Oxygen Therapy in Premature Low Birth Weight Infants is Associated with Capillary Loss and Increases in Blood Pressure: A Pilot Study

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ABSTRACT:

Low birth weight (LBW) and premature birth are known risk factors for future cardiovascular disease and in particular essential hypertension (EH). Capillary rarefaction (CR) is an established hallmark of EH and is known to occur in individuals with a history of LBW. We previously reported that LBW infants do not have CR at birth but rather increased capillary density (CD). We hypothesized that LBW infants undergo a process of accelerated CR in early life, triggered in part by oxygen therapy. We studied 26 LBW infants, of whom 10 infants received oxygen therapy, and compared them to 14 normal birth weight (NBW) infants. We measured CD at 1, 5 and 10 days after birth and again after 40 weeks-adjusted gestational age equivalent to birth at full term. We confirmed that LBW infants had higher CD at birth compared to NBW infants and found that significant structural CR occurred at term age in LBW infants who had received oxygen therapy (mean difference −22 capillaries/field, $p=0.007$) and in those who did not receive oxygen therapy (mean difference −29 capillaries/field, $p<0.001$) compared to baseline at birth. Both LBW groups showed a significant rise in BP at 40-weeks adjusted term age and the rise in systolic (mean difference 24mmHg, $p<0.0001$) and diastolic BP (mean difference 14mmHg, $p<0.001$) was more pronounced in the oxygen treated group compared to the non-oxygen group (mean difference 14 mmHg, $p=0.043$ and mean difference= 9 mmHg $p=0.056$ respectively). In conclusion, oxygen therapy in premature LBW infants may induce significant increases in their BP in early life.

Word count: 250

INTRODUCTION:

Low birth weight (LBW <2500 grams) is a recognized risk factor for the development of future essential hypertension (EH), ischaemic heart disease, diabetes mellitus, obesity and increased cardiovascular mortality in adult life [1,2]. The pathophysiological mechanisms are yet to be elucidated, however, it has been suggested that microcirculatory abnormalities and impaired tissue perfusion are implicated in the pathogenesis of these cardiovascular disorders [3]. It is thought that the suboptimal in-utero conditions that impair the foetal growth in the first instance may affect the microvasculature and result in many structural and functional abnormalities including the reduction in spatial density of capillaries or capillary rarefaction (CR) [4]. We have previously reported that much of the CR in EH is caused by the structural absence of capillaries [5]. We have also shown significant CR in patients with borderline intermittent EH and in normotensive offspring of hypertensive parents, suggesting a familial predisposition in which CR represents a primary vascular abnormality that antedates the onset of sustained elevation of blood pressure (BP) [6,7]. We recently reported the unexpected finding that LBW infants, born at term or preterm to normotensive mothers do not have CR: instead these infants have a significantly higher dermal CD at birth compared to their normal birth weight (NBW) counterparts [8]. It is becoming increasingly evident that conditions early in life can influence adult diseases. It has been reported in some studies that hyperoxia during the neonatal period may have a negative effect on the cardiovascular system [9]. We hypothesized that the high capillary density seen in the LBW infants at birth was an in-utero compensatory adaptation to unfavourable conditions and that after birth, with the availability of adequate nutrition and more importantly oxygen, an accelerated capillary remodelling then ensues [10,11]. We set out to test our hypothesis by conducting serial measurements of skin capillary density in LBW preterm infants receiving oxygen therapy.

METHODS:

Study subjects:

The study was approved by the London – Riverside Research Ethics Committee (13/LO/1449) and was conducted at St. George's University Hospitals NHS Foundation Trust, London, UK. Written informed consent was obtained from all parents. The infants in the study were recruited from the Neonatal Intensive Care Unit, Transitional Care Unit and the Postnatal Ward. We studied 26 LBW infants (<2500 grams, ≥ 30 weeks of gestation) and 14 NBW infants as controls. As this was a pilot pragmatic study, no official power calculation was done to determine sample size. We excluded infants with sepsis, chromosomal or congenital abnormalities and those requiring surgery. The antenatal history of the mothers and details of the findings during the anomaly ultrasound scan were also recorded. Dietary habits, smoking history, family history of diabetes, ischemic heart disease, stroke, hypercholesterolemia, and hypertension were obtained from both parents.

Handheld Video Capillaroscopy System (HVCS):

CapiScope Handheld Video Capillaroscopy System (HVCS, KK technology, Devon, England) was used to measure skin capillary density at the plantar surface of the infant big toe according to a previously well-validated protocol [8,12,13]. The size of the microscopic field was 0.81 mm^2, with an image size of 1280 X 1024 pixels and a field view of 1037 x 829 pixels. The optical illumination of the HVCS device was done using four 525nm light source. The live images were recorded onto a Panasonic DMR-EX99VEBK HDD recorder. Disposable sterile probe covers were used for imaging to minimize the risk of infection. Four microscopic fields, 0.81 mm^2 each, were recorded continuously for 30 seconds. The number of all capillaries (i.e., with stagnant, intermittently flowing and continuously flowing red blood cells) was counted. Basal capillary density (BCD), which represents functional capillary density, was

calculated as the mean of these four microscopic fields. We used venous congestion to maximize the number of visualized perfused skin capillaries by applying a neonatal BP cuff (Heine Gamma 7 Sphygmomanometer, Germany) around the calf muscle. The cuff was then inflated and maintained at 30 mmHg for two minutes, and further images were recorded continuously for two minutes to determine the maximal capillary density (MCD), which represents structural capillary density. Capillaries were counted off line using the CapiScope computer software (KK-Technology, Exeter, UK). Skin temperature was monitored during the study using an YSI Tele-thermometer (YSI Inc., Dayton, OH, USA).

In LBW infants not requiring oxygen therapy, capillaroscopy was performed immediately after birth (baseline measurement) provided the cardiorespiratory status was stable and repeat follow-up measurements were done after 5 days and 10 days. The baseline measurement in LBW infants requiring oxygen treatment was slightly delayed due to the medical condition of the infants. In preterm LBW infants an additional measurement of capillary density was taken at the corrected (i.e. 40 weeks) term age prior to their discharge from hospital or by visiting them at home. In the NBW group we measured capillary density at birth only before mothers were discharged from the hospital. Blood and urine samples were obtained from the infants.

Blood Pressure (BP) measurement:

We used the Welch Allyn VSM 300^{TM} series monitor to measure BP in infants at each visit while they were sleeping or feeding to avoid movement artefacts. An appropriate sized disposable Welch Allyn neonatal cuff (size 1 to 4) was used to measure the BP. All measurements were taken in the lower limb, as this was the easily accessible part to the neonate inside the incubator.

Proteome Array:

Blood samples were obtained from 13 LBW infants during their stay in the ICU by

venepuncture or heel prick method. The samples were allowed to clot for 30 minutes and after centrifugation the serum was aliquoted and stored at -80°C. 201 Cytokines were measured by Quantibody Human Cytokine Antibody Array 4000 (RayBiotech, Norcross, Georgia, USA) according to manufacturer's instructions.

Statistical analysis:

The primary endpoint was the change in maximal (structural) skin capillary density during venous congestion (MCD) between birth to adjusted age of 40 weeks postnatal life. Shapiro-Wilk test was used to assess the normality of study parameters. ANOVA test with Bonferroni correction was used for comparison of means among the groups. Student's *t*-test was used to compare the difference of capillary density measurement from baseline to the corrected term age in the preterm LBW infants. Chi-square test was used to compare proportions between the LBW and NBW mothers. Pearson correlation coefficient was used to describe the linear correlation between capillary density and birth weight and changes in BP. Statistical significance was declared when the p-value was <0.05. All statistical analysis was carried out using the IBM SPSS 22 (IBM Corporation, Armonk, NY, USA).

RESULTS:

Table 1 shows the baseline characteristics of study subjects. There were no significant differences in maternal age, body mass index, booking BP (i.e. BP measured during the first antenatal clinic visit), pulsatility index or ultrasound foetal parameters during the anomaly scan between the groups. Of the 26 LBW infants we studied, 10 received oxygen treatment either by continuous positive airway pressure, mechanical ventilation or a nasal cannula. At birth, LBW infants had a significantly higher BCD (mean difference +9.3 cap/field, 95% CI: 1.5 to 17.1, p=0.021) & MCD (mean difference +11.6 cap/field, 95% CI: 1.6 to 21.7, p=0.025)

compared to the NBW infants. Compared to their NBW counterparts, LBW infants had a significantly lower diastolic BP (mean difference -10.1mmHg, 95%CI: -17.7 to -2.6, p=0.010). (Figure) There was no significant difference in systolic BP. Pulse rate was significantly higher in LBW (mean difference 15.1±10.1 beats/min, 95%CI: 3.4 to 26.8, p=0.013). There was a significant correlation between birth weight and both BCD (r = -0.309, p=0.052), and MCD in the entire group (r =-0.355, p=0.025).

At adjusted age of 40 weeks, the LBW oxygen group showed a significant reduction in BCD (mean difference -20.4 cap/field, 95%CI: -7.2 to -33.6, p=0.009) and MCD (mean difference -20.6 cap/field, 95%CI: -3.7 to -37.5 p=0.025) compared to baseline values at birth. Similarly the LBW non-oxygen group had a significant reduction in BCD (mean difference -26.7 cap/field, 95%CI -16.3 to -37.1 p<0.0001) and MCD (mean difference -28.2 cap/field, 95%CI, -17.1 to -39.2 p<0.001). There were no significant differences in BCD and MCD between the two LBW groups. The reduction in CD was associated with a significant rise in systolic (r=-0.385, p<0.035), and diastolic BP (r=-0.361, p<0.050) in both LBW groups. The rise in systolic BP and diastolic BP was more pronounced in the LBW oxygen group (mean difference 24 mmHg, 95%CI: 4 to 11, p=0.004 for systolic BP, and mean difference 11mmHg, 95%CI: 3 to 19, p=0.014 for diastolic BP) compared to the LBW non-oxygen group (mean difference 16mmHg, 95%CI: 4 to 6, p=0.005 and mean difference 13mmHg, 95%CI: 3 to 4, p=0.01 respectively). (Table 2)

We found no significant differences in angiogenic or antiangiogenic factors between the two LBW groups. Macrophage colony stimulating factor was significantly lower in the LBW oxygen group compared to the LBW non-oxygen group (0.656±0.389 vs 0.217±0.195 pg/ml, p<0.035) but we are unable to explain the significance of this finding.

DISCUSSION:

The study demonstrates that premature LBW infants who received oxygen therapy developed significant functional and structural capillary rarefaction, which was associated with a significant increase in both systolic, and diastolic blood pressures at adjusted 40 weeks of age. We also found that LBW infants who did not receive oxygen therapy developed similar capillary rarefaction but the increase in their blood pressure was not as significant as in those who received oxygen therapy. Additionally, we confirmed our previous report that LBW infants have higher functional and structural capillary densities at birth compared to NBW infants.[8] The effect of preterm birth on microvascular development has also been highlighted by studies showing reduced retinal vascular caliber and density (independently of retinopathy of prematurity), as well as reduced cutaneous capillary density in children and young adults born very preterm.[14,15] As previously stated, it has become increasingly evident that conditions early in life can influence adult diseases; but the underlying mechanisms are unkown.[16] Recent data suggest that perinatal oxidative stress may be one of the initiating triggers in long-term programming of cardiovascular function. Our results are in agreement with several preclinical studies. In animal models, the continuous supplementation of oxygen has been shown to affect the development of microvasculature and to induce vascular obliteration and capillary rarefaction[17,18] and an increase in blood pressure.[19] Yzydorczyk *et al*, studied Sprague-Dawley pups who were exposed to 80% oxygen from 3-10 days after birth and found that in both male and female rats exposed to oxygen as newborns, systolic and diastolic BP were increased by about 15 mmHg, capillary density was reduced by 30% and the number of nephrons per kidney was decreased by 25%.[9] They suggested that neonatal hyperoxia leads in the adult rat to increased blood pressure, vascular dysfunction, capillary rarefaction, and reduced nephron number. It has been shown that the nephron numbers is decreased in adult individuals with essential hypertension [20] and in intrauterine growth–restricted infants.[21,22] Milstein et al studied sublingual microvascular vessel density, vessel diameters, and

196 microvascular flow in rabbits breathing sequential oxygen/air mixtures under normobaric and
197 hyperbaric conditions.[19] They found that normobaric hyperoxia produced significant
198 microvascular rarefaction and significant increases in systolic and mean blood pressure when
199 compared to normobaric normoxia. Of interest they found that all microcirculatory
200 abnormalities reverted back to normal values upon return to normoxia.
201 Ashton et al confirmed that 80% inspired oxygen in healthy kittens caused “vaso-obliteration”
202 of the newly formed capillaries; when the animals were returned to ambient air, a “vaso-
203 proliferative” effect was observed causing retinopathy of prematurity.[23] Oxygen
204 supplementation in humans during the neonatal period has proven adverse effects on the
205 microvascular circulation especially in the retina and the lungs. However it has been shown
206 previously that capillary density ordinarily decreases after birth in preterm infants who did not
207 receive oxygen therapy. Kroth *et al*, measured basal or functional small vessel density
208 (FSVD) in 25 preterm infants born <30 weeks old using orthogonal polarization spectral
209 imaging on their upper arm. They found that FSVD decreased at 4 weeks compared to week
210 1. However, they did not observe any significant change in blood pressure but observed a
211 negative correlation between FSVD and systolic blood pressure.[24] Similarly van Elteren *et al*,
212 measured total vascular density (TVD) using incident dark field technology in 60 preterm
213 infants born less than 32 weeks and 33 term infants during the first month of life. Similar to us,
214 they found that TVD was higher in preterm infants at birth and that there was a progressive
215 decline in TVD from birth to 28 days in preterm infants. [25]
216 Our results corroborate with Kistner et al who found that preterm-born women had significant
217 rarefaction of retinal vessels manifested as fewer numbers of vascular branching points
218 compared with normal birth weight controls. This was associated with an increased casual
219 blood pressure suggesting that being born preterm does have effects on the vascular system
220 that persist into adult life.[15]

It has been reported that premature infants have decreased antioxidant defenses and are exposed upon birth to high oxygen concentration relative to the intrauterine environment.[26] Additional oxygen therapy may therefore cause oxidative tissue damage, leading to pathologies such as retinopathy of prematurity and broncho-pulmonary dysplasia.[27] More recent studies indicate that individuals with a history of premature birth exhibit higher blood pressure levels and abnormal retinal microvasculature and parameters of cardiovascular dysfunction. [15,28] While the mechanisms linking prematurity to adult cardiovascular disorders are unknown, our data and that from others support a putative role for neonatal oxidative stress. Oxidative stress has been shown to be involved in the promotion of rarefaction through endothelial apoptosis in hypertensive rats, while treatment with antioxidants has resulted in a reduction of microvessel loss.[29]

We acknowledge the limitations in our study that include the small numbers of LBW infants treated with oxygen, but this highlights the difficulties in recruiting such infants while they are in the neonatal intensive care unit (NICU). There was also uneven ethnicity and the significant difference in the age of infants on the study day may have been a confounding factor.[30] However, we assessed BCD and MCD in NBW term infants of different ethnic backgrounds born to normotensive mothers and found no difference in capillary density between the different groups (unpublished data). There was also significant variability in the mode of oxygen therapy, duration and percentage of fractional percentage of inspired oxygen (FiO_2) received in the post-natal period. It was not possible to control for the above factors, or render them uniform for the study, as they were tailored according to the clinical needs of each preterm infant. The timing of baseline measurement in the LBW oxygen group was slightly delayed compared to the non-oxygen group because of limited access to the NICU due to the medical condition of the baby. However, the mean gestational age was closely matched during the serial measurement of capillary density between the groups. All the blood samples

were taken within the first two weeks in the NICU as we aimed for the blood sampling to coincide with routine clinical blood sampling to avoid additional discomfort to the baby. Similarly, blood pressure measurements were taken only once, again to avoid undue discomfort to the infants. We have previously observed that sequential BP measurements awaken infants and disturb them, such that second and third readings were almost always higher than the first reading. We therefore ensured that all the BP measurements were taken while the baby was sleeping or feeding to minimise any artefacts.

In conclusion, oxygen therapy in premature LBW infants in the neonatal period is associated with higher systolic and diastolic BP levels but has no significant effect on the rate of capillary reduction from birth to adjusted age of 40-weeks. Further studies are needed to investigate the humoral factors that trigger the microcirculatory changes in LBW infants during the neonatal period, which will be of importance in preventing future hypertension and cardiovascular diseases in later life.

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Figure Legend:

Changes in the capillary density (A & B), systolic blood pressure (C & D) and diastolic blood pressure (E & F) from baseline measurement to adjusted 40 weeks of age in preterm LBW infants. Both the LBW oxygen group and the LBW control group showed a statistically significant reduction in the capillary density (A&B) followed by increase in the blood pressure (C-F).

347 **Table 1: Baseline Characteristics of Study Subjects**

Variables	LBW Oxygen (n= 10)	LBW Non-oxygen (n=16)	NBW Control (n=14)	P- value ANOVA
Maternal Data:				
Age *(years)*	34±2.0	34±7.8	31±4.0	0.198
BMI *(kg/m^2)*	27.0±6.8	30.2±6.3	28.2±5.4	0.447
Blood Pressure *(mmHg)*				
Systolic	123±14	125±13	114±17	0.161
Diastolic	77±14	77±16	72±7	0.614
Ethnicity *n(%)*				
Caucasian	4 (40)	9 (56.3)	7 (50)	1.0
South Asian	2 (20)	4 (25)	5 (35.8)	1.0
Afro Caribbean	2 (20)	3 (18.7)	1 (7.1)	1.0
Mixed	2 (20)	0	1 (7.1)	1.0
HDP	5 (50)	6 (37.5)	1 (7.1)	0.059
Family history of HTN	3 (30)	8 (50)	10 (71.4)	0.324
Prenatal Data:				
Bi-Parietal diameter *(cm)*	54.2±3.8	52.3±2.4	53.2±2.4	0.258
Head circumference *(cm)*	182.5±25.9	186.8±7.3	178.8±43.2	0.780
Femur length *(cm)*	34.7±3.3	35.3±1.4	36.2±1.5	0.227
Abdominal Circumference *(cm)*	161.5±14.2	162.4±8.6	167.2±11.0	0.409
Uterine artery PI	2.79±0.89	2.53±0.74	2.42±0.56	0.640

Neonatal Data:

Birth weight *(grams)*	1645±418	1715±377	3388±558	0.0001
Gestational age *(weeks)*	32.5±1.4	33.3±1.7	39.1±2.29	0.0001
Age at Capillaroscopy *(days)*	9±6	4±4	2±1	0.001
Gestational age at analysis	33.8±1.7	34.0±1.6	39.3±2.1	0.0001
Weight on study day *(grams)*	1513±299	1740±331	3388±588	0.0001
Capillary Density *(per mm^2)*				
Basal	92±15	93±18	83±8	0.155
Maximal	97±17	101±17	87±11	0.069
Skin Temperature °C	34.8±1.5	34.9±1.2	33.7±1.2	0.106
Room Temperature°C	24.6±0.97	25.2±1.5	26.7±1.2	0.001
Blood Pressure *(mmHg)*				
Systolic	61±9	68±13	70±11	0.168
Diastolic	33±6	38±10	45±12	0.022
Mean	43±8	48±10	53±11	0.104
Heart Rate	145±15	131±17	122±14	0.007

Laboratory results:

Haemoglobin	147±20	180±26	171±21	0.022
Haematocrit	46±6.3	55±8.4	56±0.1	0.035
Bilirubin	143±70	156±40	123±1.4	0.803
pO^2	6.7±1.5	6.4±1.5	NA	0.827
pCO^2	6.3±0.9	5.9±0.8	NA	0.166

Duration of O^2 exposure (Hours)	347.8±524.8
Maximum FiO^2 given	59.6±34.2

LBW=low birth weight, NBW=normal birth weight, HDP=hypertensive disorder of pregnancy, FiO^2 = Fractional percentage of inspired oxygen.

Table 2. Comparison between LBW babies at adjusted 40th week who were treated or not treated with oxygen compared to NBW infants at term

Variables	LBW Oxygen (n= 10) (40th week)	LBW non-oxygen (n=16) (40th week)	NBW Control (n=14) (Term age)	P- value ANOVA
Capillary Density *(per mm^2)*				
Basal	69±12	66±11	83±8	0.0001
Maximal	72±11	72±9	87±11	0.005
Blood Pressure *(mmHg)*				
Systolic	85±9	81±10	70±11	0.006
Diastolic	49±7	47±8	45±12	0.742
Heart Rate	160±11	158±14	122±14	0.0001

LBW=low birth weight, NBW=normal birth weight

Summary Table:

"What is known about topic:"

- Low birth weight and premature birth are known risk factors for future cardiovascular disease and in particular essential hypertension
- Capillary rarefaction is an established hallmark of essential hypertension and is known to occur in individuals with a history of low birth weight
- It has been shown recently that low birth weight infants do not have capillary rarefaction at birth, but rather increased capillary density compared to normal birth weight
- In preclinical animal studies, oxygen therapy has been shown to induce vascular obliteration and capillary rarefaction in the new born

"What this study adds":

- Oxygen therapy in premature low birth weight infants in the neonatal period is associated with higher systolic and diastolic blood pressure levels but has no significant effect on the rate of capillary reduction
- Further studies are needed to investigate the humoral factors that trigger the microcirculatory changes in low birth weight infants during the neonatal period, which will be of importance in preventing future hypertension and cardiovascular diseases in later life

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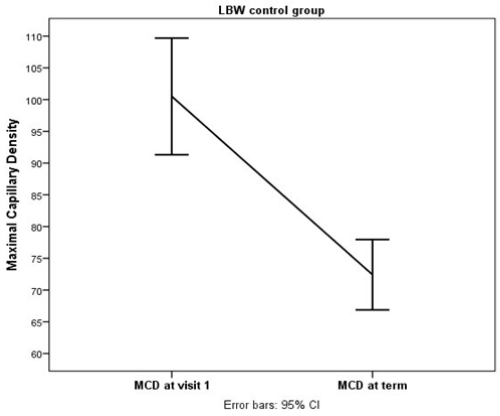
LBW control group
Maximal Capillary Density
110
105
100
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80
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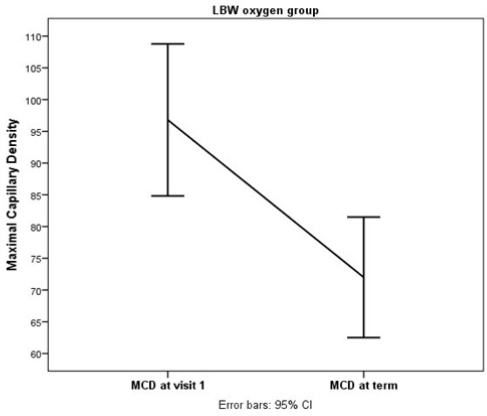
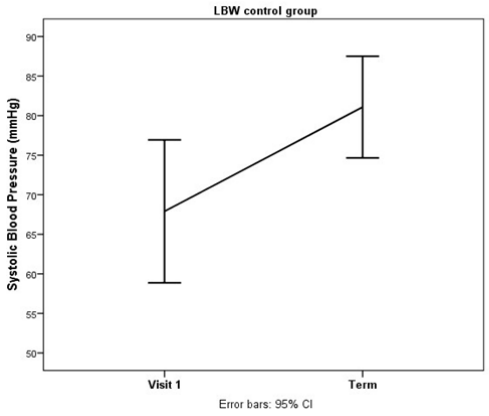
65
60
MCD at visit 1
MCD at term
Error bars: 95% CI

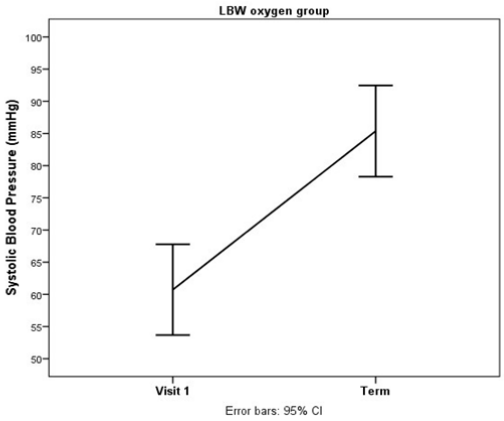
B


LBW oxygen group
Maximal Capillary Density
110
105
100
95
90
85
80
75
70
65
60
MCD at visit 1
MCD at term
Error bars: 95% CI

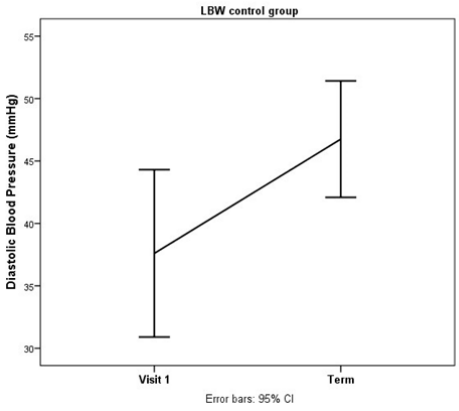
C


LBW control group
Systolic Blood Pressure (mmHg)
90
85
80
75
70
65
60
55
50
Visit 1
Term
Error bars: 95% CI

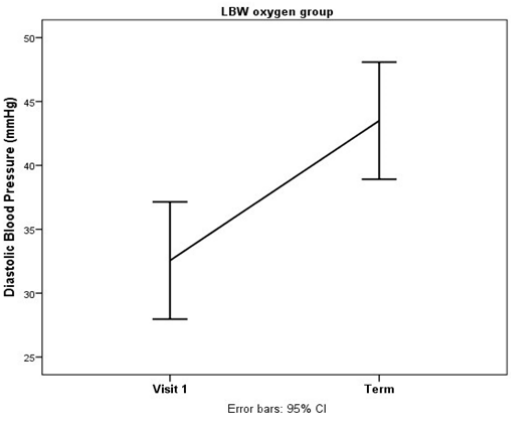
D


LBW oxygen group
Systolic Blood Pressure (mmHg)
100
95
90
85
80
75
70
65
60
55
50
Visit 1
Term
Error bars: 95% CI

E


LBW control group
Diastolic Blood Pressure (mmHg)
55
50
45
40
35
30
Visit 1
Term
Error bars: 95% CI

F


LBW oxygen group
Diastolic Blood Pressure (mmHg)
50
45
40
35
30
25
Visit 1
Term
Error bars: 95% CI