resistance during late pregnancy facilitates glucose provision to the fetus when the dietary carbohydrate input is limited, it contributes little to glucose homeostasis after food withdrawal because insulin levels are greatly suppressed. To minimize maternal use of glucose and essential amino acids, the metabolic response to starvation is augmented and accelerated [42]. As in the non-pregnant state, the progression from the postprandial to the post-absorptive state in pregnancy is associated with enhanced adipose tissue lipolysis ([43] and reviewed in [42]) and suppression of whole-body glucose utilization [9]. However, when compared with values observed when insulin concentrations are high, both the increase in lipolysis and the decline in peripheral glucose disposal caused by short-term starvation are much more marked in pregnancy [9]. Furthermore, suppression of skeletal-muscle PDHC activity occurs much more rapidly in response to starvation in pregnancy [43,44]. We have recently examined the pattern of change in whole-body glucose turnover observed in response to progressive starvation in relation to changes in glucose utilization in individual maternal muscles [9] in vivo. Our data indicate that in the non-pregnant state decreased glucose uptake/phosphorylation by both fast-twitch and slow-twitch skeletal muscles contributes to glucose sparing after acute starvation, but only oxidative slow-twitch skeletal muscles contribute to the further decline in whole-body glucose uptake caused by more prolonged starvation [9]. We have evidence that the latter is a consequence of a progressive increase in the use of lipid-derived substrates [9,27,41]. In pregnancy, despite enhanced adipose tissue lipolysis and hepatic ketogenesis on extended starvation (from 6 to 24 h), the metabolic adaptation of almost complete suppression of glucose utilization by slow-twitch oxidative muscle, normally elicited by very prolonged (24-48 h) starvation only, is already established after acute (6 h) starvation [9]. As a consequence, the capacity for further glucose sparing as starvation is extended is severely compromised. The significance of enhanced lipolysis during extended starvation in pregnancy must therefore reside in the increased provision of glycerol (for use as gluconeogenic precursor) and fatty acids (for use as ketogenic precursor), ketone bodies being used as energy substrate both by the brain and the fetus.

Impact of inappropriate maternal nutrition on fuel homeostasis during pregnancy

Despite maternal adaptations to starvation in terms of lipid–carbohydrate cross-talk, maternal starvation during late pregnancy nevertheless
impairs fetal growth [45]. This may, in part, reflect the reduced supply of essential amino acids. Consistent with this, fetal growth and the structural and functional development of several specific fetal organs, including the liver and the endocrine pancreas, is impaired if protein is restricted during pregnancy [46–49]. Studies from our laboratory examined the influence of protein restriction on maternal fuel homeostasis during late pregnancy. Our aim was to determine whether a restriction on dietary protein intake during pregnancy, via changes in maternal insulin sensitivity, leads to adverse effects on maternal fuel homeostasis that might be considered to impair the optimum provision of nutrients to the developing fetus and thereby underlie growth retardation in utero. We anticipated that, as was previously observed in unmated rats [50], protein restriction would enhance peripheral insulin sensitivity during pregnancy, thereby increasing maternal glucose disposal and reducing glucose availability to the fetus. In the post-absorptive state, glucose turnover rates (production = disposal) in late-pregnant rats were unaffected by protein restriction [51]. At the same time, the anti-lipolytic action of insulin (assessed by suppression of plasma NEFA concentrations) was attenuated [51]. Our results suggest partial substitution of fat for glucose as the energy substrate in muscles of pregnant rats as a consequence of protein restriction, and imply that protein restriction during pregnancy alters the balance of utilization of the major energy substrates — carbohydrate and fat — by the muscle mass during hyperinsulinaemia via effects exerted on the adipocyte. The impaired suppressibility of adipose-tissue lipolysis by insulin during dietary protein restriction, through increased provision of glycerol and fatty acids, restricts the necessity for marked maternal protein loss to supply protein-derived amino acids for either energy production or gluconeogenesis and, at the same time, ensures that the fetal protein supply is minimally compromised (Figure 2).

In view of the epidemiological associations between small birthweight and the development of insulin resistance in later life, we examined whether the growth retardation resulting from protein malnutrition during intrauterine and early life had any long-term influence on insulin action in adulthood during a subsequent pregnancy [52]. The female offspring of dams maintained on an 8% protein diet during pregnancy

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**Figure 2**

Maternal and fetal responses to protein restriction

Dietary protein restriction enhances lipolysis. Fatty acids are oxidized and glycerol used for gluconeogenesis. The switch in maternal muscle metabolism to favour lipid utilization, together with the enhanced glycerol supply, reduces the requirement for glucose synthesis from amino acids de novo, thereby sparing amino acids for fetal use.
and lactation were transferred to standard (20% protein) diet at weaning, and maintained on this diet until mating and during their own pregnancies, restricting the 'window' of suboptimal protein nutrition to their fetal and neonatal development. They were then studied at day 19 of their own pregnancies. We found that glucose turnover in the post-absorptive state (Ra = Rd) during late-pregnancy was significantly suppressed (by approx. 30%) in pregnant rats that had experienced early growth retardation as a result of early protein restriction [52]. This effect was observed in conjunction with a significant reduction in in vitro rates of glucose utilization in five of eight maternal tissues studied [52]. In addition, insulin action in fast-twitch skeletal muscle was specifically targeted by antecedent suboptimal protein nutrition, with a 30-40% reduction in insulin-stimulated glucose utilization [52]. Although fast-twitch skeletal muscle is of limited quantitative importance to whole-body glucose utilization after short-term starvation [27,30], it assumes greater quantitative importance as a site of glucose disposal on insulin stimulation, particularly when the capacity for storage of glucose as glycogen is increased by prior glycogen depletion (e.g. by prolonged starvation or vigorous exercise) [30,53].

**Does leptin have a role in orchestrating fetal growth to maternal nutrient status in pregnancy?**

The protein product of the Obese (OB) gene, leptin, is a 16 kDa protein known to act as an afferent satiety hormone, regulating appetite and fat deposition [54–60] via a feedback loop involving receptors in the hypothalamus (its main site of action) and choroid plexus [57,61,62]. Thus a loss of body fat leads to a decrease in leptin which, in turn, promotes food intake in excess of energy expenditure to restore body fat mass. In the absence of functional leptin (as seen in ob/ob mice), animals fail to restrain their food intake and become obese [63]. However, at the same time, such animals appear to exist in a state of perceived starvation and exhibit many of the characteristics of early starvation, notably decreased activity, hormonal abnormalities and stunted linear growth [64,65]. Adipose tissue is important in the synthesis and release of leptin [66] and, importantly, changes in adipose-tissue OB mRNA levels parallel changes in food intake [67–72]. Thus, fasting (12–48 h) reduces OB mRNA expression in adipose tissue of lean rodents, whereas feeding rapidly (3–6 h) increases it.

The ob/ob mouse, in addition to suffering from massive obesity and associated metabolic complications (hyperinsulinaemia, hyperglycaemia and diabetes) is infertile [73]. The ob/ob female’s infertility can be corrected by exogenous leptin replacement [74]. Weight loss induced by food restriction does not restore fertility [73]. It is thus implied that leptin has a vital role in reproduction. It has been proposed that the role of leptin may be to signal the attainment of the long-term energy stores critical for reproduction [73–77]. A more critical role for leptin in reproductive competence is suggested by elevated leptin levels in pregnant women [6,8], compared with non-pregnant women matched for age and body mass index (BMI) [6]. Hyperleptinaemia is particularly marked during the second and third trimesters [6], and comparable with that found in obese animals and humans [78–81]. In addition, recent studies employing RT–PCR and in situ hybridization have detected leptin mRNA and protein expression in the (murine) fetus, with particularly high levels of gene expression in cartilage/bone (vertebrae, ribs, scapula, clavicle and long bones) [7].

In the non-pregnant state, leptin concentrations are proportional to BMI [6,55,78], findings consistent with a primary role of adipose tissue in leptin production. Thus, until very recently, leptin was thought to be synthesized and secreted almost exclusively by adipose tissue. However, plasma leptin levels during pregnancy do not correlate with BMI [6]. The recent discoveries of leptin mRNA and protein expression in the placentas of several species, at levels comparable to those found in white adipose tissue [7], suggest that leptin is of fundamental importance to mammalian reproduction and highlight the possibility that the placenta may be a potential source of circulating leptin production. In support of the placenta as a potential site of leptin production during pregnancy, a human choriocarcinoma cell line (BeWo cells) secretes leptin during the process of cellular differentiation from cytotothoblasts to syncytiotrophoblasts [6].

The physiological role of maternal hyperleptinaemia during pregnancy remains enigmatic. The finding of elevated maternal leptin concentrations during pregnancy [6–8], in conjunction with an additional cohort of feto-placental tissues which contain leptin receptors [7], sug-
ggest that high leptin levels are necessary to optimize fetal growth and/or development. The presence of mature leptin protein in several tissues of the fetus contrasts with the absence of leptin in the corresponding adult tissue, and suggests that leptin may be involved in the growth and development of the fetus. An important question is therefore to what extent food restriction or inadequate nutrition during pregnancy may modify leptin levels, and that lowered leptin levels, impair the growth and development of the fetus. In view of the selective effect of protein restriction to enhance lipolysis and retard fetal growth in late pregnancy, it is tempting to suggest that protein restriction during pregnancy may modify leptin levels, and that lowered leptin levels restrict the growth and development of the fetus to a sustainable rate.

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Pre- and Post-Partum Nutrition and Metabolism
Mechanisms of maternofetal exchange across the human placenta


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Understanding of the mechanisms of maternofetal solute and water exchange across the placenta is an absolute prerequisite for understanding the nutrition of the fetus. For most of pregnancy there are two main cellular layers separating the maternal and fetal blood in the placenta, the syncytiotrophoblast and the fetal capillary endothelium [1]. The former is the transporting epithelium of the placenta and, uniquely in the human body, forms a true syncytiotrophoblast. The fetal capillary endothelium has permeability properties typical of continuous capillaries elsewhere, i.e. it has high permeability to small solutes but restricts the diffusion of large molecules such as albumin [2]. Evidence to date suggests that there are four primary mechanisms of solute transfer across the human placenta: flow-limited diffusion, paracellular/transcellular diffusion, transport protein-mediated transfer and endocytosis/exocytosis.

Flow-limited diffusion is the mechanism by which small hydrophobic molecules (e.g. the respiratory gases) move across the placenta. They have high solubility in the plasma membrane so that they are able to utilize the whole surface area of the placenta for diffusion. Therefore their rate of transfer is more dependent on their rate of delivery to and carriage away from the exchange surface (i.e. on the uteroplacental and fetoplacental blood flows) than it is on the permeability of the placenta to them [1].

There is now good evidence that hydrophilic molecules are also able to diffuse across the placenta. Furthermore, such diffusion may contribute a relatively large proportion of the total unidirectional fluxes in each direction (maternofetal and fetomaternal) [3] although its contribution to net flux (the sum of the two unidirectional fluxes) may still be quite small, depending on the prevailing driving force for diffusion, i.e. the electrochemical gradient. The routes of such diffusion remain controversial but probably include transcellular movement across the plasma membrane (especially for small solutes less than 200-300 $M_\text{r}$, although at a much lower rate than for hydrophobic molecules [4] and paracellular movement through extracellular water-filled pores or channels in the syncytiotrophoblast and fetal capillary endothelium. This latter route is considered in more detail below.

The transfer of some hydrophilic molecules will also be catalysed by the presence of specific transporter proteins in the microvillus (maternal-facing) and basal (fetal-facing) plasma membranes of the syncytiotrophoblast. These may operate only in the direction of favourable electrochemical gradients or may utilize ATP (directly or indirectly) to transport molecules against such gradients. Transporter proteins may impose directionality on transfer, because they transport against electrochemical gradients and/or because of asymmetry in their distribution between microvillus and basal plasma membranes [1].

Large molecules such as immunoglobulin G appear to be transferred across the syncytiotrophoblast and, perhaps, the fetal capillary endothelium by endocytosis at the maternal-facing plasma membrane, vesicular diffusion across the cytosol, and exocytosis at the fetal-facing plasma membrane. Although easy to conceptualize, this...