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Insulin and branched-chain amino acid depletion during mouse preimplantation embryo culture programmes body weight gain and raised blood pressure during early postnatal life

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ABSTRACT

Mouse maternal low protein diet exclusively during preimplantation development (Emb-LPD) is sufficient to programme altered growth and cardiovascular dysfunction in offspring. Here, we use an in vitro model comprising preimplantation culture in medium depleted in insulin and branched-chain amino acids (BCAA), two proposed embryo programming inductive factors from Emb-LPD studies, to examine the consequences for blastocyst organisation and, after embryo transfer (ET), postnatal disease origin. Two-cell embryos were cultured to blastocyst stage in defined KSOM medium supplemented with four combinations of insulin and BCAA concentrations. Control medium contained serum insulin and uterine luminal fluid amino acid concentrations (including BCAA) found in control mothers from the maternal diet model (N-insulin + N-bcaa). Experimental medium (three groups) contained 50% reduction in insulin and/or BCAA (L-insulin + N-bcaa, N-insulin + L-bcaa, and L-insulin + N-bcaa). Lineage-specific cell numbers of resultant blastocysts were not affected by treatment. Following ET, a combined depletion of insulin and BCAA during embryo culture induced a non sex-specific increase in birth weight and weight gain during early postnatal life. Furthermore, male offspring displayed relative hypertension and female offspring reduced heart/body weight, both characteristics of Emb-LPD offspring. Combined depletion of metabolites also resulted in a strong positive correlation between body weight and glucose metabolism that was absent in the control group. Our results support the notion that composition of preimplantation culture medium can programme development and associate with disease origin affecting postnatal growth and cardiovascular phenotypes and implicate two important nutritional mediators in the inductive mechanism. Our data also have implications for human assisted reproductive treatment (ART) practice.

1. Introduction

Undernutrition is a worldwide concern affecting not only countries with developing and emerging economies but also populations in countries with a high human development index [1]. Human epidemiological studies have revealed that undernutrition during the prenatal period can increase the risk of developing non-communicable diseases (NCDs) in adulthood [2,3]. Indeed, experimental research in animal models has provided strong evidence that prenatal undernutrition can program the occurrence of altered phenotypes (e.g. increased blood pressure) in postnatal life [3]. This unfavourable programming is the basis for the developmental origins of health and disease (DOHaD) hypothesis [2]. Such an adverse programming can be induced at several developmental stages during the prenatal period, including the preimplantation phase of embryo development [4].

In a murine model of protein restriction it was shown that dams fed with a low protein diet (9% casein) exclusively during the preimplantation period (days 0–3.5 of embryonic development; Emb-LPD) produced offspring that displayed altered phenotypes during postnatal life, including increased postnatal growth, high blood pressure, vascular dysfunction and hyperactive behavior [5–7]. This animal model of undernutrition has also revealed that compensatory mechanisms exist to maintain viable growth of the developing fetus by altering cellular characteristics of the placental lineages. For instance, compared to the

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control group (18% casein), protein-restricted females (i.e. Emb-LPD) produced blastocysts with a higher number of cells in the trophectoderm (TE) [8], augmented endocytic activity in TE cells [9], and increased spreading capacity during in vitro outgrowth formation [8]. Later in gestation, ectoplacental cones collected at embryonic day 8 (E8.5) from Emb-LPD females and cultured in vitro for 24 h displayed an increased spreading area along with decreased number of secondary trophoblast giant cells [10]. Similarly, in the primitive endoderm lineage and derivative yolk sac placenta, increased endocytic activity is stimulated by maternal protein restriction [5,9]. These changes are associated with an increased fetal:placental weight ratio due to development of larger fetuses with smaller placentas [10]. These phenotypic alterations seem to be induced at the blastocyst stage, around the time of cell lineage determination, since recipients fed with normal levels of protein receiving protein-restricted embryos through embryo transfer produced conceptuses with increased weight [5].

In the Emb-LPD murine model, decreased levels of insulin in blood and branched-chain amino acids (BCAA) in uterine luminal fluid (ULF) were detected at the time of blastocyst formation and coincided with a reduced blastocyst mTORC1 signal mediated through these metabolites [8]. In vitro experiments have revealed that exposure to insulin and amino acids (AA) during the preimplantation period can affect not only early embryo development [11–13] but also fetal growth [14–16]. However, the possible long-term effects of fluctuations of these nutritional mediators during the preimplantation period on postnatal development are currently unknown. This type of research is critical for the elucidation of the mechanisms behind the adverse programming of chronic disease during prenatal undernutrition. Hence, in the present study we test the hypothesis that insulin and/or BCAA depletion during preimplantation embryo development can act as inductive factors of altered phenotypes during postnatal life. Using an in vitro embryo culture (IVEC) and embryo transfer (ET) model we provide evidence that exposure to low levels of insulin and BCAA exclusively during preimplantation embryo development is sufficient to alter body weight gain and blood pressure during early postnatal life in mice.

2. Materials and methods

2.1. Animals

Outbred MF1 mice under UK Home Office Licence were bred in-house (Biomedical Research Facility, University of Southampton) on a 0700–1900 light cycle. Experimental procedures were conducted using protocols approved by, and in accordance with, the UK Home Office Animal (Scientific Procedures) Act 1986 and local ethics committee at the University of Southampton. All males and females used for embryo production or ET were fed with standard chow and water ad libitum at all times (i.e. mating, pregnancy and lactation).

2.2. Embryo collection

Non-superovulated virgin MF1 females (7–8.5 weeks) were mated (1:1) overnight with MF1 males. Presence of copulation plugs was checked the following morning and regarded as a sign of successful mating. Plug-positive females were considered to be on embryonic day 0.5 (E0.5) at midday on the day the vaginal plug was detected. Pregnant females were caged in groups of two to four. Mice were killed by cervical dislocation and oviducts were immediately dissected on E1.5 to collect two-cell embryos. Oviducts were placed in warm (37 °C) saline solution (BR0053G, OXOID, UK) and then transferred to an empty petri dish where they were gently flushed under a stereomicroscope with 0.5 ml of H6 medium supplemented with 4 mg/ml bovine serum albumin (BSA), (A3311, Sigma, UK) [17]. Embryos were then washed with fresh H6-BSA to remove debris.

Table 1
Amino acid composition of control medium (N-insulin + N-bcaa).

Amino acid	Concentration (mM)
Alanine	3.8
Arginine	0.2
Asparagine	0.1
Aspartic ac id	1.8
Cysteine	0.2
Glutamic acid	4.7
Glutamine	1.4
Glycine	2.7
Histidine	0.1
Isoleucine	0.2
Leucine	0.3
Lysine	0.5
Methionine	0.2
Phenylalanine	0.1
Proline	0.1
Serine	1.0
Taurine	14.7
Threonine	0.7
Tryptophan	0.06
Tyrosine	0.2
Valine	0.5

2.3. In vitro embryo culture

Two-cell embryos were randomly allocated to different concentrations of insulin and BCAA in groups of 11–15 in 30 μl drops and cultured up to the blastocyst stage for 66 h. Microdrops were covered with mineral oil (M8410, Sigma, UK) and incubated under 5% CO_2 in air at 37 °C. The basic in vitro culture medium was potassium simplex optimized medium (KSOM) where BSA was omitted and ethylenediaminetetraacetic acid (EDTA) at non-toxic concentrations (0.01 mM) was added to allow a chemically defined milieu for embryo culture [18,19]. The control KSOM medium was supplemented with serum insulin levels and uterine luminal fluid amino acid (AA) concentrations, including BCAA, found in pregnant (E3.5) MF1 mice fed with normal levels of protein (18% casein) [8]. The concentrations of insulin (1 ng/ml) and BCAA (valine = 0.46 mM, isoleucine = 0.21 mM, leucine = 0.32 mM) used in the control medium were termed “normal” (N-insulin + N-bcaa) and represented 100% of the levels found in vivo. The concentrations of amino acids used are shown in Table 1. The control group was compared with three experimental groups where insulin and BCAA were either low (50%) or constant (100%), giving the following combinations: L-insulin + N-bcaa, N-insulin + L-bcaa, and L-insulin + N-bcaa (Fig. 1). Our in vitro model intended to mimic our in vivo model of protein restriction where the Emb-LPD treatment reduced maternal serum insulin by approximately 40% and BCAA concentrations in ULF by approximately 30% at the time of blastocyst formation (E3.5) [8]. To remove H6-BSA medium, embryos were washed three times in their respective culture medium before in vitro culture.

2.4. Differential cell staining in blastocysts

Differential nuclear labelling was carried out in expanded blastocysts (Fig. 1) based on the protocol developed by [20] with some modifications as previously described [21]. Briefly, zona pellucidae were removed with warm (37 °C) acid Tyrode's solution (T1788, Sigma, UK) followed by 15–20 min washing in H6-BSA. Zona-free blastocysts were incubated 10 min in 10% trinitrobenzenesulfonic acid solution (TNBS, P-2297, Sigma, UK) at room temperature. Embryos were then washed three times with H6-PVP, incubated for 10 min in 0.4 mg/ml goat anti-dinitrophenyl (anti-DNP) antibody (D9781, Sigma, UK) in H6-PVP at room temperature, washed again three times with H6-PVP and incubated in 50 μl drops of reconstituted (1:10 dilution with H6-BSA) Low-Tox® guinea pig complement (CL4051, Cedarlane, Canada)