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RESEARCH ARTICLE

Effects of Maternal Under-nutrition on the Coronary Arterioles in Sheep Fetuses

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ABSTRACT

The maternal nutritional status during pregnancy and even before conception may lead to cardiovascular risk in luminal diameters. In present study, effects of maternal under-nutrition on the wall thickness, wall thickness to luminal diameter ratio and related parameters of coronary arterioles of sheep fetuses were investigated. One group was raised on high diet and the other on low maintenance diet. The tissue samples were taken from ventricular myocardium, fixed in neutral buffered formaldehyde solution and processed by using paraffin tissue preparation techniques. Round arterioles were selected from different microscopic fields of the prepared tissue sections. The coronary arterioles were measured with image analysis software, Image J®. Live weight of well-nourished sheep (68.7±3.67 kg) and birth weight of lambs (6.55±0.43 kg) was significantly increased as compared to undernourished sheep (56.8±3.04 kg) and lambs (5.85±0.65 kg), respectively. Intima media thickness (IMT) of coronary arterioles was significantly higher in undernourished sheep fetuses (11.51±0.54 µm) than well-nourished sheep fetuses (10.21±0.64 µm). IMT to luminal diameter ratio was also significantly higher in undernourished group fetuses (0.59±0.08 µm) than well-nourished group fetuses (0.35±0.12 µm). However, perimeter of coronary arterioles was significantly reduced in fetuses of undernourished sheep (124.22±7.38 µm) than fetuses (153.10±7.38 µm) obtained from well-nourished sheep. The results revealed that maternal under-nutrition increases the IMT and decreases the diameter and perimeter of coronary arterioles in sheep fetuses.

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INTRODUCTION

Cardiovascular diseases (CVDs) are one of the major causes of mankind mortalities. The nutritional and different environmental factors during pregnancy and even prior to conception contribute to cardiovascular risks and impaired tissue growth (Torrens *et al.*, 2009; Tao and Dahl, 2013). Among the various CVDs, coronary artery disease (CAD) is responsible for atherosclerosis. Studies upon the carotid and aortic arteries have shown that thickness of tunica intima and tunica media/intima media thickness (IMT) can help predicting the risk of CVDs (Koklu *et al.*, 2007; Painter *et al.*, 2007). Epidemiological studies have also shown links between impaired maternal nutrition and compromised fetal growth to increase the incidence of cardiovascular diseases in adulthood and late stages of life (Cormac, 2012) supporting the DoHAD (Developmental origins of health and disease) concept regarding the fetal origin of various diseases.

The coronary arteries are thick muscular arteries, responsible to supply heart. Walls of these arteries, like the elastic arteries are formed by three main layers: tunica intima/interna, tunica media and tunica externa. In human coronary arteries (during adult life), the combined thickness of intima and media ranges from 0.21-1.20 mm. The tunica intima comprises a thickness range of 0.10-0.89 mm (Kume *et al*., 2005), while individual thickness of tunica media has not been reported. Values of coronary IMT have not been reported in many other species. The arterioles have a tunica intima, like arteries, with an endothelium, a thin subendothelial layer (absent in smallest arterioles) and an internal elastic membrane (disappears in the smallest arterioles). Contrary to arteries, arterioles contain only 1-3 layers of smooth muscle cells, while external elastic membrane may be absent (Eurell and Frappier, 2006).

Adequate maternal nutrition is critical for the proper fetal growth and development (Blackmore *et al.*, 2012;

Lloyd *et al.,* 2012; Sami *et al*., 2013). Epidemiological studies have shown links between impaired maternal nutrition and compromised fetal growth with increased incidence of cardiovascular diseases in adulthood (Gruber *et al.,* 2012). In rats, maternal under-nutrition in pregnancy has been linked with high blood pressure in the resistance arteries of the offspring and the thermogenic capacity (Torrens *et al*., 2006; Lanham *et al.,* 2012; Lie *et al.,* 2013). It has also been found that maternal under-nutrition influences IMT of the aortic and carotid arterial walls (Painter, 2006; Skilton *et al*., 2006; Herrera *et al.,* 2012). Moreover, IMT of arterial wall has been found crucial in determining the susceptibility to CVDs. However, no report is available in accessible literature about the effects of maternal under-nutrition on coronary IMT. Also that small resistance artery structure has been found as an independent predictor of cardiovascular disease (Mathiassen *et al*., 2007). Therefore, in the present study, effect of maternal under-nutrition on coronary arteriolar IMT, perimeter, vascular diameter, luminal diameter and IMT; luminal diameter ratio was determined.

MATERIALS AND METHODS

The present study was conducted to determine the effects of maternal under-nutrition of offspring of sheep. For this purpose estrus synchronization of randomly selected ewes (n= 20) was carried out from a commercial flock of 2,900 Romney ewes. The ewes were bred using semen from one of four Suffolk rams of similar size. The 20 small ewes having single pregnancy (transabdominal ultrasonographic examination) at 50 days post-insemination were separated. From day 21 post-insemination, the ewes were randomly kept in two different groups. In one group the ewes were maintained on high (H) nutritional regimens and the other on low nutritional regimens. The sheep kept in high (H) nutritional regimens were offered clover (high energy value) and the ryegrass (low nutritive value) was offered to low nutritional regimens until 140 days of pregnancy. The clover being higher in nutrient contents was preferred than the ryegrass (Van Dorland *et al.,* 2007). The ewes of high nutritional regimens were also provided 14 million Joules metabolizeable energy and 10% proteins per head per day (MJ ME/head/day). However, the sheep of poor nutritional regimens got only 11 MJ ME/head/day (Wagg, 2008). At day 140 of pregnancy, these 20 singleton-bearing ewes were weighed and then euthanized to collect the fetuses. The born lambs were also weighed and their hearts were collected for further investigation (Firth *et al*., 2008).

Prior to sampling nitroglycerine (vasodilator) was injected at a physiological pressure into the coronary arteries to keep them in a maximal state of dilation. The hearts were collected from the fetuses of two groups and weighed. The tissues were collected from ventricular myocardium and fixed in neutral buffered formaldehyde within half an hour after killing the fetus. The fixed tissues were processed by using the paraffin tissue preparation techniques. Sections were cut and stained with hematoxylin and eosin stain (Qureshi *et al.,* 2013). For histological analysis 100 round arterioles (50 from each group, five per fetus) were selected randomly from different microscopic fields of the each tissue and their images were captured with Ziess Axiophot photo-microscope. Arterioles were differentiated from small coronary arteries by the number of smooth muscle layers in tunica media (1-3 layers of smooth muscle cells in arterioles) (Eurell and Frappier, 2006). To reduce the error margin only circular sections were selected. The measurements of coronary arterioles were made with image analysis software, Image J, version 1.41 (Rasband, 2008). The luminal perimeter and vascular perimeter was directly measured with Image J using automatic edge detection. For statistical analysis the mean values of the all the parameters of each animal were subjected to statistical analysis. Live ewe weights, fetal body weights and absolute heart weights of the two groups were compared by student T-test.

RESULTS

The birth weights of the fetuses and live weights of sheep kept on higher nutritional regimens (Group H) were significantly increased than the ewes which were maintained on low nutritional regimens (Group L) indicating the clear impact of under-nutrition. Absolute heart weights of fetuses obtained from group H were higher than those of group L. However, the difference was statistically non-significant. The results showed significant statistical difference for luminal diameter of arterioles between the two groups (Table 1). In group H arterioles of the fetuses had more than 38% larger luminal diameter than the fetuses of group L. Also the arterioles in group H fetuses were more than 38% larger in luminal perimeter than group L.

The coronary arterioles in group H had significantly larger vascular perimeter than that of group L. The results revealed that vascular perimeter of arterioles in group H was about 18% larger than those of group L. The mean vascular diameter arterioles of group H was also significantly larger (<18%) than arterioles of group L (Table 1).

There was a significant difference between the IMT (Fig. 1 and 2) of two groups (Table 1). Mean value of IMT of group L was 11% higher than of group H. The IMT: luminal diameter ratio also significantly different between the two groups, was in the fetuses of group L than those of group H (Table 1).

DISCUSSION

The live weight of ewes and birth weight of fetuses were significantly higher in well-nourished animals when compared to the fetuses of undernourished animals. However, heart weight of fetuses of well-nourished and undernourished sheep was not significantly different from each other. The decreased weight of ewes and birth weight of fetuses in present experimental study could be due to effect of maternal under-nutrition on fetal growth. Similar findings have already been reported in different animals (Blackmore *et al.,* 2012; Liu *et al.,* 2013). The lower body weight and organ weight due to poor nutrition has also been reported (Gruber *et al.,* 2012). The results showed that the IMT of coronary arterioles was significantly higher in under-nourished fetuses (11.51±0.54 μm) than

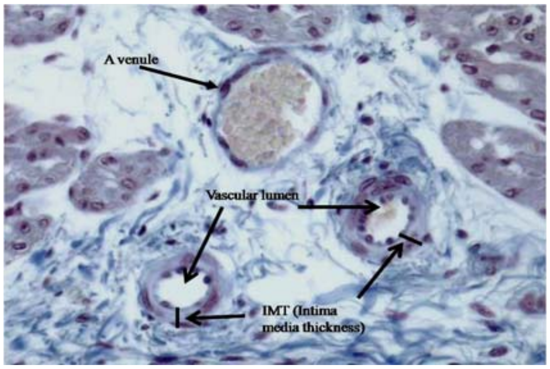

Fig. 1: Light micrograph showing IMT, luminal diameter of arterioles and a venule. 200X; H & E

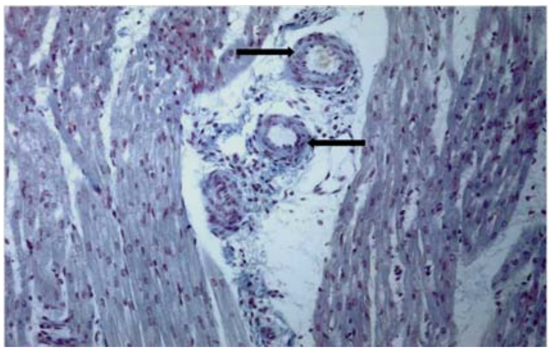

Fig. 2: A light micrograph of myocardium showing arterioles. Stain H&E, 200X

Table 1: The heart weight, birth weight of lambs born and live weight of singleton bearing small ewes (after parturition) with two different nutritional statuses

Parameter	Units	Fetuses from		P value
		High nutritional regimen	Low nutritional regimen	
Absolute heart weight	g	50.8±5.8	43.6±6.6	0.053
Birth weight of lambs	kg	6.55±0.43	5.85±0.65	0.007
Live weight of sheep	kg	68.7±3.67	56.8±3.04	0.001
Luminal Diameter	µm	26.96±1.92	16.54±1.92	0.002
Intima Media Thickness (IMT)	µm	10.21±0.64	11.51±0.54	0.045
IMT: luminal diameter ratio		0.35±0.12	0.59±0.08	0.001
Luminal perimeter	µm	84.65±6.04	51.94±6.04	0.002
Vascular Perimeter	µm	153.10±7.38	124.22±7.38	0.001
Vascular Diameter	µm	48.76±2.35	39.56±2.35	0.001

well-nourished fetuses (10.21±0.64 µm) in the present study. Previously it has been reported that sustained hypertension results an increased wall to lumen ratio (IMT) in small resistance arteries. Studies on various effects such as atherosclerotic lesions in the aortic root, dyslipidaemia and cardiac dysfunction in fetuses due to maternal under-nutrition have been reported (Blackmore *et al.*, 2012; Lloyd *et al.*, 2012). The increased IMT in present study could also be due to hyperplastic growth and re-modelling, an adaptive response to increased blood pressure (Heagerty *et al*., 1993). Previously due to poor caloric values decreased length of capillaries, volume and subcellular compartments of cardiomyocytes and interstitium have been reported (Gruber *et al.,* 2012). However, the results of present study regarding increased IMT are contrary to the findings of previous study (Painter *et al*., 2007) about carotid and femoral IMT in humans. They reported that femoral and carotid IMT was thinner in 58 years old people which were exposed to famine during gestation (during the Second World War) than the unexposed ones. Thus maternal under nutrition during pregnancy causes a decrease in carotid and femoral IMT, but an increased prevalence of CAD around 58 years of age (Painter *et al*., 2007). But, no reports could be found in accessible literature about how the maternal under-nutrition affects coronary arterial and arteriolar IMT during pregnancy in developing fetuses of different species. However, in rats due to hypoxic pregnancy increase in wall thickness and wall-to-lumen area ratio of the fetal aorta has been reported (Herrera *et al.*, 2012). Effect of maternal under-nutrition and intrauterine growth restriction has also been reported on aortic IMT. Aortic IMT was found higher in new born animals with intrauterine growth restriction (Skilton *et al*., 2005, Koklu *et al*., 2006). But Skilton *et al*. (2006) has also reported that maternal under-nutrition (low protein) reduces aortic IMT in rat pups. Apparently, in human babies, broad-spectrum intrauterine under-nutrition due to placental insufficiency might be a cause of increased aortic wall thickness. However, the maternal dietary protein restriction may be responsible for reduced aortic IMT in the rat model (Skilton *et al*., 2006).

In the present study, the luminal perimeter in the coronary arterioles of under-nourished group fetuses was significantly lower than well-nourished group. This indicates that under-nutrition of ewes, from day 21-140 has decreased the luminal perimeter of coronary arterioles in the fetuses. No literature was available to compare these findings.

The luminal diameter of coronary arterioles of H group fetuses (26.96±1.92 µm) was significantly higher than those of L group (18.54±1.92 µm). Similar variation has been reported between the hypertensive and normotensive rats for luminal diameters of different arteries. Luminal diameter has been reported in the mesenteric (178-222 µm), cerebral (87 µm) and femoral (167 µm) resistance arteries of hypertensive rats. In the normotensive rats luminal diameters of mesenteric, cerebral and femoral arteries have been recorded as 194-265, 102 and 199 µm, respectively (Bund *et al.*, 1991; Deng and Schiffrin, 1992; Thybo *et al.*, 1992). This suggests that decreased luminal diameter in coronary arteries could be due to hypertension. No report was available to compare the nutritional effect on the diameter of coronary arteriole.

Conclusion: This study supports the fact that maternal nutritional status is critical for fetal development in general and cardiovascular development in particular. Maternal under-nutrition not only decreases the birth and heart weight by compromising the fetal growth but it decreases the luminal diameter and increases the blood wall thickness/ IMT of coronary arterioles too.

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