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Maternal nutritional restriction during gestation impacts differently on offspring muscular and elastic arteries and is associated with increased carotid resistance and ventricular afterload in maturity

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Abstract

Background: Intrauterine undernutrition could impact offspring left ventricle (LV) afterload and arterial function. The changes observed in adulthood could differ depending on the arterial type, pathway and properties studied. **Aim:** To analyze whether undernutrition during early and mid-gestation is associated with changes in cardiovascular properties in adulthood. **Methods:** Pregnant ewes were assigned to one of the two treatment groups: (1) standard nutritional offer (high pasture-allowance, HPA; $n = 16$) or (2) nutritional restriction (50–75% of control intake) from before conception until day 122 of gestation (≈85% term) (low pasture allowance, LPA; $n = 17$). When offspring reached adult life, cardiovascular parameters were assessed in conscious animals (applanation tonometry, vascular echography). **Measurements**: Peripheral and aortic pressure, carotid and femoral arteries diameters, intima-media thickness and stiffness, blood flow, local and regional resistances and LV afterload were measured. Blood samples were collected. Parameters were compared before and after adjustment for nutritional characteristics at birth and at the time of the cardiovascular evaluation. **Results:** Doppler-derived cerebral vascular resistances, mean pressure/flow ratio (carotid resistance) and afterload indexes were higher in descendants from LPA than in descendants from HPA ewes ($p < 0.05$). Descendants from LPA had lower femoral diameters ($p < 0.05$). Cardiovascular changes associated with nutritional restriction during pregnancy did not depend on the offsprings' nutritional conditions at birth and/or in adult life. **Conclusion:** Pregnant ewes that experienced undernutrition gave birth to female offspring that exhibited increased carotid pathway resistances (cerebral microcirculatory resistances) and LV afterload when they reached the age of 2.5 years. There were differences in the impact of nutritional deficiency on elastic and muscular arteries.

Introduction

A deficient intake of energy and nutrients during pregnancy that may be associated with intrauterine growth restriction (IUGR) and low birth weight (LBW)[1,2] is an important health problem in underdeveloped countries. Studies in humans and animal models have demonstrated that IUGR, LBW and/or accelerated postnatal weight gain (frequently, but not exclusively associated with IUGR and LBW) could result in an increased prevalence of cardiovascular risk factors and augmented cardiovascular risk.[1–8] On the other hand, if nutritional deficiency is limited to an early stage of pregnancy, as frequently occurs in humans due to the intervention of social support programs, it could not be associated with alterations in birth weight or in post-natal growth.[7,9–11] Data on the cardiovascular impact of nutritional deficiency during pregnancy without IUGR, LBW or accelerated weight gain are scarce and many issues await to be assessed.

Available information regarding the impact of nutritional restriction on the cardiovascular system of offspring shows limitations and controversies. These could be partially explained by methodological factors. First, most studies have used *in-vitro* techniques to analyze vascular tissues collected from foetuses, new-borns or young adults (i.e., rat or sheep isolated vessels).[3–5,12–14] However, the functional impact of undernutrition on the cardiovascular system can only be accurately assessed by evaluating the system under real hemodynamic conditions. Second, general studies have focused on a single property, in a specific histological (i.e., muscular)[14] or functional (i.e., resistance, conductance) arterial type, but undernutrition could have dissimilar effects on different arteries and arterial properties.[4,5,15] Therefore, results obtained in a certain artery should not be extrapolated to other arteries or arterial pathways. Similarly, alterations in the arterial system may not necessarily affect left ventricle (LV) function (and vice

versa).[16] Third, available data were mostly obtained from foetal or neonatal tissues, and then, the potential impact of undernutrition in adulthood was theoretically analyzed.[14] However, some consequences of intrauterine undernutrition could be observed only in adult life.[17] Finally, while male offspring have shown to be highly susceptible to intrauterine nutritional restriction, the impact of such restriction on female offspring is not completely accepted. Although in some studies nutritional restriction affected the cardiovascular system of female offspring, it did not in others (i.e., females did not develop hypertension, or if they did, it was not as frequent as in males).[18] Further studies are necessary to determine whether the cardiovascular system of female offspring is affected by nutritional restriction during pregnancy. In this context, the studies should consider (1) evaluations in live conscious animals (cardiovascular system working under physiological conditions), (2) the assessment of LV functional capability together with structural and functional properties of different arterial types (i.e., elastic and muscular; conductance and resistance vessels) and (3) the analyses of adult individuals.

We hypothesized that intrauterine nutritional restriction results in impairment of female offspring arterial structure and function observed in adult life. The impact would differ depending on the arterial type, pathways and vascular properties studied, and it would be associated with changes in LV load. In this context, the main objective of this study was to analyze whether nutritional restriction during early and mid-gestation is associated with changes in structural and functional cardiovascular properties in adult female offspring.

Methods

Pregnant ewes (mothers) and female descendants: high and low pasture allowance

This study was performed at the Experimental Station Bernardo-Rosengurtt (32°S, 54°W) from March 2013 to April 2016 (autumn). Thirty-three multiparous Corriedale ewes weighing 46.9 ± 0.9 kg (mean ± standard error of mean [SEM]) and with a body condition score of 2.8 ± 0.1 (1:emaciated to 5:obese)[19] were included and studied in a completely randomized-block design. Ewes grazed on 32 hectares of natural pastures, in three blocks divided into two plots (units) by electric fences. Each treatment was repeated in three plots. Ewes grazed continuously and had free-access to water. From the same cohort of sheep (mothers and descendants), data related to physiological changes and behavioral issues (i.e., ewe–lamb bonding and behaviors at lambing and at weaning) and their association with pasture allowance during pregnancy were previously published.[20,21]

From 23 days before insemination until 122 days of gestation, ewes (mothers, "M" prefix) were randomly assigned to one of the two nutritional management methods: (1) normal feeding or high native pasture allowance (M-HPA) or (2) nutritional restriction or low pasture allowance (M-LPA).[20,21] Hence, the "control" group (M-HPA) was constituted by ewes who had normal availability of nutrients during pregnancy, and the under-nourished group of mothers (M-LPA) by ewes exposed to a nutritional restriction (~50–75% of the usual amount of forage offered) during approximately the first 2/3 of pregnancy. Animals from the M-HPA group had access to 10–12 kg/dry matter/100 kg of body weight per day and those from the M-LPA group had access to 5–8 kg dry matter/100 kg of body weight per day. Forage availability was estimated monthly using the double-sampling method[22] and forage allowances were adjusted using "put-and-take" ewes.[20,21] Ewes were shorn on day 122 of gestation, and supplemented with 200 g of rice bran plus 50 ml of crude glycerine (77% of glycerol)/animal/day from the week before shearing until lambing. After shearing, ewes were placed in a paddock with *Festuca arundinacea* prairie, where they grazed *ad libitum*. Therefore, before and after the nutritional intervention, M-HPA and M-LPA had access to the same pastures. Body weight and condition score were estimated monthly from 23 days before conception until lambing. Gestational age and birth weight were measured and the birth weight/gestational age ratio was quantified.

All the ewes and their lambs were maintained as a single group for 90 days when lambs were weaned. Then, all female lambs continued grazing together in the same pastures. Sixteen ewes (11 born as single lambs and 5 twins) born from M-HPA (descendants, "D" prefix, or offspring from HPA mothers, "D-HPA" group) and 17 ewes (11 born as singles and 6 twins) born from M-LPA (descendants or offspring from LPA mothers, "D-LPA" group) underwent cardiovascular evaluation when they were 2.5 years old. Body weight, length and condition score were measured at the time of cardiovascular evaluation. The day before cardiovascular evaluation, blood samples were obtained and total cholesterol, glucose, protein and albumin concentrations were determined using an automated chemistry analyzer (WienerLabBT-3000 Plus/CB-350i, Argentina) (Table 1).

Cardiovascular evaluation of D-HPA and D-LPA ewes

Studies were conducted while the animal was resting quietly in a sling, lying down in dorsal decubitus, being conscious, without anaesthetic administration.[23] To avoid isolation (an important stressor in sheep), a companion ewe was continuously present during the evaluation. Evaluation started once the animal was quiet, and hemodynamic variables (i.e., blood pressure [BP], heart rate) were stable. During the cardiovascular studies, researchers did not know the group of the studied ewes belonged to (blinded evaluation).

Evaluation included: (1) peripheral BP (pBP); (2) central (aortic) BP (cBP), aortic wave-derived parameters and LV afterload; (3) common carotid and femoral arteries (CCA and CFA) beat-to-beat diameter waveforms, intima-media thickness and local stiffness; (4) CCA and CFA blood flow velocity levels, patterns and velocity-derived indexes and (5) CCA and CFA characteristic (local) impedance, carotid and femoral pathways (regional) peripheral resistances (Fig. 1a–d). A detailed explanation of the studies and parameters is shown in the Supplementary Methodology.

Peripheral blood pressure

Non-invasive oscillometric pBP measurements (HEM-4030; Omron-Healthcare, USA) were obtained using a cuff placed around the metatarsus of the upper pelvic limb or above the carpus on the upper thoracic limb.[24] Systolic (pSBP) and diastolic (pDBP) pressures were recorded. Then, peripheral pulse (pPP) and mean BP (MBP) pressures were calculated: $pPP = pSBP - pDBP$ and $MBP = pDBP + (pPP/3)$.

Central (aortic) pressure, wave-derived parameters and ventricle afterload

Aortic cBP and wave-derived parameters were assessed (SphygmoCor-CvMS-v.9, AtCor-Medical, Australia) from carotid or central (reference method) and peripheral (femoral) applanation tonometry (CAT and PAT, respectively) (Fig. 1a). First, right

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Table 1. Characteristics of descendant ewes groups at the time of the cardiovascular study: comparison of high (D-HPA) and low (D-LPA) pasture allowance groups

	All ($n = 33$)		D-HPA ($n = 16$)		D-LPA ($n = 17$)		p Value (D-HPA vs. D-LPA)	
	MV	SE	MV	SE	MV	SE	2-tailed	1-tailed
Age (days)	988	1	989	1	987	1	0.103	0.051
Birth weight (kg)	4.09	0.18	4.08	0.23	4.10	0.27	0.976	0.488
Gestational age (day)	148	0	147	0	148	1	0.233	0.116
Ratio "BW/GA" (10^{-2} kg/day)	2.77	0.12	2.78	0.16	2.75	0.17	0.909	0.454
Body weight (kg)	45.32	0.74	44.53	1.11	46.06	0.99	0.314	0.157
Body condition scoring	3.48	0.06	3.47	0.10	3.50	0.07	0.799	0.400
Body length (cm)	125.22	1.16	125.60	1.83	124.88	1.52	0.765	0.383
Heart rate (beats/minute)	77	3	75	3	80	4	0.378	0.189
Peripheral SBP (mmHg)	139	4	134	6	144	5	0.243	0.122
Peripheral DBP (mmHg)	103	3	99	5	108	4	0.189	0.095
Peripheral PP (mmHg)	36	4	35	5	36	7	0.873	0.437
Glucose (mg/dL)	56.2	1.6	55.1	1.6	57.3	1.6	0.080	**0.040**
Total cholesterol (mg/dL)	77.1	1.9	74.1	1.9	80.1	1.9	0.090	**0.045**
Total protein (g/dL)	7.0	0.1	6.9	0.1	7.1	0.1	0.320	0.160
Albumin (g/dL)	3.3	0.1	3.3	0.1	3.3	0.1	0.650	0.325

Values expressed as mean value (MV) and standard error of mean (SE). D-HPA and D-LPA: descendants (offspring) from mother exposed to High and Low Pasture Allowance during pregnancy, respectively. BW: Birth weight. GA: Gestational age. SBP, DBP, PP: systolic, diastolic and pulse blood pressure, respectively. Body condition scoring (BCS): score 1 = emaciated to 5 = obese. A p value < 0.05 was considered significant.

CCA BP waveforms were recorded using CAT and calibrated to pDBP and MBP. Then, central systolic, diastolic, end-systolic and pulse pressure (cSBP, cDBP, cESP and cPP) values were obtained from CCA waveforms.[16,25] Central aortic augmented pressure (cAP) and augmentation index (cAIx = cAP/cPP) were quantified (Fig. 1b).[16] LV afterload complementary indexes were calculated: ejection duration, diastolic duration (ms), relative ejection duration (RED = ejection duration/pulse period), ejection duration*cSBP product, RED*cSBP product and subendocardial viability ratio (Fig. 1c).[16] Subendocardial viability ratio, an indicator of myocardial perfusion/workload relationship was quantified as the ratio between aortic systolic and diastolic tension-time indexes (cSTTI and cDTTI) (Fig. 1c). cDTTI is the area below diastolic aortic BP curve and cSTTI is the area beneath the systolic aortic BP curve. Right CFA BP waveforms were recorded using PAT.[16,25] Parameters similar to those obtained from CAT-derived aortic waves were obtained from PAT-derived aortic waves.

Carotid and femoral diameters, intima-media thickness and local stiffness

B-mode ultrasound (7–13 MHz linear-transducer; M-Turbo/SonoSite, USA) was used to obtain sequences of images from longitudinal CCA and CFA views. Beat-to-beat diameter waveforms were obtained using border detection algorithm.[25,26] Systolic, mean and end-diastolic diameters and arterial intima-media thickness (computed on the posterior wall at end-diastole) were quantified (Hemodyn-4M/Dinap, Argentina). Pulsate diameter was calculated as systolic diameter minus diastolic diameter. CCA and CFA local stiffness were evaluated using complimentary BP-dependent and independent parameters: pressure–strain elastic modulus, stiffness index, incremental elastic modulus and local pulse wave velocity.[16,27]

Carotid and femoral blood velocity, flow patterns and blood velocity-derived indexes

Peak systolic, mean, end-diastolic and minimum diastolic velocity levels were computed from CCA and CFA blood flood velocity waveforms (Doppler, 7–13 MHz, M-Turbo-SonoSite, USA) (Fig. 1d). The amplitude and time to early diastolic reversal peak and to the secondary forward diastolic peak were computed in the CFA. Peak systolic, mean and end-diastolic blood flows were determined from blood flow velocities and cross-sectional areas. Blood flow velocity waves were analyzed considering widely used Doppler-derived indexes: resistive Index, pulsatility index and systo-diastolic velocity ratio.[25,26,28]

Local characteristic impedance and regional peripheral vascular resistances

From pressure and flow signals,[27,29] characteristic impedance was quantified as the ratio between BP (dP/dt; mmHg/s) and blood flow (dF/dt; ml/s) changes observed early in the systolic phase (early ejection). Additionally, carotid and femoral pathways (regional) resistances were quantified as the ratio between mean BP and blood flow.

Data and statistical analysis

After confirming the normal distribution of the variables with the Kolmogorov–Smirnov test, the statistical analysis was divided into three steps. First, concordance between cBP values and aortic wave-derived parameters, obtained with CAT (reference method)

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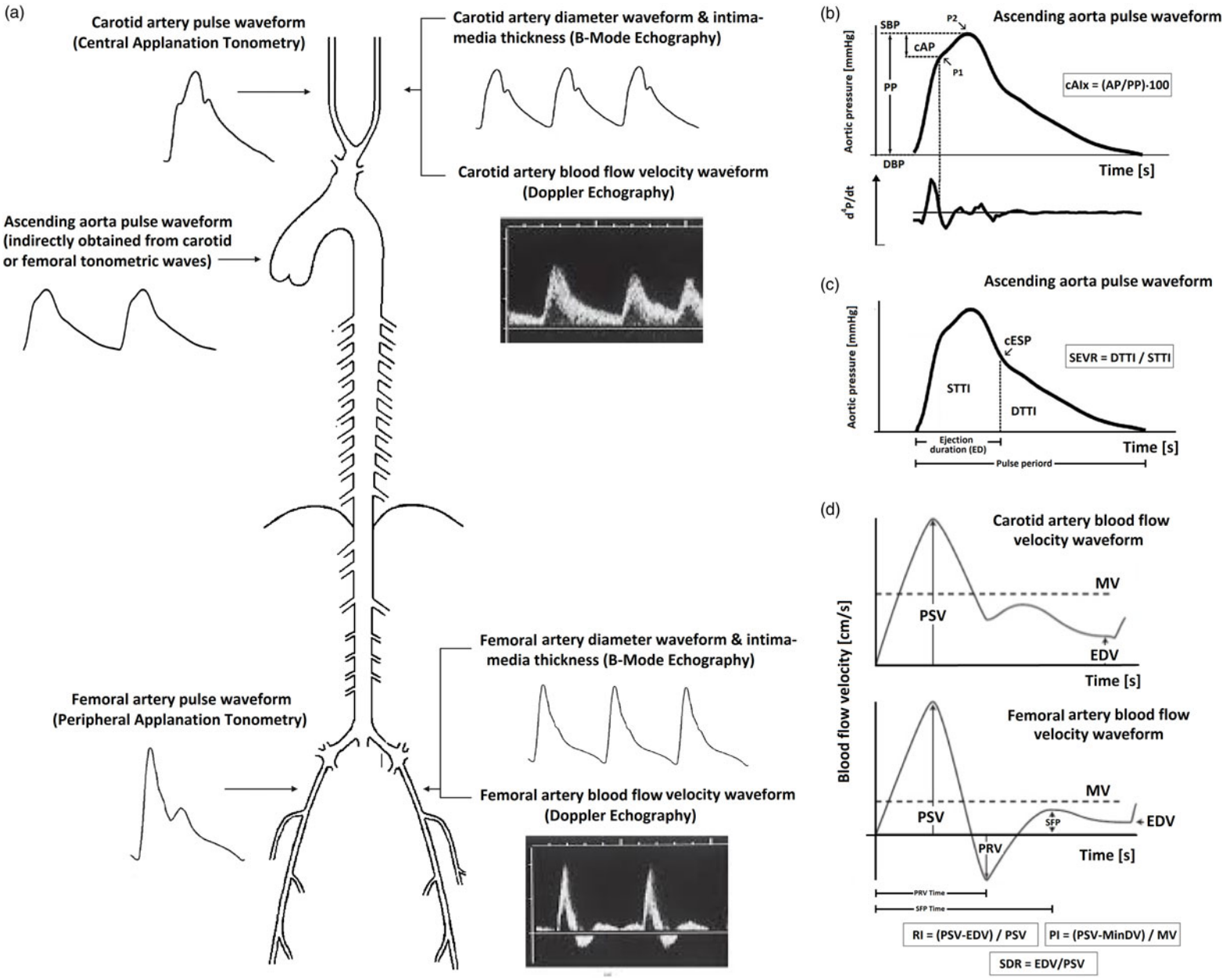

Fig. 1. (a): arterial non-invasive echographic (B-Mode and Doppler-Mode) and tonometric (applanation tonometry) records. (b, c and d): arterial parameters derived from tonometric or Doppler recordings. SBP, DBP and PP: systolic, diastolic and pulse pressure, respectively. cAP: central augmented pressure. cAIx: central augmentation index. cESP: central end systolic pressure. ED: ejection duration. cSTTI and cDTTI: systolic and diastolic tension time index, respectively. SEVR: subendocardial viability ratio. PSV: peak systolic velocity. EDV: end diastolic velocity. MV: mean velocity. PRV: peak reversal velocity. SPV: secondary forward velocity. MinDV: minimal diastolic velocity (i.e., PRV for CFA). RI: Resistive or Pourcelot Index. PI: Pulsatility or Gosling Index. SDR: Systolic-Diastolic Velocity Ratio or Index.

and PAT (alternative method), was evaluated in data collected from a subgroup of 12 ewes that showed high-quality CAT recordings and excellent PAT signals. Concordance analysis was done to evaluate the capability of obtaining accurate cBP and wave-derived parameters estimation from peripheral data, since in some animals CAT would not provide reliable results (mainly due to the thickness of the ewes' neck and the depth of the CCA). Correlation and Bland–Altman analyses were done (Supplementary Table S1 and Figure S1). Positive correlations were observed between methods (CAT and PAT) when cSBP, cDBP, cPP, cESP, subendocardial viability ratio, cSTTI and cDTTI indexes were considered ($p < 0.05$) (Supplementary Table S1). In turn, no significant correlations were observed for cAIx and cAP (Supplementary Table S1). The systematic difference (mean error) and the slope of the regression equation (proportional error) were not different from zero when cSBP, cDBP, cPP, cESP, cSTTI and cDTTI were considered (Supplementary Table S1 and Figure S1). For subendocardial viability ratio, systematic, but not proportional differences were observed. Then, central BP levels and waveforms obtained using PAT allowed arriving to reliable central parameters (except for cAP and cAIx). Considering those results and given the technical advantages, central hemodynamic and wave-derived parameters were estimated from PAT-derived aortic waveform (Tables 2 and 3).

Second, to compare treatments (D-LPA vs. D-HPA) and following the mentioned strategy (Randomized Complete Block Design), the block number was included into the model as a randomized factor. Additionally, we evaluated potential nutritional co-factors (fixed factors) that should be considered for an adequate analysis. In this sense, nutritional characteristics at the time of birth (weight, gestation age, weight/gestational age) or at the time of the cardiovascular study (body weight, length, condition score) were considered (Correlation) (Supplementary Tables S2 and S3).

Third, after analyzing data without considering cofactors, comparisons were made adjusting for body weight, length and condition score (fixed factors previously identified) (Tables 2 and 3).

Taking into account available data (mean values and standard deviations), a total of 32 ewes were the minimum required to detect a statistically significant effect of the different pasture allowances with at least 80% of power (Supplementary Methodology Table S4).[30] Hence, the study sample size ($n = 33$) was large enough to detect differences maintaining the criteria of reducing the number of experimental units. In any case, comparisons were statistically significant, indicating that the comparisons had adequate statistical power.

Analyses were carried out using MedCalc Statistical Software (Belgium). Statistical differences were considered significant when $p < 0.05$.

Results

M-HPA had greater body condition score than M-LPA (2.72 ± 0.03 vs. 2.57 ± 0.04; $p = 0.004$). The number of offspring born from each mother (single or twin), did not impact on the descendants body weight or condition score (weight: 42.9 ± 1.1 vs. 42.5 ± 1.2 kg; body condition score: 3.48 ± 0.07 vs. 3.60 ± 0.06, twins and singles, respectively). There was no association between treatment and number of offspring born from each mother (litter size). There were no differences in birth weight, gestational age or birth weight/gestational age ratio between D-HPA and D-LPA (Table 1). At the time of the cardiovascular study, body weight, length, condition score, hemodynamic and blood variables from D-HPA and D-LPA ewes did not show significant differences (Table 1).

To compare cardiovascular data from D-HPA and D-LPA groups, considering potential co-factors related to nutritional characteristics at birth or at the time of the cardiovascular study, correlation analyses between nutritional and cardiovascular variables were done (Supplementary Data Tables S2 and S3). CFA flow velocities, Doppler indexes (pulsatility index), arterial diameters and intima-media thickness were positively associated with body weight, length and/or condition score. Blood flow velocities, stiffness (stiffness index), diameters and CCA intima-media thickness were also associated with nutritional characteristics at the time of the cardiovascular study. However, except for carotid pathway resistance, cardiovascular parameters were not associated with birth weight, gestational age or birth weight/gestational age ratio. As a result, the comparative analysis between D-HPA and D-LPA groups required considering nutritional variables at the time of the study (but not at birth).

Table 2 shows comparison (D-HPA vs. D-LPA) before and after adjusting for co-factors. CCA end-diastolic velocity was lower in the D-LPA group after considering nutritional characteristics ($p = 0.029$). In agreement with that, Doppler indexes that evaluate cerebral vascular resistance (resistive index and systo-diastolic velocity ratio) were higher in the D-LPA group ($p = 0.030$ and 0.022, respectively). Mean pressure/flow ratio data further reinforced those results, since ewes from the D-LPA group showed lower mean ($p = 0.042$) and end-diastolic ($p = 0.028$) flows, and a higher resistance to blood flow in the carotid pathway (mainly after adjusting for nutritional characteristics) ($p = 0.016$). There were no significant differences in CCA characteristic impedance (mainly determined by its cross-sectional area and wall stiffness). Therefore, the higher resistances would be determined by the intracranial carotid territory (cerebral circulation).

The differences in the femoral pathway between D-LPA and D-HPA ewes were not as significant as those described for the carotid pathway. After adjusting for nutritional factors, D-LPA ewes had lower diastolic and mean diameters ($p = 0.034$ and 0.039, respectively). Despite the lower femoral diameters observed in the D-LPA ewes, CFA characteristic impedance was not lower in that group ($p = 0.089$).

Table 3 shows that cSTTI and the relative ejection duration*cSBP product (an afterload index) values were higher in ewes from the D-LPA group ($p = 0.038$ and 0.034, respectively, unadjusted comparison). These results (higher cSTTI and RED*cSBP product) indicate increased LV afterload in D-LPA ewes. It should be noted that when nutritional characteristics were included as cofactors, the differences were statistically significant for cSTTI and RED*cSBP product only with a one-tailed test, suggesting that increased load condition was influenced by the nutritional characteristics of the ewes at the time of the cardiovascular study. The time to and the amplitude of the early diastolic reverse peak and secondary forward peak did not show differences between groups (Table 2).

Discussion

Our work adds support to previous findings mainly obtained in *in-vitro* studies, as it provides original, complementary information related to the impact of nutritional conditions during pregnancy on offspring cardiovascular parameters. From non-invasive, *in-vivo* studies in conscious animals, we showed for the first time that ewes that experienced nutritional restriction (50%–75% of control intake) until day 122 of gestation (≈85% term) gave birth to female offspring that at the age of 30 months (2.5 years old)

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Table 2. Common carotid and femoral artery blood flow velocities, diameters, wall thickness, local and regional impedance characteristics: comparison of high and low pasture allowance groups

			Unadjusted comparison						Comparison adjusted by: body weight, body height, and BCS at the cardiovascular study time									
	All ($n = 33$)		D-HPA ($n = 16$)		D-LPA ($n = 17$)		Unadjusted		D-HPA ($n = 16$)				D-LPA ($n = 17$)				Adjusted[a]	
											95% CI				95% CI			
	MV	SE	MV	SE	MV	SE	P (2-tailed)	P (1-tailed)	MV	SE	Lower Limit	Upper Limit	MV	SE	Lower Limit	Upper Limit	P (2-tailed)	P (1-tailed)
Common Femoral Artery: Doppler and B-Mode parameters																		
Peak systolic velocity (cm/s)	42.45	1.47	41.32	1.31	43.52	2.58	0.455	0.228	42.12	1.58	38.79	45.45	39.02	1.66	35.53	42.51	0.215	0.108
Mean velocity (cm/s)	19.41	0.88	19.27	0.99	19.52	1.42	0.890	0.445	19.60	1.22	17.04	22.15	16.72	1.28	14.04	19.41	0.139	0.070
End diastolic velocity (cm/s)	14.64	0.63	15.04	0.85	14.26	0.94	0.539	0.269	15.24	0.87	13.40	17.07	12.08	0.92	10.16	14.00	0.029	0.014
Resistive or Pourcelot Index	0.66	0.01	0.64	0.01	0.68	0.01	0.029	0.015	0.64	0.01	0.61	0.67	0.69	0.02	0.66	0.72	0.030	0.015
Pulsatility or Gosling Index	1.48	0.06	1.39	0.04	1.56	0.09	0.126	0.063	1.39	0.09	1.20	1.59	1.67	0.10	1.47	1.88	0.062	0.031
Systolic–Diastolic Ratio or Index	2.98	0.09	2.81	0.10	3.14	0.14	0.072	0.036	2.81	0.14	2.52	3.10	3.33	0.14	3.03	3.63	0.022	0.011
Systolic diameter (mm)	5.75	0.10	5.83	0.14	5.67	0.15	0.443	0.221	5.89	0.17	5.52	6.25	5.82	0.18	5.44	6.20	0.814	0.407
Diastolic diameter (mm)	5.63	0.10	5.70	0.13	5.56	0.15	0.468	0.234	5.75	0.17	5.39	6.12	5.70	0.18	5.32	6.08	0.842	0.421
Mean diameter (mm)	5.67	0.10	5.75	0.13	5.60	0.15	0.459	0.230	5.80	0.17	5.43	6.16	5.74	0.18	5.36	6.12	0.833	0.416
Pulsatile diameter (mm)	0.12	0.01	0.13	0.01	0.12	0.01	0.336	0.168	0.13	0.01	0.11	0.15	0.12	0.01	0.10	0.14	0.541	0.270
Intima-media thickness (mm)	0.31	0.01	0.305	0.01	0.317	0.01	0.495	0.248	0.317	0.01	0.30	0.34	0.298	0.01	0.28	0.32	0.223	0.111
Peak systolic blood flow (ml/s)	11.00	0.44	11.04	0.52	10.96	0.72	0.927	0.464	11.58	0.66	10.22	12.93	10.32	0.59	9.10	11.53	0.175	0.088
Mean blood flow (ml/s)	1.52	0.07	1.61	0.09	1.43	0.10	0.185	0.093	1.68	0.10	1.48	1.89	1.39	0.09	1.20	1.57	0.042	0.021
End diastolic blood flow (ml/s)	3.62	0.16	3.80	0.20	3.44	0.25	0.262	0.131	3.91	0.22	3.46	4.35	3.21	0.19	2.81	3.61	0.028	0.014
Carotid pathway PVR (mmHg/ml/s)	91.97	4.85	81.50	5.50	101.14	7.09	0.037	0.019	78.68	7.01	64.21	93.14	103.76	6.29	90.78	116.73	0.016	0.008
Systolic dP/dt (mmHg/s)	225.61	15.61	238.96	20.48	213.05	23.58	0.413	0.207	243.57	27.22	187.39	299.74	220.27	24.41	169.88	270.65	0.539	0.270
Systolic dF/dt (ml/s)	66.41	3.83	63.71	3.88	68.95	6.54	0.497	0.249	68.33	6.36	55.20	81.46	65.09	5.71	53.32	76.87	0.715	0.357
Zc (mmHg/ml/s)	3.75	0.36	3.80	0.30	3.70	0.65	0.887	0.444	3.67	0.63	2.37	4.96	3.95	0.56	2.79	5.12	0.741	0.371
Common Femoral Artery: Doppler and B-Mode parameters																		
Peak systolic velocity (cm/s)	56.50	2.35	50.61	2.84	62.05	3.22	0.012	0.006	52.43	3.64	44.93	59.93	61.81	3.02	55.57	68.05	0.065	0.033
Early diastolic reverse peak (cm/s)	−16.72	1.00	-15.92	1.59	-17.47	1.27	0.449	0.225	-16.81	1.74	-20.39	-13.23	-17.21	1.44	-20.18	-14.23	0.867	0.434
Time to early diastolic reverse peak (ms)	279.17	4.03	281.92	6.49	277.06	5.22	0.565	0.282	280.08	6.94	265.75	294.40	277.01	5.77	265.09	288.92	0.743	0.372
Secondary forward peak (cm/s)	18.05	1.05	15.96	1.32	20.02	1.49	0.050	0.025	16.77	1.74	13.19	20.36	19.84	1.44	16.86	22.82	0.198	0.099
Time to secondary forward peak (ms)	447.17	5.64	449.23	9.70	445.59	6.89	0.762	0.381	448.83	9.95	428.30	469.36	444.94	8.27	427.87	462.02	0.773	0.386
Mean velocity (cm/s)	15.95	1.14	15.88	1.75	16.01	1.54	0.958	0.479	16.34	1.85	12.51	20.16	16.32	1.54	13.14	19.51	0.997	0.498
End diastolic velocity (cm/s)	7.71	0.61	7.55	0.54	7.87	1.09	0.798	0.399	7.29	1.01	5.21	9.38	8.09	0.84	6.35	9.82	0.563	0.281
Resistive or Pourcelot Index	0.86	0.01	0.85	0.01	0.87	0.02	0.161	0.081	0.85	0.02	0.81	0.89	0.87	0.02	0.84	0.90	0.488	0.244
Pulsatility or Gosling Index	5.12	0.35	4.66	0.39	5.55	0.56	0.202	0.101	4.88	0.62	3.59	6.16	5.41	0.52	4.34	6.48	0.527	0.264

Systolic–diastolic Ratio or Index	13.89	3.87	7.28	0.73	20.11	7.25	0.097	0.048	9.25	7.01	-5.21	23.72	18.93	5.83	6.89	30.96	0.312	0.156
Systolic diameter (mm)	4.29	0.05	4.31	0.07	4.27	0.07	0.688	0.344	4.40	0.06	4.26	4.53	4.22	0.05	4.11	4.33	0.055	0.027
Diastolic diameter (mm)	4.04	0.05	4.06	0.06	4.01	0.07	0.595	0.298	4.16	0.06	4.03	4.29	3.97	0.05	3.86	4.08	0.034	0.017
Mean diameter (mm)	4.12	0.05	4.15	0.07	4.10	0.07	0.625	0.313	4.24	0.06	4.11	4.37	4.05	0.05	3.94	4.16	0.039	0.020
Pulsatile diameter (mm)	0.25	0.01	0.24	0.01	0.26	0.01	0.492	0.246	0.24	0.01	0.21	0.27	0.26	0.01	0.23	0.28	0.502	0.251
Intima-media thickness (mm)	0.20	0.00	0.203	0.00	0.205	0.00	0.658	0.329	0.206	0.00	0.20	0.21	0.203	0.00	0.20	0.21	0.537	0.269
Systolic blood flow (ml/s)	8.17	0.36	7.45	0.51	8.86	0.47	0.051	0.026	8.04	0.58	6.84	9.24	8.65	0.48	7.65	9.64	0.441	0.220
Mean blood flow (ml/s)	0.67	0.05	0.68	0.08	0.66	0.06	0.835	0.418	0.73	0.08	0.56	0.90	0.66	0.07	0.52	0.80	0.529	0.265
Diastolic blood flow (ml/s)	0.97	0.08	0.98	0.07	0.97	0.13	0.973	0.486	0.98	0.13	0.70	1.26	0.98	0.11	0.75	1.21	0.997	0.499
Femoral pathway PVR (mmHg/ml/s)	223.60	15.10	219.10	26.11	227.84	16.78	0.780	0.390	220.73	26.24	166.57	274.89	229.41	21.83	184.36	274.46	0.807	0.403
Systolic dP/dt (mmHg/s)	401.94	27.50	432.87	35.58	372.82	41.35	0.280	0.140	454.59	47.45	356.65	552.52	376.93	39.47	295.46	458.40	0.233	0.116
Systolic dF/dt (ml/s)	65.52	3.06	59.66	4.19	71.03	4.12	0.062	0.031	64.56	5.08	54.08	75.04	69.22	4.22	60.51	77.94	0.498	0.249
Zc (mmHg/ml/s)	6.61	0.56	7.82	0.89	5.48	0.59	0.037	0.019	7.80	0.87	6.01	9.60	5.74	0.72	4.25	7.24	0.089	0.044

Values expressed as mean value (MV) and standard error of mean (SE). D-HPA and D-LPA: descendants (offspring) from mother exposed to high and low pasture allowance during pregnancy, respectively. a: Adjusted *p* value: based on estimated marginal means. Covariates appearing in the model are evaluated at the following values: Body weight = 44.609 Kg., Body length = 126.48 cm., Body Condition Scoring (BCS) = 3.48. Zc: characteristic impedance. PVR: peripheral vascular resistances. CI: confidence interval. A *p* value <0.05 was considered significant.

exhibited structural and functional cardiovascular alterations compared to control (D-HPA) ewes. First, D-LPA ewes had higher peripheral vascular resistances and Doppler-derived indexes in the carotid pathway (cerebral). In turn, when structural parameters were analyzed, the impact of nutritional factors was observed on muscular (resistance arteries, CFA) rather than on elastic (conductance arteries, CCA) arteries (Table 2). Second, the vascular changes observed in D-LPA ewes were accompanied by detrimental changes in LV afterload (Table 3). Third, the detrimental vascular changes observed in D-LPA ewes were not associated with birth weight and/or nutritional conditions at the time of evaluation.

Our results add to previous findings from *in vitro* studies. Increased vascular tone, reduced vasodilator capacity and impaired endothelium-dependent vascular responses (conditions that could result in increased vascular resistance) have been observed in association with nutritional interventions (i.e., rats under a low-protein diet during pregnancy).[13,14,31–33] In this study, we did not evaluate endothelium-dependent or -independent vascular responses, but we analyzed functional parameters associated with them in conscious ewes for the first time. In this regard, D-LPA ewes had increased peripheral resistances in the carotid pathway, which are usually associated with endothelial-dependent and independent capability to maintain an adequate basal vasodilator tone ("dilated" microcirculation). It is to note that the increase in regional resistances was observed considering two independent and complementary approaches: blood flow velocity-derived indexes and the relationship between mean BP and flow (Table 2). Resistive index is an indicator of "peripheral" resistances, almost independent of large arteries resistances. In turn, CCA characteristic impedance ("local CCA resistance") mainly depends on local arterial stiffness (Table 3). Looking at our findings, the increased vascular resistances in D-LPA ewes may be explained by resistive factors associated with the microcirculation and small peripheral arteries.

Carotid pathway resistances were higher in D-LPA than in D-HPA ewes, while there were no differences in the femoral resistances between groups. On the other hand, nutritional restriction was associated with changes in structural parameters (diameter) only when muscular resistance arteries (CFA) were considered, but not when elastic conductance arteries (CCA) were analyzed. Then, our work supports the concept that nutritional restriction during pregnancy could impact differently on offspring conductance and resistance arteries.[3]

There were no regional differences in the vascular impact of nutritional restriction during pregnancy when arterial (CCA and CFA) stiffness was analyzed. Furthermore, disregarding the parameter considered (pressure dependent or independent), there were no differences in arterial stiffness between D-LPA and D-HPA ewes. This means that there were no differences in arterial stiffness when considering: (a) potential differences in pBP and cBP (stiffness index), (b) the arterial wall intrinsic stiffness (incremental elastic modulus) and (c) the arterial segment as a three-dimensional structure (pulse wave velocity). At least in theory, the lack of changes in arterial stiffness associated with nutritional restriction could be explained by the increase in nutrient availability during late pregnancy, when structural and functional properties of medium and/or large arteries are established. The extracellular matrix, an arterial stiffness determinant (mainly, elastin and collagen), is formed during the late gestation and the early postnatal period.[3] Smooth muscle cells modulate the extracellular matrix development to achieve the biomechanical requirements of systemic arteries, signalling changes associated with the

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Table 3. Common carotid and femoral artery stiffness, central (aortic) blood pressure levels and wave-derived left ventricle afterload parameters: comparison of high and low pasture allowance groups

			Unadjusted comparison						Comparison adjusted for body weight, body length and BCS at the cardiovascular study time									
	All ($n = 33$)		D-HPA ($n = 16$)		D-LPA ($n = 17$)		Unadjusted		D-HPA ($n = 16$)				D-LPA ($n = 17$)				Adjusted[a]	
											95% CI				95% CI			
	MV	SE	MV	SE	MV	SE	P (2-tailed)	P(1-tailed)	MV	SE	Lower Limit	Upper Limit	MV	SE	Lower Limit	Upper Limit	P (2-tailed)	P (1-tailed)
Common Carotid Artery: Arterial stiffness																		
Pressure-strain EM (mmHg)	1539.23	175.72	1515.26	187.58	1561.79	297.94	0.896	0.448	1533.09	220.28	1075.00	1991.18	1365.53	239.14	868.21	1862.86	0.623	0.311
Beta-Index (peripheral pressure)	14.66	1.85	14.65	1.72	14.68	3.27	0.993	0.497	14.83	1.81	11.07	18.59	11.51	1.96	7.43	15.59	0.241	0.120
Beta-Index (central pressure)	11.83	1.50	11.90	1.55	11.77	2.57	0.965	0.482	12.13	1.71	8.58	15.68	9.68	1.85	5.82	13.54	0.358	0.179
Incremental EM (Einc; 10^7 dyn/cm^2)	2.01	0.23	2.10	0.33	1.90	0.32	0.657	0.329	2.08	0.34	1.38	2.79	1.92	0.37	1.16	2.68	0.755	0.378
Local Pulse wave velocity (cm/s)	684.48	30.72	693.78	44.33	673.62	43.80	0.749	0.375	696.54	45.69	601.54	791.55	670.40	49.60	567.25	773.54	0.711	0.355
Common Femoral Artery: Arterial stiffness																		
Pressure-strain EM (mmHg)	690.03	63.98	742.98	92.99	640.20	89.12	0.431	0.215	723.85	85.76	545.49	902.20	581.77	93.11	388.14	775.40	0.289	0.145
Beta-Index (peripheral pressure)	5.13	0.50	5.69	0.70	4.60	0.70	0.279	0.140	5.57	0.65	4.21	6.92	4.04	0.71	2.57	5.51	0.140	0.070
Incremental EM (Einc; 10^7 dyn/cm^2)	1.22	0.09	1.32	0.13	1.13	0.14	0.341	0.170	1.32	0.14	1.03	1.60	1.06	0.15	0.75	1.37	0.232	0.116
Local pulse wave velocity (cm/s)	521.20	18.84	540.44	25.89	503.10	27.30	0.329	0.164	536.52	27.39	479.56	593.47	494.83	29.73	433.00	556.67	0.329	0.165
Central pressure and pulse wave analysis-derived parameters																		
Central (aortic) SBP (mmHg)	132.94	3.66	128.11	5.55	137.78	4.50	0.196	0.098	144.32	7.31	129.12	159.52	156.79	7.93	140.29	173.29	0.275	0.138
Central (Aortic) DBP (mmHg)	103.89	3.23	99.67	4.88	108.11	3.99	0.200	0.100	113.26	6.64	99.44	127.08	129.45	7.21	114.45	144.45	0.125	0.062
Central (aortic) PP (mmHg)	29.06	3.18	28.44	4.37	29.67	4.87	0.854	0.427	31.07	3.42	23.96	38.19	27.33	3.71	19.61	35.06	0.481	0.240
Central STTI (mmHg/ms)	3195	124	2934	124	3442	197	0.038	0.019	2954	190	2559	3348	3464	206	3036	3892	0.092	0.046
Central DTTI (mmHg/ms)	4685	141	4574	224	4789	177	0.458	0.229	4565	253	4040	5090	4920	274	4349	5490	0.367	0.184
Central SEVR (%)	150.27	4.61	157.06	6.22	143.88	6.55	0.155	0.077	155.54	7.35	140.24	170.83	145.87	7.98	129.27	162.48	0.398	0.199
Central (aortic) pulse period (ms)	797.36	22.57	821.75	34.60	774.41	29.20	0.304	0.152	823.42	36.32	747.89	898.95	773.76	39.43	691.76	855.76	0.380	0.190
Central (aortic) ejection duration (ms)	295.33	5.48	293.31	6.86	297.24	8.65	0.727	0.364	290.88	8.85	272.72	309.04	298.05	8.29	281.03	315.07	0.566	0.283
Central (aortic) diastolic duration (ms)	502.00	19.07	528.50	28.51	477.06	24.79	0.183	0.092	526.76	30.33	463.69	589.83	477.78	32.93	409.31	546.25	0.301	0.151
Ejection duration/pulse period ratio (%)	37.67	0.80	36.13	0.82	39.12	1.28	0.059	0.030	36.38	1.10	34.09	38.67	38.81	1.19	36.32	41.29	0.162	0.081
Diastolic duration/pulse period ratio (%)	62.33	0.80	63.88	0.82	60.88	1.28	0.059	0.030	63.62	1.10	61.33	65.91	61.19	1.19	58.71	63.68	0.162	0.081
Ejection duration * cSBP product (ms*mmHg)	44231	1566	42435	2255	45921	2158	0.273	0.137	42129	2428	37147	47111	46252	2276	41583	50921	0.234	0.117
Relative ejection duration * cSBP product (mmHg%)	5648	212	5193	215	6077	331	0.034	0.017	5267	305	4640	5893	6062	286	5476	6650	0.072	0.036

Values expressed as mean value (MV) and standard error of mean (SE). D-HPA and D-LPA: descendants (offspring) from mother exposed to high and low pasture allowance during pregnancy, respectively. EM: elastic modulus. Einc: Incremental elastic modulus. SEVR: subendocardial viability ratio. STTI and DTTI: systolic and diastolic tension time index, respectively. SBP, DBP and PP: systolic, diastolic and pulse blood pressure, respectively. a: Adjusted p value: based on estimated marginal means. Covariates appearing in the model are evaluated at the following values: Body weight = 44.609 kg, body length = 126.48 cm, Body Condition Scoring (BCS) = 3.48. CI: confidence interval. A p value <0.05 was considered significant.

hemodynamic conditions (i.e., BP and flow within a vessel).[34] Elastin, an extracellular matrix component associated with the arterial stiffness at low BP working conditions, rapidly accumulates during late gestation and the early neonatal period, and is later slowly degraded with aging. Collagen, a stiff component of the extracellular matrix, accumulates in association with increases in biomechanical load (i.e., high BP), aging and disease.[34]

The vascular changes observed in D-LPA ewes were accompanied by detrimental changes in LV afterload indexes. In previous studies, LV load was indirectly evaluated by pBP levels (measured on a limb). However, from both, physiological and pathological points of view, aortic BP is the real direct pressure load imposed on the LV during early systole. Consequently, LV structural and functional properties are more associated with cBP than with pBP.[16,35] As a result, an accurate evaluation of LV load would require considering cBP together with the ventricle frequency (heart rate).

It has been demonstrated that experimental undernutrition of pregnant animals is associated with pBP increase in the offspring.[14,31,36,37] In humans, children of mothers who had thin triceps skinfolds in early pregnancy and low weight-gain during pregnancy have increased pBP. In turn, pBP levels in middle-aged men and women are associated with maternal carbohydrate and protein intake during pregnancy.[36] In this context, our study showed (for the first time) that D-LPA ewes had higher cSTTI and RED*cSBP levels than D-HPA ewes, which means that during "systolic time" BP levels developed by the LV were higher (to overcome aortic pressure), and myocardial oxygen consumption during ejection work was greater in D-LPA ewes. In this sense, the greater the cSTTI and/or RED*cSBP product, the higher the LV load.[16] The hemodynamic and vascular characteristics observed in the D-LPA ewes are in agreement with a condition of increased LV load and impaired LV-arterial coupling. Our findings agree with and complement results reported by Cleal *et al.*[12] These authors reported that intra-uterine nutritional restriction resulted in increased interventricular septum and mean LV wall thickness in 2.5-year-old sheep. Both our functional findings and the structural results showed by Cleal *et al.*[12] suggest that LV load is increased in D-LPA ewes. D-LPA and D-HPA ewes did not show differences in the arrival time or in the amplitude of the early diastolic reverse peak and secondary forward peak. Therefore, the increased LV afterload in D-LPA ewes would not be explained by enhanced reflections from the posterior hemi-body.

Finally, it is noteworthy that the detrimental changes observed in the arterial system of the D-LPA offspring were not strictly associated with the birth weight and/or the nutritional conditions at the time of the cardiovascular evaluation. Our results agree with available data showing that different foetal growth patterns could result in a similar birth size,[3] highlighting the limitations of birth weight as a single reliable indicator of the intrauterine nutritional experience.[36] Moreover, it has been suggested that nutritional deficiency could have permanent detrimental effects if it occurs in a sensitive period during the intrauterine development. Furthermore, some effects could only be observed late in life.[36] It can be proposed that placental or foetal compensatory mechanisms that develop in response to maternal nutritional restriction could preserve foetal growth (and hence birth weight), but the consequences of nutritional deficiency during pregnancy may be evident later. Thus, the adoption of a biological "strategy" that ensures intrauterine growth could not ensure well-being in adulthood.[39] In this context, although D-LPA ewes did not show external evidence (anthropometric and nutritional parameters) of the exposure to undernourishment, they had cardiovascular alterations associated with intrauterine nutritional restriction.

Methodological considerations: strengths and limitations

In order to make an adequate interpretation and evaluation of our findings and to analyze strengths and limitations of our experimental approach, some methodological aspects should be considered. There are several factors that make the use of a conscious sheep model a strength. First, there are multiple similarities between human and sheep pregnancy, especially regarding placental development, metabolic function and nutrient transport, making ovine models useful.[7,40] Second, ovine and human systemic arteries working conditions and responses to vasoactive agents are quite similar. In this regard, it is noteworthy that our group has experience in evaluating ovine and human cardiovascular systems *in-vivo*using both, invasive and non-invasive approaches.[25–27,41,42] Third, sheep are practically non-exposed to modifiable cardiovascular risk factors (unlike other animals who consume pro-atherogenic diets), which could modify the association between nutritional interventions and cardiovascular parameters. Then, potential confusing factors that could explain our cardiovascular findings were reduced. Furthermore, to increase the strength of our results, we also made statistical adjustments for potential confusing variables. Taking all these into account, it should be noted that the applicability of our findings to human arteries (and pregnancies) requires confirmation.

It should also be considered that the impact of nutritional deficiency on the arterial system could differ depending on the offspring sex.[18,43] In fact, it is currently well accepted that males are highly susceptible to intrauterine nutritional restriction. The inclusion of only females made it possible to work with a homogeneous population, decreasing intra-treatment factors of variation. However, we are aware that it could also represent a limitation, and undoubtedly, analyzing and comparing data from both, males and females, would have been interesting, enriching the work and increasing our knowledge of the impact of nutritional interventions during pregnancy on the cardiovascular system of the offspring. Considering the available information, differences may be even greater in males. However, results cannot be directly extrapolated to male sheep.

In our work, animals were exposed to nutritional restriction only during early and mid-gestation. The model aimed to simulate a real situation mainly observed in humans in developing countries where low accessibility to food in the first two thirds of pregnancy frequently occurs. As pregnancy progresses (second half or final third), many mothers are actively assisted (recruited) by social support programs. Fall described that nutritional interventions in under-nourished pregnant women usually start in the second or third trimester.[44] Therefore, interventions frequently result in newborns without evidence of IUGR or LBW, despite their exposure to intrauterine nutritional restrictions.[44] It is widely known, from the developmental point of view that during the first and second trimesters, the mother is in an anabolic stage (i.e., pregnancy-associated fat accumulation), in the last trimester, she changes to a catabolic stage in which the foetal growth is maximum.[42] Furthermore, during mid-to-late gestation, the foetus undergoes rapid growth.[45] Although, at least in theory, re-feeding or supplementation during the last stage of pregnancy ensures an adequate body growth (weight and height), alterations in organs and vital systems (i.e., cardiovascular system) could persist or develop later. Even though there are many studies on the impact of intrauterine malnutrition on offspring (children) with IUGR and/or LBW,

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works assessing the impact of malnutrition on descendants who do not show these conditions are scarce. In this context, our study contributes to understanding the impact that nutritional restriction during the first two thirds of pregnancy has on the cardiovascular system of offspring who did not show IUGR or LBW. Other intervention models (i.e., different nutritional restriction severity or patterns and/or interventions during a different period) could associate different alterations. Previous studies showed that foetal responses to changes in maternal nutrition may have immediate benefit for the foetus, but in the long-term it could be detrimental if the postnatal nutritional offer does not match "that predicted by the foetus" on the basis of its prenatal environment. Cleal *et al.*[12] observed that the mismatch between pre- and postnatal nutritional environments was associated with impaired cardiovascular function in adult sheep, which was not observed when these environments results were similar ("predictive adaptive responses" hypothesis). In our model, the mismatch between pre- and post-natal nutritional environments was reduced (similar nutrient availability for both groups late in pregnancy and in the post-natal period).

The carotid artery pressure waveform obtained from applanation tonometry is used as a surrogate for the aortic pressure waveform. High-quality pressure waveforms are often easier to obtain in peripheral arteries than in carotid arteries. The use of a specific generalized transfer function allows the aortic pressure waveform to be derived from the peripheral artery pressure waveform. However, the transfer function used by the SphygmoCor system (the device used in this study) was developed and validated in human studies and, to the best of our knowledge, it was not known whether a generalized transfer function could be applicable to the ovine vasculature. Considering the difficulties in obtaining high-quality carotid registers using tonometry, as a first step in our analysis we evaluated whether peripheral registers would allow equivalent levels of central pressure and parameters derived from pulse waves to be obtained. Then, considering the results of the equivalence analysis and given the technical advantages, central hemodynamic and wave-derived parameters were estimated from PAT-derived aortic waveform.

Finally, a joint analysis of our findings showed that an adequate evaluation of the impact of intrauterine nutritional restriction and its impact on the offspring cardiovascular system requires a comprehensive and multiparametric assessment of cardiac and vascular properties, using different techniques and methodological approaches. Different arteries and arterial pathways, as well as both central and peripheral hemodynamic parameters should be considered. If this is not the case, the association between intra-uterine malnutrition and cardiovascular alterations could be under- or overestimated.

Conclusions

Structural and functional cardiovascular parameters were non-invasively assessed *in-vivo* (conscious animals) in adult ewes with and without intra-uterine exposure to nutritional restriction. Widely used and validated (gold standard) methodological approaches enabled us to evaluate peripheral and central BP, wave-derived parameters, carotid and femoral arteries diameters, wall thickness and stiffness, blood flow levels, local and regional (peripheral) blood flow resistances and LV afterload. At 30 months of age, female offspring exposed to intrauterine nutritional restriction (without evidence of IUGR or LBW) showed higher carotid pathway arterial resistances (cerebral microcirculatory resistances) and LV afterload than those exposed to control nutritional offer. The impact of intrauterine nutritional restriction varied depending on the artery and/or arterial property considered (structural vs. functional), and would not depend on nutritional conditions at birth or in adult life.

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Conflicts of Interest. None.

Ethical Standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guides on the care and use of laboratory animals (Guide for the Care and Use of Laboratory Animals published by the US National Research Council, National Academy Press, Washington, DC, 1996, and Comisi;n Honoraria de Experimentaci;n Animal, Universidad de la República, Uruguay) and has been approved by the institutional committee (Comisi;n de Ética en el Uso de Animales, Facultad de Agronomía, Universidad de la República, Uruguay; Ethics committee approval: CEUA-Fagro. 020300-001929-17/111130-001469-13/111130-001856-13).

Supplementary Material. To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S2040174419000230

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