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Is the fetal origins hypothesis of diabetes supported by animal research?
A systematic review and meta-analysis of the evidence

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ABSTRACT

The fetal programming hypothesis states that fetal undernutrition during pregnancy results in permanent changes in the offspring’s metabolism. A large number of animal studies have evaluated the effect of fetal undernutrition on later susceptibility to type 2 diabetes with varying results.

Aim: We systematically reviewed the existing animal literature examining effects of prenatal undernutrition on glucose and insulin metabolism.

Methods: An electronic search was performed in Medline and Embase to identify all articles that reported studies investigating the effect of fetal undernutrition on plasma insulin, plasma glucose and beta cell mass in animal models. Summary estimates of the effect of undernutrition on mean glucose concentration, insulin level, and beta cell mass were obtained through meta-analysis.

Results: The search resulted in 1827 articles, of which 117 were potentially eligible, based on title and abstract, and 49 met the selection criteria and were included in the review. Prenatal protein restriction increased plasma glucose concentrations (0.42 mmol/l (95% CI 0.07 to 0.77)). Both general undernutrition and protein restriction reduced plasma insulin concentrations (general undernutrition: -0.03 nmol/l (95%CI -0.04 to -0.01), protein restricted: -0.04 nmol/l (95%CI -0.08 to 0.00)) and beta cell mass (general undernutrition: -1.24 mg (95% CI -1.88 to -0.60), protein restriction: -0.99 mg (95% CI -1.67 to -0.31)). In all cases, heterogeneity was significant.

Conclusions: Despite significant heterogeneity, evidence from experiments in different species suggests that prenatal undernutrition – both general or protein restriction – results in increased glucose and reduced insulin concentrations as well as beta cell mass in later life.
INTRODUCTION

In the early 1990s, a cohort study of 64-year-old men in Hertfordshire revealed an inverse association between birth weight and glucose concentrations and insulin resistance\(^1\). Subjects with the lowest birth weights were 6 times more likely to develop type 2 diabetes or impaired glucose tolerance than those with highest birth weights. These findings led to the ‘fetal origins hypothesis’, stating that fetal adaptations to reduced nutrient supply predispose to impaired glucose tolerance and type 2 diabetes in adult life\(^2\). Since, more than 40 studies in populations across the world have investigated the association between size at birth and later risk of type 2 diabetes\(^3\). A systematic review of human studies on birth weight and type 2 diabetes confirmed an inverse relationship between birth weight and type 2 diabetes\(^4\).

Birth weight, however, is only a proxy for poor maternal nutrition during gestation. Animal models allow us to experimentally study the effects of maternal undernutrition during gestation on glucose and insulin metabolism. While the number of animal studies is increasing, many different models are used, ranging from large species as sheep to small rodent models. The intervention studies include a variety of different dietary regimens, varying from undernutrition during only part of gestation, to undernutrition during the entire pre- and early postnatal life. The conclusions of these studies have been diverging, with some offering support for the hypothesis, while others do not. These inconsistencies might be due to the differences in dietary regimens or strains or species of animals used. Therefore we systematically reviewed the literature on fetal undernutrition and glucose and insulin metabolism in animal studies and used meta-analysis to obtain summary estimates of the effects of maternal nutrition during gestation on plasma glucose, insulin and beta cell mass.

METHODS

Search strategy

We performed a search in the electronic databases Medline (1951-January 2011) and Embase (1980-January 2011) to identify all articles that reported on fetal undernutrition and plasma insulin, plasma glucose and beta cell mass as diabetes-related outcomes in experimental animal studies. The search terms ‘undernourished’, ‘(fetal) malnutrition’, ‘famine’, ‘starvation’, ‘caloric restriction’, ‘protein restriction’, ‘low protein diet’, ‘low calorie diet’, ‘pregnancy’, ‘diabetes’, ‘glucose metabolism’, ‘glucose’, ‘insulin metabolism’, ‘insulin’ and ‘beta cell mass’ were used. Only articles written in English were included. After screening of titles and abstracts, two reviewers independently examined full text articles and extracted data on study characteristics, quality and results. Reference lists of reviews and relevant papers were hand searched.
Study selection
We included studies that provided data describing outcomes in experimental animal models of prenatal undernutrition that reported on plasma glucose, plasma insulin or beta cell mass as measures of outcome. Prenatal undernutrition included low protein malnutrition and general caloric malnutrition. Studies had to report outcomes in comparison to control animals that were born to a mother that was normally fed throughout pregnancy. Eligibility was evaluated independently by two readers. Disagreements were resolved in consensus discussions.

Data extraction
Two reviewers independently extracted information on study design, exposure period, animal species and type of undernutrition. To assess methodological quality, data on allocation concealment, randomization, blinding and sample size calculation were extracted. When more than two experimental groups were formed, we focused on the experimental group with malnutrition as early in pregnancy as possible and preferably limited to pregnancy alone. When outcome in offspring was measured at multiple time points, we chose the oldest age at which the measurements were taken. When multiple groups were measured at different ages, both age groups were included. If results were only displayed graphically, outcome was read as precise as possible. Studies that reported results as mean and standard deviation or standard error, and number of animals per group were used for meta-analysis. Data on plasma glucose, plasma insulin and beta cell mass were converted to mmol/l, nmol/l and mg, respectively.

Statistical analysis
Data were analyzed using Review Manager Version 5.0. To examine potential publication bias we constructed funnel plots. We examined the possible heterogeneity in results across studies by calculating the $I^2$ statistic.

Summary estimates of the effects of undernutrition were obtained using a random effects model for meta-analysis, which accounts for both within- and between-study variability. Separate estimates were obtained for model type (protein or general malnutrition) and outcome measure (plasma glucose, plasma insulin and beta cell mass). The summary effects were expressed as mean difference with 95% confidence intervals (CI). When significant statistical heterogeneity was detected, the sources of heterogeneity were explored and subgroup analyses were performed for different species, animal sex, different experimental regiments or in animals of different ages at time of measurement. To evaluate the robustness of our results against influential studies, a leaving-one-out sensitivity analysis was performed.
RESULTS

The search resulted in 1827 articles, of which 117 were considered potentially eligible after screening titles and abstracts (MV and ST). After reading full text articles (MV and either DY, RP or ST), 49 primary studies met the inclusion criteria and were suitable for data extraction (Figure 1). Twenty-six studies reported on protein restricted undernutrition, one using a mouse model, and twenty-five using a rat model. Twenty-four reported on general (caloric) undernutrition, one study using guinea pigs, two on a mouse model, five using a sheep model, and 16 studies on rats.

Figure 1 Literature search results for publications reporting on prenatal undernutrition with regard to glucose and insulin metabolism.

Methodological aspects

Only one study reported blinding of the investigator. Randomization was reported in twenty-four studies, either randomization to the dietary regimen or randomly selecting the pups that were studied from the litters. None of the studies reported a sample size calculation or methods for concealment of allocation. Funnel plots of all six outcomes showed symmetrical scattering of the study results around the summary estimate. There was no evidence of a small study effect or publication bias.
Plasma glucose after prenatal low protein diet

Twenty-two primary animal studies provided data for meta-analysis (464 undernourished animals, 464 controls). Twenty-one studies were performed using rats7-16,18-21,23-29, one using a mouse model4. Using the random effects model we found a higher mean plasma glucose level in prenatally undernourished animals compared to the control group: a mean difference of 0.42 mmol/l (95% CI 0.07 to 0.77) (Figure 2). The results showed statistically significant heterogeneity (I² 89%). The heterogeneity persisted even after separately pooling fasting values, stratifying for the sex of the offspring, or limiting the analysis to Wistar rats only. Offspring of low protein undernourished adults that were older than 6 weeks of age had a 0.54 mmol/l higher plasma glucose level (95% CI 0.16 to 0.92) compared to control offspring. But glucose concentrations measured at day 0 were lower in undernourished offspring compared to controls with a mean difference of -0.62 mmol/l (95% CI -1.34 to 0.11). In both cases, heterogeneity was substantial, with an I² of 89% and 69% respectively.

Figure 2 Forest plot of mean differences and 95% CIs in plasma glucose concentrations (mmol/l) after prenatal low protein undernutrition in all animal studies. Study-specific mean differences were combined by using a random-effects model. SD, standard deviation. UN, undernourished.
Plasma glucose after prenatal general malnutrition

Twenty studies provided data on plasma glucose in offspring after prenatal caloric malnutrition. Twelve studies had been performed in rats\(^7\),\(^8\),\(^13\),\(^38\),\(^41\),\(^43\)-\(^47\),\(^49\),\(^51\), one in mice\(^31\), one in guinea pigs\(^30\), and 5 using a sheep model\(^33\)-\(^37\). In total, 301 undernourished animals were described, compared to 339 controls. The mean plasma glucose level was 0.05 mmol/l higher (95%CI -0.14 to 0.24) in undernourished animals compared to controls (Figure 3). The meta-analysis showed statistically significant heterogeneity (I\(^2\) 84%). Subgroup analysis of rodent models only, stratifying for species, fasting values or sex, did not remove heterogeneity. Undernourished animals measured at day 0 had a significantly lower plasma glucose level, -0.49 (95%CI -0.87 to -0.11) mmol/l (I\(^2\) 78%) as opposed to rodents older than 6 weeks, which had a higher plasma glucose level: 0.25 (95%CI 0.04 to 0.46) mmol/l (I\(^2\) 79%). Meta-analysis of the effects on sheep only (71 undernourished animals, 79 controls) showed no significant difference in glucose concentrations, with a mean difference of 0.03 mmol/l (95%CI -0.31 to 0.26) (I\(^2\) 43%).

Figure 3 Forest plot of mean differences and 95% CIs in plasma glucose concentrations (mmol/l) after prenatal general undernutrition in all animal studies. Study-specific mean differences were combined by using a random-effects model. SD, standard deviation. UN, undernourished.
Plasma insulin after prenatal low protein

Data for meta-analysis were available from nineteen experimental studies. One study used a pig model5, one used a mouse model4, and the remaining 17 studies were performed in a rat model7,8,10,11,13-19,21,24-26,28,29. The meta-analysis, using data from 377 low protein undernourished animals and 382 controls, showed a lower mean plasma insulin level in undernourished offspring compared to control offspring, with a mean difference of 0.04 nmol/l (95%CI -0.08 to 0.00) (I² 95%) (Figure 4). The heterogeneity persisted after separately pooling animals according to species, sex or age or separately analyzing fasting values.

Figure 4 Forest plot of mean differences and 95% CIs in plasma insulin concentrations (nmol/l) after prenatal low protein undernutrition in all animal studies. Study-specific mean differences were combined by using a random-effects model. SD, standard deviation. UN, undernourished.

Plasma insulin after prenatal general malnutrition

In the meta-analysis we could include data from 21 studies, obtained in 330 undernourished animals and 358 controls. Fourteen experiments were conducted in rats7,8,12,13,38,39,43-49,51, 4 in sheep33,35-37, 2 in mice31,32 and one in guinea pigs30. The mean plasma insulin level was 0.03 nmol/l lower (95%CI -0.04 to -0.01) in the undernourished group compared to control animals, I² 86% (Figure 5). The heterogeneity remained after stratification by fasting values, sex, rodent species or age.
Figure 5 Forest plot of mean differences and 95% CIs in plasma insulin concentrations (nmol/l) after prenatal general undernutrition in all animal studies. Study-specific mean differences were combined by using a random-effects model. SD, standard deviation. UN, undernourished.

In rats at day 0, there was no significant effect of prenatal undernourishment on plasma insulin, with a mean difference of 0.23 nmol/l (95%CI -0.67 to 0.21) ($I^2$ 91%). However, adult undernourished rats had a lower plasma insulin level than controls, with a mean difference of 0.04 nmol/l (95%CI -0.07 to -0.01) ($I^2$ 91%). The four sheep studies (66 undernourished animals, 74 controls) did not show any difference in the mean fasting plasma insulin level (0.00 nmol/l; 95%CI -0.01 to 0.01, $I^2$ 4%)\textsuperscript{33,35-37}.

**Beta cell mass after prenatal low protein**

Five rat studies reported beta cell mass of offspring (94 undernourished, 92 control animals)\textsuperscript{6-8,13,22}. The beta cell mass was lower in the undernourished offspring compared to control offspring, with a mean difference of -1.24 mg (95%CI -1.88 to -0.60) (Figure 6). There was statistically significant heterogeneity, $I^2$ 97%.
Beta cell mass after prenatal general malnutrition

The 9 studies on rats (91 undernourished and 91 control animals)\textsuperscript{7,8,13,38,40,42-44,49} showed a reduction in beta cell mass of 0.44 mg (95%CI -0.75 to -0.13) in undernourished animals compared to controls. The results showed statistically significant heterogeneity (I\textsuperscript{2} 94\%) (Figure 7).

Sensitivity analysis

In a series of sensitivity analysis, we evaluated the robustness of our findings by repeating the analyses a number of times, each time leaving one study out of the meta-analysis. If a study appears to be an outlier, with results very different from the rest of the studies, then its influence will become apparent, as the result without the study would be very much different from the...
result of the meta-analysis of all the studies. All sensitivity analyses, for each of the six outcome measures evaluated, confirmed the stability of our analysis. No influential individual study could be identified.

**DISCUSSION**

Although heterogeneity in all meta-analyses was significant, the results suggest that both general and low protein undernutrition during gestation results in increased glucose and reduced insulin concentrations and beta cell mass in the offspring. These findings generally support the fetal origins hypothesis.

The most marked effect of prenatal undernutrition —both general and low protein— was found on beta cell mass. Undernourished offspring had a significant decrease in beta cell mass, the effect was stronger in the low protein group. Prenatal low protein diet also had a significant effect on plasma glucose concentrations, which were higher in undernourished offspring.

The effect of prenatal general malnutrition depended on the time at which glucose metabolism was studied. When data from newborn rodent offspring were pooled separately, these offspring had a significantly lower plasma glucose level, as opposed to adult offspring which had higher glucose concentrations. In the low protein models the same effect of age was seen, although the effect was not significant in newborn offspring. This shows that prenatal undernutrition leads to lower glucose concentrations directly after birth, while after normal postnatal diet, glucose concentrations rise more than in control animals. Biologically, this phenomenon may be similar to the hypoglycaemia that is often observed among infants who are small for gestational age. Higher glucose concentrations at later age are consistent with the findings of glucose intolerance in people prenatally exposed to the Dutch famine. Both low protein and general undernutrition models showed a slight decrease of plasma insulin concentrations, which is consistent with reduced insulin production through decreased beta cell mass.

Meta-analyses of animal studies are known to show significant heterogeneity. In line with this, we found severe statistical heterogeneity in our meta-analyses, and we have to be cautious when interpreting the mean differences. Many different animal models have been used to study the effects of prenatal undernutrition. We find it defendable to pool results of all animal models together, since consistency of the results would indicate that the same effects may apply to different species including humans.

Exploring potential sources of heterogeneity, the subsequent subgroup analyses conducted for animal model, species, age of the animals at investigation and protocol (fasted or not), only accounted for a small part of the heterogeneity. Heterogeneity could also have been caused by the fact that some of the articles that we included were not originally designed to investigate the effect of prenatal undernutrition on plasma glucose and insulin levels or beta cell mass as
primary outcome. This could be an explanation for the great variety in group sizes in the studies we identified.

Methodological heterogeneity was one of the major reasons for the heterogeneity observed. The methodological quality of most reported studies was poor, with only one study reporting blinding of the investigators\textsuperscript{34}, and less than half of the included studies reporting randomization of the animals. None of the studies reported a sample size calculation. In contrast to human studies, randomization, blinding, sample size calculation and planned analysis were not standard. Animal studies that did not report randomization and blinding have been shown to be more likely to report a difference in study groups than studies that did use these methods\textsuperscript{58}. Quality of animal studies could be improved by standardized reporting.

The findings from animal research in this review are in line with evidence from human studies. A prospective cohort study in India showed significantly lower cord blood insulin concentrations in babies born from malnourished mothers, compared to controls. In that study malnourishment was defined as a BMI of less than 17 kg/m\textsuperscript{2} \textsuperscript{59}. In subjects prenatally exposed to the Leningrad siege between 1941 and 1944, there was no difference in concentrations of fasting and 2 hour plasma glucose during an oral glucose tolerance test compared to unexposed subjects. In utero exposed subjects also did not have different plasma insulin concentrations or an excess of known diabetes or glucose intolerance\textsuperscript{60}.

Three studies have reported on the long term effects of prenatal exposure to the Dutch famine of 1944-45\textsuperscript{56,57,61}. Glucose tolerance was decreased in subjects that were prenatally exposed to famine when measured at both age 50 and 58 years\textsuperscript{56,57}. In a subset of participants, an intravenous glucose tolerance test was performed. The results showed impaired glucose tolerance in prenatally exposed subjects, especially those exposed in mid and early gestation. This effect was suggested to be caused by an insulin secretion defect\textsuperscript{61}. Similarly, in adult men and women prenatally exposed to the Chinese famine (1959-1961) there was an increased prevalence of hyperglycemia defined as increased fasting plasma glucose, impaired glucose tolerance or a previous diagnosis of type 2 diabetes\textsuperscript{62}.

In summary, this systematic review shows that the results from animal experiments support the fetal origins hypothesis: prenatal undernutrition leads to a disturbed glucose and insulin metabolism and a decrease in beta cell mass in later life.
REFERENCE LIST


