Available online at www.sciencedirect.com
SCIENCE DIRECT®
Growth Hormone & IGF Research 15 (2005) 291–299
www.elsevier.com/locate/ghir

Maternal nutrient restriction during early to mid gestation up-regulates cardiac insulin-like growth factor (IGF) receptors associated with enlarged ventricular size in fetal sheep

Feng Dong [a], Stephen P. Ford [b], Cindy X. Fang [b], Mark J. Nijland [c], Peter W. Nathanielsz [c], Jun Ren [a,*]

[a] *Division of Pharmaceutical Sciences and Center for Cardiovascular Research and Alternative Medicine, University of Wyoming, Laramie, WY 82071-3375, United States*
[b] *Department of Animal Science and the Center for the Study of Fetal Programming, University of Wyoming, Laramie, WY 82071-3375, United States*
[c] *Center for Pregnancy and Newborn Research, University of Texas Health Sciences Center at San Antonio, San Antonio, TX 78299, United States*

Received 1 May 2005; revised 26 May 2005; accepted 28 May 2005
Available online 19 July 2005

Abstract

Intrauterine undernutrition is associated with a high incidence of cardiovascular diseases in adulthood. We previously showed that maternal nutrient restriction during early to mid gestation produces ventricular enlargement, although the mechanism is unknown. We examined myocardial expression of insulin-like growth factor I (IGF-1), IGF-2, IGF binding protein 3 (IGFBP-3), IGF-receptor 1 (IGF-1R) and IGF-2R in fetal sheep with maternal undernutrition. Multiparous ewes were fed with 50% (nutrient-restricted, NR) or 100% (control-fed, C) of NRC requirements from day 28 to 78 of gestation. Some of NR and C ewes were euthanized on day 78, and the rest were fed 100% NRC requirements from day 79 to 135 of gestation. At necropsy on day 78 or day 135 of gestation, gravid uteri were recovered. mRNA expression of IGF-1 and IGF-2 in ventricles were measured with RT-PCR, and protein expression of IGF-1R, IGF-2R, IGFBP-3 was quantitated with Western blot. Crown-rump length was reduced and left ventricle was enlarged in NR fetuses on day 78. At day 135 after re-alimentation, ventricular weights were similar between the two groups although ventricular wall thicknesses were greater in NR than C fetuses. No difference was found in IGF-1, IGF-2 or IGFBP-3 levels between the NR and C groups at either gestational age. Protein expression of IGF-1R and IGF-2R in the left ventricle and IGF-1R in the right ventricle was significantly elevated in the NR group on day 78 of gestation. Only IGF-1R expression remained elevated after late gestational re-alimentation in association with increases in ventricular wall thickness. Our study suggest that maternal undernutrition from early to mid gestation may change the expression of IGF-1R and IGF-2R in fetal myocardium, and play a role in cardiac ventricular enlargement in fetal sheep.

Keywords: Fetal; Nutrition; Gestation; IGF; IGF receptor

1. Introduction

Changes in maternal nutritional status during pregnancy often results in permanent structural and functional deficits in fetal as well as postnatal growth, which predisposes the fetus to detrimental pathological consequences in both intrauterine and extrauterine life [1–4]. Gestational undernutrition especially during the first to second trimesters of pregnancy may directly enhance the propensity of cardiovascular, metabolic and endocrine diseases in later postnatal life [5–7]. It was demonstrated that unsupplemented ewes on rangeland lose a significant amount of weight during early

* Corresponding author. Fax: +1 307 766 2953.
E-mail address: jren@uwyo.edu (J. Ren).

1096-6374/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ghir.2005.05.003

to mid gestation. Even after nutrient supplementation later in gestation, the overall health condition of their offspring is still compromised [7–10]. This gestational undernutrition-triggered pre- or postnatal defect is consistent with the critical role of the first half of gestation for fetal development [6,11]. Despite these clinical and agricultural observations, the precise mechanism responsible for abnormal fetal growth and physiological function as a result of maternal undernourishment remains poorly elucidated.

Several hormones and growth factors have been indicated to participate in the nutrition deficiency-related fetal and possibly postnatal physiological dysfunction. Insulin-like growth factors (IGF), namely IGF-1 and IGF-2, which possess metabolic, mitogenic and differentiative actions, play an important role in fetoplacental growth throughout the gestational period. Maternal undernutrition during periconceptual and gestational periods has been shown to cause significant falls in fetal insulin, IGF and IGF binding protein-3 (IGFBP-3) levels in conjunction with enhanced IGFBP-2 in sheep [4,12]. Such alterations of IGF cascade are speculated to play a significant role in intrauterine undernutrition-associated compromised fetal growth and fetal programming [4,12]. Nevertheless, conflicting results have indicated that birth weight rather than maternal nutrition may serve as the key determining factor for IGF-1 levels in sheep [13]. Both IGF-1 and IGF-2 genes (*Igf-1* and *Igf-2*) are expressed in fetal tissues from the earliest stage of pre-implantation development to the final phase of tissue maturation just prior to birth, with *Igf-2* gene expression being more abundant than expression of the *Igf-1* gene [14]. IGF-1 and IGF-2 have been shown to determine both normal and abnormal growth and development of many organ systems including heart and vasculature [15–19]. Mutation of the IGF-1 receptor or deletion of the *Igf-1* gene retards intrauterine and postnatal growth while overexpression of the *Igf-2* gene stimulates fetal overgrowth [20]. IGFs are believed to determine not only the growth of individual fetal tissues but also uptake/utilization of nutrients by fetal/placental tissues. Expression of IGF genes is developmentally regulated in a tissue-specific manner and may be altered by nutritional and endocrine conditions in utero [17,21]. In general, the *Igf-1* gene is more responsive to these stimuli than the *Igf-2* gene. In addition, the effects of IGFs on fetoplacental growth can be amplified or attenuated by the IGFBPs, which are themselves regulated by nutritional and endocrine signals [22]. The *Igf-2* gene appears to provide the constitutive drive for intrauterine growth via its placental and paracrine actions on fetal tissue while the *Igf-1* gene regulates fetal growth and development in relation to the nutrient supply [22]. It is possible that maternal undernutrition may impact the fetal IGF system and therefore affect organogenesis and fetal development during critical stages of gestation, the period of rapid cell division [6]. Although several reports have been seen with regards to gestational nutrient restriction in the ewe [23–25], no special attention has been given to ventricular development and cardiac IGF cascade. Therefore, the aim of this study was to analyze the impact of an early and prolonged nutrient restriction on fetal cardiac growth, development and expression of IGF-1, IGF-2, IGFBP-3 and receptors for IGFs.

2. Materials and methods

2.1. Experimental animals

All animal procedures were approved by the Animal Care and Use Committee at the University of Wyoming (Laramie, WY). On day 20 of pregnancy, 30 multiparous ewes (mixed breeding) were weighed to allow individual diets being calculated on a metabolic body weight basis (weight$^{0.75}$). The diet consisted of a pelleted beet pulp (79.7% total digestible nutrients, TDN, 93.5% dry matter, DM and 10.0% crude protein). Rations were delivered on a DM basis to meet the total TDN required for maintenance for an early pregnant ewe (NRC requirements) [26]. A mineral–vitamin mixture [51.43% sodium triphosphate, 47.62% potassium chloride, 0.39% zinc oxide, 0.06% cobalt acetate and 0.50% ADE vitamin premix (8,000,000 IU vitamin A, 800,000 IU vitamin D_3 and 400,000 IU vitamin E per pound); amount of vitamin premix was formulated to meet the vitamin A requirements] was included with the beet pulp pellets to meet nutritional requirements. On day 21 of gestation, all ewes were placed in individual pens and fed with control rations. On day 28, ewes were randomly assigned to a control-fed (C) group [100% NRC requirements [26] which included 100% mineral–vitamin mixture] or a nutrient-restricted (NR) group (fed 50% NRC requirements which included 50% mineral–vitamin mixture). Beginning on day 28 of gestation, and continuing at seven day intervals, ewes were weighed and rations adjusted for weight gain (i.e., increased the amount of feed) or loss (i.e., decreased the amount of feed). On day 45 of gestation, the numbers of fetuses carried by each ewe was determined by ultrasonography (Ausonics Microimager 1000 sector scanning instrument; Ausonics Pty Ltd., Sydney Australia). Randomly selected C and NR ewes were euthanized on day 78, and the remaining C and NR ewes were fed the control diet from day 79 to day 135, and then euthanized (gestation length ~150 days). At sacrifice, each ewe was weighed and was given an overdose of sodium pentabarbitol (Abbott Laboratories, Abbott Park, IL) and exanguinated, and the gravid uterus quickly removed and weighed.