Fetal oxygen and glucose utilization of uncomplicated monochorionic twins: adapting to the intrauterine environment.

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Abstract

Introduction: Monochorionic twins (MC) develop under unique intrauterine conditions and show a high risk of compromise during fetal life. Here we describe umbilical vein blood flow (UVBF) and fetal oxygen and glucose utilization in uncomplicated MC twins and investigate possible differences within twin-pairs according to birth-order.

Methods: Prospective single-center study on 48 uncomplicated MC twins enrolled at the time of elective cesarean delivery. Ultrasound measurements of UVBF for Twin 1 and Twin 2 labelled according to birth-order were performed before spinal anesthesia. Umbilical arterial and venous blood samples were collected for each twin after fetal delivery, and fetal oxygen and glucose deliveries and uptakes were computed.

Results: All twins were delivered within 2 minutes from one-another under steady-state conditions. Birthweight and umbilical cord gas analyses were within physiological ranges for all twins. Second-born twins showed significantly lower UVBF, measured before delivery, and lower median birthweight compared to first-borns. Moreover, median values of estimated fetal oxygen and glucose consumption were lower in second compared to first uncomplicated MC twins.

Discussion. Uncomplicated monochorionic twins show different birthweight, oxygenation and metabolic rates based on their position in utero, hinting at pre-existing conditions possibly deriving by uneven vascular and metabolic distribution of the two placental territories. The innovative findings of this study emphasize the biological uniqueness of these pregnancies and prompt further physiological studies on monochorionic twins and placenta metabolism.

Kewwords: monochorionic twin; oxygen uptake; glucose uptake; umbilical vein; twin delivery.

Highlights:

- Estimation of fetal oxygen and glucose metabolism is feasible in human pregnancy
- Monochorionic twins reduce their metabolic needs to endure in a poor environment
- Second-born twins show lower birthweight, oxygenation and metabolic rates
- Monochorionic placenta may have uneven metabolic functions

Introduction

Monochorionic (MC) twins are at increased risk of perinatal morbidity and mortality compared to both singletons and dichorionic twins [1-4]. One of the main determinants of this increased risk is the unique placental angioarchitecture allowing free sharing of circulating blood volumes between MC twins through vascular anastomoses [5,6]. Moreover, there is some evidence that, compared to first-born, second-born twins are at higher risk of lower birth weight, perinatal mortality and neonatal morbidity, especially respiratory distress syndrome, independently from chorionicity and mode of delivery [7-11].

Umbilical vein blood flow (UVBF) is an estimate of both fetal and placental circulating blood volumes that can be reliably assessed by means of Doppler ultrasound technology [12-16]. In singleton pregnancies, longitudinal observations have been made, and UVBF was found to be a major determinant of fetal growth, with an early and significant reduction in fetuses affected by intrauterine growth restriction (FGR) [12,13,17-19]. In MC twins, this parameter was mainly analyzed in pregnancies complicated by twin-to-twin transfusion syndrome (TTTS) and revealed significantly higher UVBF in recipient twins, and a transient increase in donor twins after laser treatment [20-23].

After many years of experiments on the pregnant sheep model, fetal delivery and consumption of oxygen (O_2) and glucose have been recently estimated in a reliable and non-invasive way also in the human fetus, providing important insights into fetal homeostasis [14,24-26]. We recently reported that human FGR fetuses have a strikingly reduced delivery and uptake of both O_2 and glucose compared to appropriately grown controls [26]. This field of investigation is quite new in human fetal physiology but of utmost interest given the established correlation between fetal developing environment and the individual’s short- and long-term health. No data currently exist on the intrauterine metabolic environment of monochorionic twins.

The present study investigates UVBF, oxygen and glucose uptake, as well as arterial and venous oxygenation and acid-base balance in uncomplicated MC twins evaluated at the time of elective cesarean delivery, under conditions that most nearly represent the intrauterine fetal metabolic state. Furthermore, we test the hypothesis that the birth order could have an impact on the metabolic and oxygenation rates within MC twin-pairs.

Materials and methods

Study Population

This is a prospective study performed at the Department of Woman, Mother and Child of the Buzzi Children's Hospital according to a research protocol approved by the Institutional Review Board of the Hospital (study code: MCpls_1).

Uncomplicated monochorionic twin pregnancies monitored at the Fetal Therapy Unit of Buzzi Children's Hospital were enrolled, between October 2019 and October 2021. Chorionicity was defined at first-trimester ultrasound (US) examination and confirmed after birth with macroscopic analysis of the placenta [6] and membranes and by histopathological examination. Pregnancies were dated according to crown-rump length (CRL) measurement in the first trimester [27]. Both twins were monitored longitudinally throughout pregnancy with detailed evaluation of fetal anatomy and biometry, amniotic fluid volume and Doppler evaluation of umbilical and cerebral blood flows (GE E8- Philips EPIQ5) every 2 weeks until 36-37 weeks according to international guidelines [27].

Uncomplicated MC twin pregnancies with delivery at V. Buzzi Children's Hospital at an appropriate gestational age (GA) (36-37.0 weeks according to our protocol for MC pregnancies) by means of elective cesarean section for maternal request or non-vertex presentation of the first twin were enrolled. Patients were excluded if complicated by selective FGR, TTTS, twin anemia-polycythemia sequence, major anatomical and/or genetic anomalies, discrepancy of amniotic fluid, fetal death of one twin, spontaneous preterm labour as well as maternal diabetes that needed pharmacological treatment. Lack of availability of a complete neonatal follow up was also an exclusion criteria. Written informed consent was obtained from all pregnant women before inclusion in the study.

Data collection

Maternal and pregnancy characteristics as well as birth details and neonatal outcomes were obtained from clinical reports.

The day of elective cesarean section each woman underwent an ultrasound scan performed by the same specialists that had followed her pregnancy and that subsequently performed the cesarean section (DC, ML, SF, AL). At that scan, the twins were labelled as 'Twin 1' being the twin closer to the cervix and the first in order to be born, and 'Twin 2' being the second twin to be born.

For each twin, UVBF was calculated during fetal quiescence and in the absence of breathing movements. The methodology for computing UVBF has been previously described in details by Barbera and co-workers [12]. Briefly, the mean diameter of the umbilical vein is determined on a free loop of the umbilical cord by obtaining a perpendicular view of a longitudinal section of the cord, and thereafter by averaging three consecutive measurements of inner-to-inner diameters to the nearest one tenth of a millimeter [12-16].

Mean velocity estimation was performed by rotating the same image by 90°, and multiplying the average of three consecutive measurements of UV peak velocity times 0.5, assuming a parabolic velocity profile. Maximum UV velocity was measured with the use of 10 seconds time epochs, a velocity range of 10-20 cm/sec and by placing the Doppler sample in the center of the lumen of the vessel, with a Doppler beam angle closest to zero [12-16].

Absolute Umbilical venous blood flow was then calculated according to the following equation:

UVBF (mL/min) = mean velocity (cm/seconds) · vessel area (cm2) · 60.

Each examination was performed using a 5 MHz convex probe (HS50 Samgung Healthcare Global), approximately within one hour from the surgical procedure, before the induction of anesthesia. All patients underwent spinal anesthesia and none had any secondary effects, such as maternal severe hypotension. Our group already reported no significant differences in mean UVBF measured before and after the induction of anesthesia in uncomplicated singleton pregnancies [24].

After delivery, each placenta was injected with color dye according to a technique previously described [6]. After the procedure, a picture of the treated placenta along with a measuring tape was taken with a high-resolution digital camera (Figure 1). All the pictures were retrospectively analyzed with Image J 1.49 g (National Institute of Health, USA), in order to measure the area of each vascular territory to allow estimation of the placental sharing [6].

Birthweight discordance was calculated as (birthweight of larger twin – birthweight of smaller twin)/birthweight of larger twin × 100%. The discordance of the estimated placental share and UVBF were computed with the same formula.

Oxygen, glucose and acid-base analyses

After fetal delivery, umbilical arterial and venous blood samples were withdrawn from a doubly clamped segment of the cord for each twin. All samples were collected in heparinized syringes that were analysed within a few minutes. Blood gases (pO2 and pCO2), pH, hemoglobin concentration and O2 saturation, lactate and glucose concentrations were measured using a modern spectrophotometer RAPIDPoint 500e automatic system (Siemens Healthineers).

The following calculations were then performed, as previously described [24,26]:

O_2 content (mmol/L) was calculated as:

Hemoglobin (g/L) · O_2 saturation (%) · 0.05982

Umbilical O_2 uptake (µmol/min) was calculated according to the Fick principle:

UVBF (mL/min) · D (UV−UA) O_2 Content (mmol/L)

Fetal O_2 delivery (mmol/min) was calculated as:

UVBF (mL/min) · UV O_2 Content (mmol/L)/1000

Fetal O_2 extraction (%) was calculated as:

[D (UV−UA)/UV] O_2 Content · 100

Umbilical Glucose/ O_2 metabolic quotient was calculated as:

[D (UV−UA) glucose concentration (mmol/L)/D (UV−UA) O_2 Content (mmol/L)] · 6

Umbilical Glucose Uptake (µmol/min) was calculated as:

UVBF (mL/min) · D (UV−UA) glucose concentration (mmol/L)

Since the blood flow is related to the size of the supplied mass, we normalized UVBF, umbilical O_2 and glucose uptakes as well as O_2 delivery to neonatal birth weight.

The data that support the findings of this study are available from the corresponding author on reasonable request.

Statistical analysis

Data were anonymously recorded in a database and subsequently analysed with the SPSS statistical package (IBM SPSS Statistics 27, Armonk, NY). All variables were tested for normality by means of Shapiro-Wilk test. Since absolute UVBF and some metabolic analytes (namely: umbilical artery and vein pH and lactates, and glucose uptake) resulted not normally distributed, and given the limited sample size of the population, non parametric tests were adopted for statistical comparisons and the data are presented as median and interquartile range (IQR).

The Wilcoxon signed-rank test for matched-pairs was used for statistical comparisons between Twin 1 and 2 of the same MC pregnancy.

Correlations describing the strength and direction of the relationships between two variables were assessed using the Spearman correlation coefficient because of skewed data and possible nonlinear relationships.

For all comparisons, a *p* value <0.05 was considered significant.

Results

Twenty-four MC pregnancies were enrolled. General characteristics of the study population are presented in Table 1. The mean gestational age at delivery was 36.4 weeks (IQR 36.0-37.0). The participants were mainly of Caucasian origin (83.3%) and primiparous (66.6%), with a median pre pregnancy BMI of 21.5 kg/m2 (IQR 19.7-23.1), and a median gestational weight gain of 14.0 kg (IQR 12.0-20.2) (Table 1).

Satisfactory Doppler parameters were obtained before cesarean section from all fetuses within few minutes.

Twin neonates were born with a median time-interval of 2 minutes from one-another (IQR 1-2) and showed median birthweight of 2377 grams (IQR 2160-2678), within normal centiles for the gestational age (Table 2).

Median discrepancy in birthweight and placental sharing of vascular territory was 10.8% (3.8-18.5) and 16.1% (9.8-23.7), respectively (Table 1).

Whole population of uncomplicated MC twins

Table 2 ad Table 3 present neonatal outcome, umbilical artery and vein blood gases and acid-base balance results, UVBF and estimated fetal oxygen and glucose metabolic rates for the whole population and according to birth-order.

Overall, both first and second-born MC twins showed normal oxygenation and acid-base balance at birth.

The median value of UVBF was 174.7 mL/min (IQR 138.0-247.3) and 76.9 mL/min/Kg (IQR 59.5-91.4) when normalized for neonatal body weight.

UVBF was significantly correlated to neonatal weight (Spearman correlation coefficients 0.452, p 0.002). After normalization for the neonatal weight, the UVBF/Kg resulted positively correlated to umbilical O_2 and glucose uptakes (Spearman coefficients 0.77 and 0.94, respectively, $p<0.001$). No correlations were found between UVBF/Kg and maternal characteristics such as maternal BMI or haemoglobin levels, fetal gender or proxies of neonatal outcome at birth as pH, Apgar scores and admission to neonatal intensive care unit.

The median fetal O_2 delivery was 875.9 µmol/min (IQR 580.2-1352.4) and the median O_2 delivery normalized for neonatal weight was 347.7 µmol/min/Kg (IQR 245.0-516.7). Median umbilical O_2 uptake was 359.8 µmol/min (IQR 220.0-638.3) and 152.3 µmol/min/Kg (IQR 95.0-246.3) after normalization for neonatal weight.

Umbilical O_2 uptake was significantly correlated to UVBF, fetal O_2 delivery, and umbilical glucose uptake (Spearman coefficients 0.77, 0.72 and 0.34, respectively, $p<0.05$) (Figures 2a and 2b).

Median MC twins glucose delivery was 573.1 µmol/min (IQR 436.0-865.1) and 260.3 µmol/min/Kg (IQR 187.5-341.4) after normalization for neonatal weight, while median umbilical glucose uptake was 87.9 µmol/min (IQR 48.7-151.4) and 36.6 µmol/min per Kg (IQR 21.7-56.5).

Umbilical glucose uptake was positively correlated to UVBF, umbilical O_2 uptake (Figure 2c) and fetal extraction and to the umbilical glucose/O_2 metabolic quotient (Spearman coefficients 0.94, 0.34, 0.46 and 0.53, respectively, $p<0.05$). No correlation was found with maternal BMI and weight gain, neonatal or placental weight, or fetal glucose concentration.

The median umbilical glucose/O_2 metabolic quotient was 1.29 (IQR 0.95-1.71).

Differences in MC twins according to birth order

Tables 2 ad 3 present the comparison between first- and second-born twins for neonatal outcome, umbilical artery and vein blood gases and acid-base balance, UVBF and estimated oxygen and glucose metabolic rates.

Second-born MC twins had significantly lower median birthweight and UVBF, both absolute and weight-normalized, than the first twin (Tables 2 and 3; Figure 3). The reduced UVBF seemed to be mostly related to a reduced UV diameter rather than to changes in UV flow velocity (Table 3).

No significant correlation was found between inter-twin discrepancies of placental vascular territory and of UVBF (Spearman coefficients 0.31, p 0.24).

Second-born twins showed worse oxygenation profiles compared to first-borns, mostly for UV values. As shown in table 2, significantly lower pH, pO_2, O_2 saturation, O_2 content and higher pCO_2 median values were found, while glucose, haemoglobin and lactate concentrations were not significantly different between Twin 1 and 2 (Table 2).

O_2 delivery as well as estimated O_2 and glucose uptakes (both absolute values and per Kg) were also significantly lower in second-born twins (Table 3 and Figure 3).

A significant positive correlation between values of Twin 1 and Twin 2 was found for UV diameter, birthweight, venous O_2 content, pH and pCO_2, arterial and venous lactate, haemoglobin and glucose concentrations (Spearman correlation coefficients 0.56, 0.48, 0.54, 0.62, 0.72, 0.67, 0.77, 0.59, 0.58, 0.84, 0.92, respectively, $p<0.05$). The other variables of the oxygenation profile, as well as estimated umbilical O_2 and glucose consumption and UVBF (both absolute and weight-normalized values) were not significantly correlated between twins of the same MC pair.

Discussion

To our knowledge, this is the first study that estimates fetal O_2 and glucose consumption in uncomplicated monochorionic twins, a condition characterized by a unique placenta with shared territories by the two fetal circulations.

Together with overall median values for uncomplicated MC twins, we report intriguing differences in umbilical blood flow, oxygenation and metabolic rates and in birth weights observed between the two twins of the same mother according to birth order, representing a different spatial intrauterine environment.

In our study, average absolute umbilical blood flow was 174.7 mL/min, lower than the mean absolute UV flow previously reported in singletons, ranging from 196 to 263 ml/min [25,26,28-30]. This was expected, since the median GA in our study was lower (36.4 weeks GA) and so was the fetal weight. Indeed, we have previously reported that UVBF is positively related to gestational age, placental weight, and fetal weight [26]. In uncomplicated singleton pregnancies, the progressive and exponential increase of absolute UVBF throughout gestation is mainly due to UV size increase rather than flow velocity, but after normalization for fetal weight UVBF appears substantially stable with a slight decrease along pregnancy [12,16,29].

Our results show that, after normalization for neonatal weight, median UVBF in MC twins (76.9 ml/min/kg) is quite similar to results for uncomplicated singletons at 38-39 weeks, with a mean UVBF per Kg of 68-78 ml/min [26,29,30].

Interestingly, second-born twins showed significantly lower median birthweight together with lower absolute and size-normalized UVBF compared to the first-born twins. This finding recalls the pathophysiologic mechanism observed in FGR fetuses, where the reduced UVBF represents an early event able to determine an adaptation of the fetal mass to the reduced oxygen and nutrients support from the placenta [13].

When studying circulating blood flows in MC twins it is of greatest importance to keep in mind that blood flow dynamics are not stable but may fluctuate due to vascular anastomoses, mainly artero-arterial [22]. Indeed, in monochorionic placentas, deep artero-venous anastomoses permit unidirectional transfer of blood volume from one twin to another, while superficial artero-arterial and veno-venous anastomoses allow bidirectional exchange and rapid equilibration of blood volumes between twins. Nevertheless, in uncomplicated MC twins at their term as in our population, we can assume relatively steady haemodynamics between twins in the majority of the cases.

Despite comparable perinatal clinical outcome, second-born twins also showed significant differences in oxygenation and acid-base balance compared to the first-born ones, even if all values were within the normal limits.

This finding is in agreement with previous reports on twin pregnancies after both vaginal delivery and planned cesarean section [31]. In vaginal delivery second-born twins show worse oxygenation and acid-base balance with metabolic acidosis that worsens in case of birth time-interval exceeding 30 minutes [31-33]. In our study, a major impact of the birth time-interval on the acid-base status of the second-born twin cannot be completely excluded. However, the low birth time-interval (2 minutes) under relatively stable conditions lowers this effect.

Fetal oxygen rates

Umbilical oxygen uptake reflects its utilization for fetal metabolic functions since the fetus has no long-term storage of O_2 [14,24]. No data exist on O_2 consumption in MC twins. As previously reported by our group in singletons [24], we could demonstrate, in a relatively steady state condition at the time of elective cesarean delivery, a striking positive correlation between umbilical O_2 delivery and fetal O_2 utilization also in MC twins. This finding demonstrates that MC twins adapt their metabolic rate to the availability of oxygen from the placental supply. Data from experimental animal models and recent findings on human pregnancy show that placental oxygen uptake may represent a limiting factor in the delivery of oxygen to the fetus [26,34-36] but the specific metabolic needs of a monochorionic placenta are unknown.

In our study, second-born MC twins showed significantly reduced O_2 delivery and uptake, both for the absolute and the weight-normalized values, in the order of a 50% reduction.

In a previous study we observed median O_2 delivery per Kg in a term singleton fetus of about 354.9±35.1 μmol/min/kg, compared to 179.7±26.1 μmol/min/kg in fetuses affected by FGR [26]. Looking at our present findings, we can observe that first-born MC twins have relatively high values of O_2 delivery per Kg, but even second-born MC twins show higher values compared to FGR fetuses. These data demonstrate that the placenta of an uncomplicated monochorionic twin pregnancy can provide a proper transfer of oxygen to the fetuses.

Animal studies have shown that fetal O_2 uptake expressed on a weight-specific basis appears similar among mammals, despite considerable differences in gestational age, birthweight and experimental methodology [14]. In uncomplicated singletons under experimental settings similar to our study, median O_2 uptake per neonatal weight was found to be consistently around 250 μmol/min/Kg [24,26,34], slightly higher than our finding of 225.0 μmol/min/Kg (IQR 105.6-335.1) in first-born twins and much higher that the value of 123.3 μmol/min/Kg (IQR 71.1-191.7) in second-borns. Given the proper O_2 supply, lower fetal O_2 utilization in second-born MC twins could indicate a specific metabolic profile of these fetuses, characterized by lower oxidative metabolic rates compared to healthy singletons.

Human FGR fetuses and animal experimental models of chronic hypoxia have shown that the fetus adapts to the adverse intrauterine environment by decreasing O_2 consumption in order to reduce its metabolic rate [26,36]. Similar mechanisms may be employed by MC twins to survive in peculiar intrauterine conditions given by the shared placenta and uterus.

Glucose consumption and metabolic quotient

The estimation of fetal glucose consumption has been largely reported in pregnant sheep models and, very recently, in human pregnancies [14,25,26]. Glucose represents the primary source of energy in fetal life with no demonstrated fetal gluconeogenesis in steady state conditions, leading to major dependency from placental supply [25,37,38].

On the other hand, the human placenta can release glucose in the fetal circulations [25,39]. Michelson and colleagues recently suggested that fetal glucose delivery and consumption are balanced against the placental needs for this substrate, and that placental glucose consumption is a key modulator of materno-fetal transfer of glucose [25]. Hence, high placental needs for glucose limit fetal glucose delivery and consumption. Moreover, previous studies on animal and human pregnancies showed that the utilization of glucose depends on the availability of oxygen [26,40-42]. Indeed, under conditions of mild chronic hypoxia, as in women living at high altitudes, fetal glucose consumption was found to be reduced and it was hypothesized that a greater placental consumption would take place to preserve oxygen supply to the fetus [42]. Similar mechanisms were reported in FGR fetuses [26,40,41].

In the current study we report a positive correlation between fetal utilization of oxygen and glucose also in MC twins, again underlying the close interdependence between oxygen and glucose metabolism during intrauterine life. However, second-born twins showed significantly lower glucose delivery and uptake (with a reduction of about 30 and 25 % for size-weighted values, respectively) compared to first-born twins. Overall, the glucose uptakes values found in the present study appear lower compared to previous findings in uncomplicated singletons, both for absolute and for weight-normalized median values [25,26], and appear much similar to the values we recently reported in FGR fetuses [26]. This finding seems to mimic experimental and in vivo conditions of mild chronic hypoxia, where a reduction in glucose consumption is the consequence of a reduced oxygenation [40,42,43], but the energetic demands of a monochorionic placenta need to be further investigated. In our study, the median glucose/O_2 metabolic quotient was not different in first and second-born twins, and it was similar to the value we recently found in term healthy singletons [26]. The glucose/O_2 metabolic quotient represents a measure of oxidative metabolism of glucose that is independent from the computation of UVBF [14]. Previous studies from our group have shown significantly higher glucose/O_2 metabolic quotients in FGR compared to appropriately grown fetuses [26]. This valuable comparison reveals that uncomplicated MC twins, despite lower O_2 and glucose uptakes compared to healthy singletons, seem to preserve a well-balanced aerobic metabolism of glucose, differentiating themselves from the hypoxic FGR.

Strengths and limitations

MC twin pregnancies constitute an excellent model for perinatal research since they allow optimizing many confounding variables being the twins genetically identical, of the same sex, at the same gestational age and grown in the same maternal environment.

The main strengths of the study are the prospective design and the fact that all cases enrolled were strictly monitored in a third level referral center from early pregnancy to the postnatal period with detailed documentation of maternal, fetal and neonatal parameters. Moreover, the experimental data were obtained by feto-maternal specialists experienced in this innovative field of research.

The normalization of blood flow parameters per kilogram of real neonatal weight represents a further strength of the study, since the use of ultrasound-based estimation of fetal weight to normalize metabolic parameters to the estimated fetal mass implies unavoidable errors, especially when measuring two twins at advanced GA.

A possible limitation is represented by the potential influence of the surgical procedure and the time interval in the birth of the two twins. However, this is not avoidable in human pregnancies, and we tried to limit this potential bias by a very short delivery time interval (2 minutes). Moreover, the differences in birthweight and in umbilical vein blood flow are independent from the surgical procedure and support the findings of the study. A further possible limitation of the present study is the relatively small sample size that may hamper statistical comparisons of biological variables.

Conclusions

We present for the first time the values of UVBF and of estimated fetal O_2 and glucose consumption in uncomplicated MC twins at the time of elective cesarean delivery, under conditions that approximate as closely as possible the undisturbed fetal physiological state.

Despite potential methodological differences, the comparison with the available data on singletons seems to suggest the existence of compensatory mechanisms adopted by MC twins to reduce their metabolic rate and survive in less favorable intrauterine conditions. Moreover, second-born monochorionic twins resemble FGR fetuses, but show different metabolic patterns.

There is still a great deal more to understand whether the deviation from singletons' physiology represents a pathologic process or a physiologic adaptive response intrinsic in MC twins, and our present findings seem to suggest the existence of different mechanisms rather than the chronic-hypoxia model that characterizes the pathophysiology of FGR fetuses.

Moreover, our study demonstrates significant differences in oxygenation and metabolic rates between the two twins of the same uncomplicated MC pregnancy according to birth order. The short birth time-interval in a steady-state condition and the presence of parameters that were independent from delivery (e.g. UVBF and birthweight) hint at pre-existing conditions, which is intriguing given the same environment shared by the identical twins and the shared placenta with supposed homogeneous metabolic needs.

A potential explanation is that the diversities related to the birth-order might be due to the lower umbilical cord insertion of first-born twins, with a greater proximity to the supply from uterine arteries. This could possibly determine, along gestation, differences in perfusion and function of the two placental territories that would ultimately lead to different oxygenation and metabolic profiles between the twins of the same MC pair. While the design of the current study did not allow to validate this hypothesis, further research focusing on the metabolic characteristics of the two placental territories is underway. Moreover, it will be valuable to investigate the impact of a hypometabolic state on the epigenetic adaptation of monochorionic twins, especially if second-born, with its possible short and long-term consequences.

This study was approved by the Institutional Review Board of the Hospital (Comitato Etico Milano Area 1, study code: MCpls_1, final approval 10.22.2019).

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TABLES

Characteristics	Monochorionic twin pregnancies (24)
Age, years	34.0 (28.0-35.7)
Ethnicity, n (%)	Caucasian 20 (83.3) Asian 4 (16.7)
Nulliparity, n (%)	16 (66.6)
Assisted reproductive technology, n (%)	5 (20.8)
Body mass index, kg/m^2	21.5 (19.7-23.1)
Gestational weight gain	14.0 (12.0-20.2)
Gestational diabetes on diet, n (%)	3 (12.5)
Hemoglobin at delivery, g/L	12.0 (10.9-12.9)
Gestational age at delivery, weeks.days	36.4 (36.0-37.0)
Fetal sex (female:male), n	22:26
Placental weight, grams	800 (662-937)
Placental vascular anastomoses per placenta, n	6 (2.5-10)
AV	4 (2-8)
AA	1 (1-1)
VV	0 (0-1)
Placental territory discordance, %	16.1 (9.8-23.7)
Birthweight discordance, %	10.8 (3.8-18.5)

Table 1. General characteristics of the study population.

Median and (IQR) are presented where appropriate.

AV: artero-venous anastomoses; AA: artero-arterial anastomoses; VV: veno-venous anastomoses.

		Overall 48 twins	**First Twin** 24 twins	**Second Twin** 24 twins	**p value**
	Birthweight, grams	2377 (2160-2678)	2585 (2191-2753)	2333 (2110-2578)	**0.015**
	Apgar 5 min	10 (9-10)	10 (9-10)	10 (9-10)	0.32
	NICU admission, %	9 (18.7)	4 (16.6)	5 (20.8)	0.70
Umbilical artery	**pH**	7.32 (7.30-7.34)	7.33 (7.31-7.35)	7.31 (7.29-7.34)	**0.026**
	Lactate, mmol/L	2.0 (1.7-2.3)	2.0 (1.7-2.2)	2.1 (1.7-2.3)	0.337
	pO_2, mmHg	15.9 (12.7-19.4)	16.3 (14.6-20.1)	15.0 (12.0-18.0)	0.276
	pCO_2, mmHg	48.9 (44.7-52.2)	48.4 (44.9-52.3)	50.4 (44.2-52.0)	0.833
	Hb, g/L	14.1 (13.1-15.7)	14.0 (13.2-15.9)	14.3 (13.0-15.5)	0.866
	satO_2, %	31.0 (21.0-45.0)	31.5 (27.3-47.3)	30.0 (19.0-44.0)	0.360
	O_2 content, mmol/L	2.74 (1.78-3.88)	2.84 (2.15-4.03)	2.71 (1.64-3.45)	0.420
	Glucose concentration, mmol/L	51.0 (45.0-56.0)	51.0 (45.0-57.5)	51.0 (45.3-56.0)	0.678
Umbilical vein	**pH**	7.37 (7.34-7.38)	7.37 (7.36-7.38)	7.35 (7.33-7.37)	**0.002**
	Lactate, mmol/L	1.8 (1.6-2.2)	1.8 (1.6-2.2)	1.9 (1.7-2.3)	0.087
	pO_2, mmHg	22.8 (20.6-26.0)	23.8 (22.0-29.3)	21.5 (16.7-25.1)	**0.006**
	pCO_2, mmHg	41.3 (37.8-43.9)	41.0 (37.4-42.8)	43.4 (37.8-46.7)	**0.006**
	Hb, g/L	14.1 (13.2-16.1)	14.1 (13.5-16.2)	14.1 (12.9-15.8)	0.477
	satO_2, %	59.0 (51.0-66.0)	62.0 (55.0-73.0)	55.0 (37.0-62.0)	**0.025**
	O_2 content, mmol/L	5.19 (4.21-6.07)	5.39 (4.45-6.40)	4.89 (2.63-5.78)	0.025
	Glucose concentration, mmol/L	61.0 (54.0-65.5)	61.0 (55.0-64.0)	60.5 (52.0-66.0)	0.560

Table 2. Neonatal outcomes, umbilical venous and arterial hemogasanalyses and oxygen content of the population of 48 uncomplicated monochorionic twins, as a whole and separated according to birth-order.

Median and (IQR) are presented where appropriate.

The p-value refers to the comparison between twin 1 and 2, and bold numbers indicate significant differences.

NICU: neonatal intensive care unit; O_2: oxygen; Hb: haemoglobin.

	Overall 48 twins	**First Twin** 24 twins	**Second Twin** 24 twins	**p value**
UV diameter, cm	0.66 (0.62-0.76)	0.72 (0.64-0.79)	0.64 (0.60-0.69)	**0.000**
UV velocity, cm/sec	17.2 (14.7-20.8)	18.9 (15.3-21.8)	16.5 (13.8-19.2)	0.211
UVBF, mL/min	174.7 (138.0-247.3)	210.6 (170.9-284.4)	159.1 (119.5-177.2)	**0.001**
UVBF per Kg, μmol/min/Kg	76.9 (59.5-91.4)	79.8 (66.7-121.7)	65.6 (52.6-79.2)	**0.007**
O_2 delivery, μmol/min	875.9 (580.2-1352.4)	1334.6 (886.7-1775.4)	625.9 (413.9-843.0)	**0.001**
O_2 delivery per Kg, μmol/min /Kg	347.7 (245.0-516.7)	504.6 (347.7-745.1)	265.0 (203.0-340.9)	**0.010**
O_2 uptake, μmol/min	359.8 (220.0-638.3)	525.9 (280.5-832.4)	286.8 (172.9-421.8)	**0.013**
O_2 uptake/kg, μmol/min/kg	152.3(95.0-246.3)	225.0 (105.6-335.1)	123.3 (71.1-191.7)	**0.031**
Fetal O_2 extraction, %	44.3 (30.2-55.9)	46.5 (34.8-55.7)	44.3 (23.5-58.2)	0.605
glucose delivery, μmol/min	573.1 (436.0-865.1)	746.9 (544.6-1022)	496.7 (385.7-596.3)	**0.002**
glucose delivery/kg, μmol/min/kg	260.3 (187.5-341.4)	286.0 (203.2-417.4)	203.2 (174.3-278.0)	**0.010**
glucose uptake, μmol/min	87.9 (48.7-151.4)	93.8 (61.8-197.6)	74.8 (42.7-98.1)	**0.02**
glucose uptake/kg, μmol/min /kg	36.6 (21.7-56.5)	41.1 (27.3-77.9)	30.9 (18.0-44.6)	**0.007**
Glucose/O_2 metabolic quotient	1.29 (0.95-1.71)	1.30 (1.01-1.63)	1.29 (0.72-1.99)	0.650

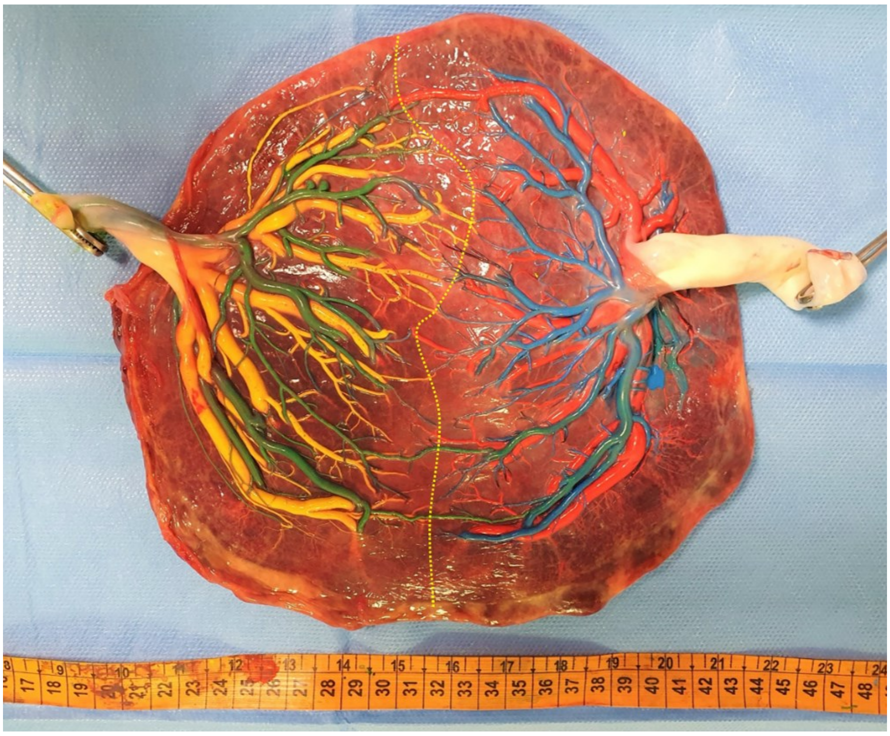
Table 3. Umbilical vein blood flow, oxygen and glucose utilization estimated in 48 uncomplicated monochorionic twins (whole population and according to birth-order).

Median and (IQR) are presented where appropriate

The p-value refers to the comparison between twin 1 and 2, and bold numbers indicate significant differences.

UVBF: umbilical vein blood flow; O_2: oxygen; min: minute.

FIGURES



[color] Figure 1. Monochorionic placenta of an uncomplicated twin pregnancy. In blue and green the arteries, in red and yellow the veins. The yellow dotted line indicates the vascular equator that separates the two placental territories.

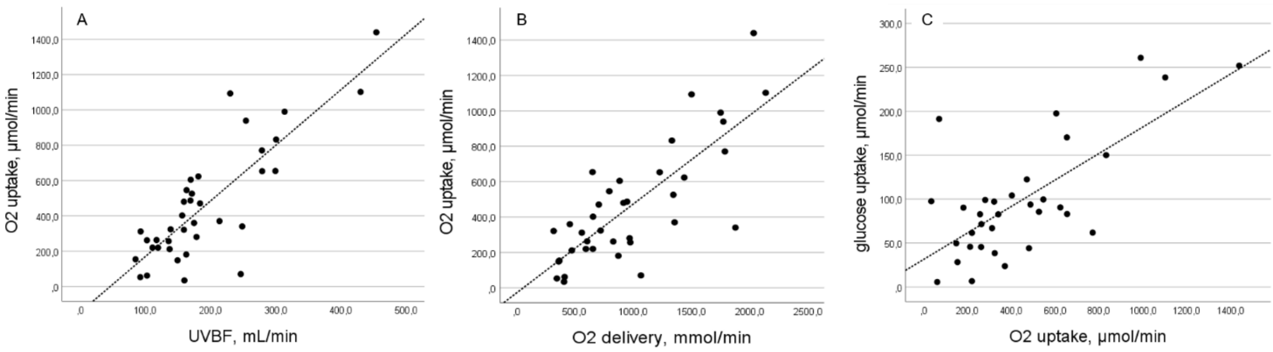


Figure 2. Correlation between: A) umbilical vein blood flow (UVBF) and umbilical oxygen (O_2) uptake (Spearman correlation coefficient of 0.79, $p < 0.001$); B) umbilical O_2 delivery and O_2 uptake (Spearman correlation coefficient of 0.72, $p < 0.001$); C) umbilical O_2 and glucose uptake (Spearman correlation

coefficient of 0.70, $p < 0.001$) in uncomplicated monochorionic twins at the time of planned cesarean delivery.

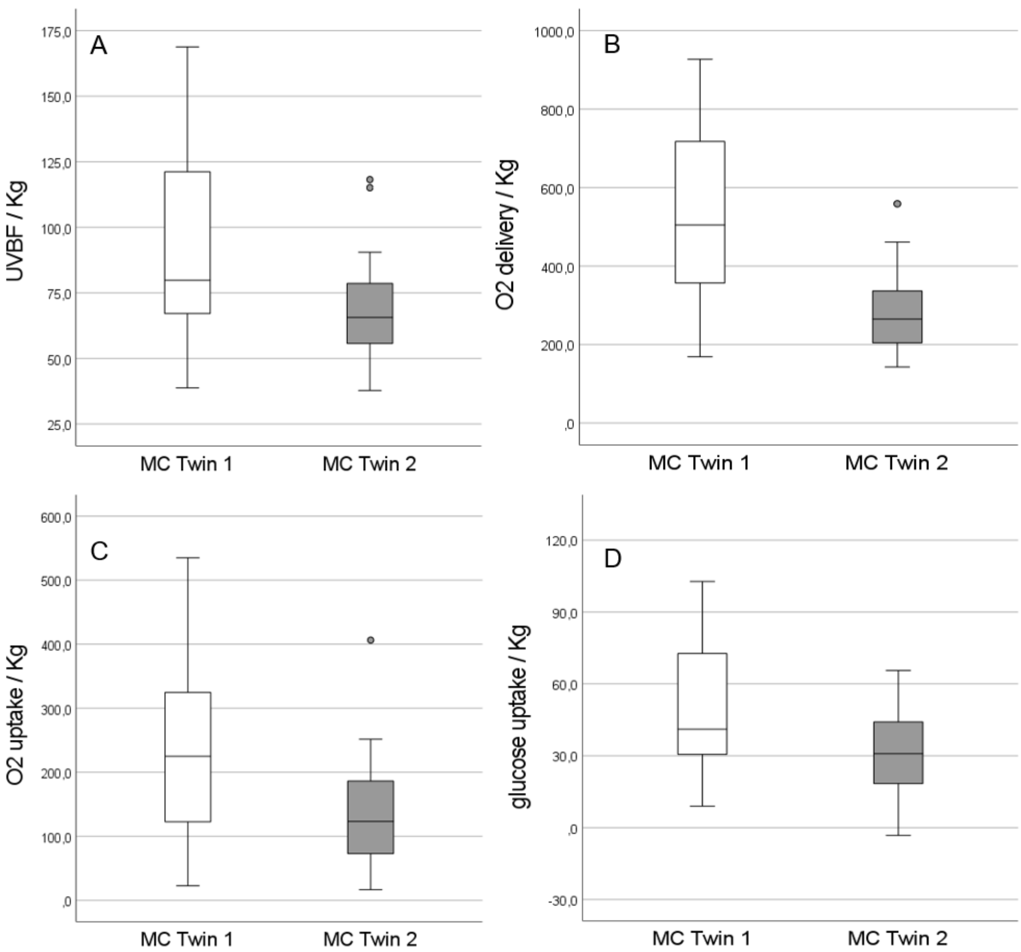


Figure 3. Comparison of: A) umbilical vein blood flow (p 0.01); B) umbilical oxygen delivery (p 0.01); C) umbilical oxygen uptake (p 0.21); D) umbilical glucose uptake (p 0.02) normalized for neonatal weight between first (Twin 1) and second-born (Twin 2) uncomplicated monochorionic twins.