Intrauterine programming of leptin

Jason Elliot Ekert, B.Sc. (Hons)

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Department of Obstetrics and Gynaecology
Adelaide University
South Australia

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ABSTRACT

Many epidemiological studies published over the last ten years have indicated that environment during pregnancy affects adult phenotype and health of offspring. The permanent postnatal effects caused by environmental factors during human pregnancy have been termed *in utero* programming. Babies which are shorter or lighter at birth show catch-up growth during early postnatal life, develop reduced insulin sensitivity and increased risks of obesity. As adults, they also have higher incidence of diabetes and cardiovascular disease and increased concentrations of leptin in their blood. Leptin is a polypeptide produced by adipose tissue and secreted into blood, that acts to suppress appetite and increase energy expenditure. The guinea pig and pig were evaluated as experimental animal models in which to investigate mechanisms of *in utero* leptin programming in humans. Adipocyte development is more advanced at birth in guinea pigs, pigs and humans than in rodents.

The first aim was to determine whether leptin is expressed in adipose tissue of pigs and guinea pigs as is the case in humans. A leptin cDNA fragment was produced from guinea pig adipose RNA and found to have a nucleotide sequence with greater than 80% identity to leptin genes of human, rat, mouse, pig and cow. Leptin mRNA was detected in several adipose sites in the guinea pig and expression was higher in the adult than the fetus. A partial leptin cDNA was also produced from pig adipose tissue RNA and found to have a nucleotide sequence identical to that concurrently published for porcine leptin cDNA. Leptin mRNA was also detected in subcutaneous adipose tissue of pigs. The pig was chosen to investigate leptin programming because an assay for measuring leptin protein in plasma from this species was available whereas an equivalent assay in the guinea pig was not available.

Increased leptin production in humans is associated with obesity and larger adipocytes. Obesity associated with larger adipocytes in adult rats can be programmed by undernutrition during the first two-thirds of pregnancy. A study of long term outcome from the Dutch famine in the winter of 1944-1945 also found that nutritional restriction during the first half of pregnancy was associated with increased adult obesity in offspring. This lead to the concept that the fetus is susceptible to programming of tissues and endocrine systems during certain phases that affects the subsequent adult phenotype. The relationship between birth weight and adult leptin levels could be hypothetically due to altered adipocyte development in growth-retarded fetuses.

The second quarter of gestation is a critical period for adipocyte developent in the pig, a period of adipocyte commitment and development. A change in adipocyte numbers or characteristics during this period could lead to permanent changes in leptin production postnatally. I therefore hypothesised that the leptin axis in offspring is programmed by maternal nutrition during pregnancy and investigated whether leptin production in offspring is altered by maternal nutrition during the second quarter of pregnancy in pigs. I found that body weight at birth and at ~8.5 weeks of age was unaffected by the level of feed during this period of pregnancy. However, leptin mRNA abundance in adipose tissue ($p=0.015$) and plasma leptin concentration ($p=0.01$) were higher in progeny from mothers provided with more feed in the second quarter of pregnancy. Leptin protein concentration in plasma was correlated with leptin mRNA abundance in adipose tissue in these animals.

Growth hormone treatment during pregnancy alters maternal metabolism, especially increasing maternal glucose. This mimics diabetic pregnancy in humans which increases the transfer of glucose to the fetus. The increased glucose delivery to the fetus at a critical stage of adipocyte development might affect adipocyte development or endocrine systems that regulate leptin production. I hypothesised that maternal hyperglycemia would alter leptin programming. To determine whether maternal glucose or other ciculating metabolites are involved in intrauterine leptin programming, pigs were treated with growth hormone in the second quarter of pregnancy and the effects on maternal metabolites and progeny levels of leptin and leptin-regulating hormones were measured. Treatment with GH increased maternal plasma insulin, IGF-I and glucose concentrations. Weight of offspring at birth was not affected. GH treatment ($p<0.005$) during the second quarter of pregnancy increased plasma leptin concentrations in 61 day old progeny. Treatment with GH in pregnancy also increased triiodothyronine ($p=0.002$) and estradiol ($p=0.002$) and decreased IGF-II concentrations ($p=0.009$) in plasma from 61 day progeny.

Programming of postnatal leptin production by maternal environment in pregnancy is likely to be due to an increase in the availability of glucose to the fetus. A direct mechanism of programming leptin expression could be through glucose altering the UDP-*N*-acetylglucosamine pathway in the preadipocytes in the fetus or indirectly through the actions of fetal insulin or insulin-like growth factor-I on fetal adipocyte maturation. Also leptin may be programmed indirectly through the actions of fetal or placental leptin, insulin or insulin-like growth factor-I on fetal hypothalamic maturation.