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Validation studies of a fluorescent method to measure placental glucose transport in mice

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ABSTRACT

Introduction: Proper placental function is essential for optimal fetal growth *in utero*. Placental transfer of nutrients to the fetus can be measured using radiolabelled tracers, but non-radioactive methods have potential advantages. This study aimed to develop a fluorescence-based method to measure placental glucose transport in mice.

Methods: Time course and localisation of the IRDye 800CW 2-deoxyglucose were recorded (Lumina IVIS Live Imaging System) following tail vein injection into anaesthetised late pregnant mice. Fluorescent signals in placental and fetal tissues were assessed after injecting conscious dams with 10 nmol IRDye 800CW 2-deoxyglucose (3, 30, 60, 120 min) or vehicle. Specificity of dye uptake was determined by comparing uptake of IRDye 800CW conjugated to 2-deoxyglucose or carboxylate, at 2 and 24 h. Finally, we assessed relationships of fetal size and umbilical blood flow velocities with relative dye uptake.

Results: In late pregnant mice, uterine fluorescent signal localised rapidly over placentas and remained consistent for > 1 h. Signal intensity in whole and homogenised tissues increased in fetuses and decreased in placentas after 3 min and stabilised by 30 min post-injection. Relative fetal dye uptake at 2 and 24 h was greater in littermates with the highest compared to lowest placental efficiency; signals were similar for 2-deoxyglucose- or carboxylate-conjugated dyes. Relative fetal dye uptake correlated positively with fetal weight and placental efficiency and negatively with umbilical artery resistance indices.

Conclusions: Fetal uptake of IRDye 800CW correlates with markers of placental blood flow and fetal growth, but does not specifically measure placental glucose transport.

1. Introduction

One of the major determinants of intrauterine growth is the placental supply of nutrients to the fetus, which occurs primarily by diffusion and transporter-mediated transfer [1]. The capacity of the placenta to facilitate this nutrient exchange depends on its size, morphology and blood flow as well as the abundance and activity of nutrient transporters [2,3]. There is now considerable evidence that poor placental function is a major contributor to restricted fetal growth [4]. Although the placenta can adapt morphologically and functionally to fetal signals of nutrient demand [5–7], inadequate placental function can result in intrauterine growth restriction (IUGR) wherein the fetus fails to reach its genetically pre-determined growth potential [8,9]. Low birthweight (LBW, < 2.5 kg) and IUGR fetuses are at a higher risk of fetal mortality and morbidity, both perinatally and later in life [10–14].

One of the most widely used methods of assessing placental efficiency is the measurement of placental nutrient transporter expression and activity. Isolated placental vesicles from human pregnancies complicated by IUGR typically exhibit decreased placental activity of system A amino acid transporters [15]. While not differentially expressed in placentas from human IUGR and normally-grown fetuses [16], placental protein abundance of the facilitated glucose transporter

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solute carrier family 2 member 1 (Slc2a1, previously known as GLUT1) is lower in experimentally-induced rat IUGR pregnancies at late gestation compared to sham controls [17]. Conversely, in non-manipulated murine pregnancy, small placentas are able to support fetal growth by increasing the expression of nutrient transporters when compared with larger placentas [5]. Near term, at 19 days after mating (e19), although not at e16, the lightest placentas had 20–40% higher expression of *Slc2a1* and the placental solute carrier family 38, member 2 (Slc38a2/SNAT2), an isoform of System A amino acid transporter, compared to the heaviest placentas within the litter [5]. Compared with the heaviest placenta, active transfer of [^{14}C]methyl aminoisobutyric acid (MeAIB) per gram of placenta was ~40–50% greater in the lightest placentas of the litter, at both e16 and e19, when mouse fetuses are growing most rapidly in absolute terms [5]. However, despite greater transported expression of *Slc2a1*, materno-fetal transfer of [^{14}C]glucose per gram placenta did not differ between the lightest and heaviest placentas in the litter [5]. Along with alterations in placental morphology, such changes in placental function are considered to be adaptations that aim to match nutrient delivery to fetal demand. As a result of these alterations, around 30% more fetus is produced per gram of placenta by the lightest than the heaviest placenta in the litter in a normal mouse litter [5]. Consequently, the fetal:placental weight (FW:PW) ratio is used as a marker of placental efficiency [5,6,18].

While measuring protein levels can provide an indication of transport capacity, direct assessment of nutrient transfer requires the use of labelled substrates. Alterations in the passive and active placental transport of solutes in experimental models of IUGR has been demonstrated by quantifying the maternofetal transfer of radiolabelled tracers including [^{14}C] inulin, [^{3}H] methyl-D-glucose (MG) and [^{14}C] aminoisobutyric acid (AIB) [7,19,20]. An alternative approach to this use of radiolabelled substrates is to use fluorescently labelled compounds. These offer a number of potential advantages over radioisotopes, including fewer constraints on handling and disposal, and the availability of many emission spectra and therefore ability to measure greater numbers of substrates simultaneously [21]. Additionally, live course imaging can be performed by tracking the movement of the label in real-time as well as obtaining better signal localisation and stability [22]. One such commercially available fluorescently labelled substrate is IRDye 800CW 2-DG Optical Probe (LI-COR Biosciences, Lincoln, NE, Lincoln, NE). The fluorescent dye is conjugated to 2-deoxyglucose (2-DG), which is a non-metabolisable glucose analogue that remains within cells after phosphorylation [23]. Uptake of IRDye 800CW 2-DG in human tumour cell lines with high and low metabolic rates and in mouse adipocytes is dose-dependent and can be blocked by incubation with unlabelled glucose, 2-DG or monoclonal antibodies raised against Slc2a1 [24]. This probe has been previously used to study *in vitro* and *in vivo* glucose uptake in cancer research, including in rodents [24,25]. To date, there are no non-radioactive methods available to study placental transport. Therefore, the current study aimed to develop and validate a fluorescence-based method to measure placental glucose transport in mice using the IRDye 800CW 2-DG.

2. Methods

2.1. Ethics and animal management

Experiments One and Two: Experimental procedures were approved by the University of Adelaide Animal Ethics Committee (M-2014-168) and carried out in accordance with the Australian code of practice for the care and use of animals for scientific purposes [26]. C57BL/6J virgin female mice aged 10 weeks were obtained from the Animal Resource Centre, Perth and housed at ~23 °C with 12 h:12 h light:dark lighting cycle (lights on 06:00 h). Access to water and meat-free rat and mouse diet (14.0 MJ/kg, 20% protein, Speciality Feeds, Glen Forrest, Australia) was *ad libitum* and mice were weighed daily throughout the experiment. Estrous cycle stages were confirmed by daily monitoring of vaginal cell types collected by gently flushing the vagina with 10 μL of saline [27]. To generate timed pregnancies, females in either proestrus or early estrus were placed overnight with a mature adult CBAF1 male. Pregnancy was determined by the presence of a vaginal plug (day 0.5 after mating, e0.5) and/or weight gain, and confirmed at post-mortem.

Experiment Three: CBAF1 virgin female mice aged 10–12 weeks were obtained from the Animal Resource Centre, Perth and housed at ~23 °C on a 12 h:12 h light:dark lighting cycle (lights on 0700 *h*), with *ad libitum* access to Teklad global soy protein-free extruded rodent diet (irradiated; 13 MJ/kg, 18.4% protein, Envigo, Huntingdon, UK). Mice were weighed daily throughout the experiment. Timed pregnancies were generated and confirmed as described above, with commencement of breeding delayed until ~17–20 weeks of age due to a shift of animal facilities.

Experiment Four: CBAF1 virgin female mice aged 10–12 weeks were obtained from the Animal Resource Centre, Perth and housed and bred under the same conditions as those described above for experiment three.

2.2. Experiment 1 – live imaged time course

In order to confirm our ability to detect fluorescent signals over the conceptus regions and the timeframe for the appearance of signals, pregnant mice aged 15–18 weeks at e17.5 were anaesthetised by intraperitoneal injection of 20 mg/mL Avertin (2,2,2-tribromoethanol and tert-amyl alcohol; Sigma-Aldrich, Missouri, United States) immediately after being injected with 10 or 20 nmol of fluorescently-labelled 2-DG (LI-COR Biosciences) dye (n = 2/dose) diluted in a total volume of 50–100 μL phosphate buffered saline (PBS), intravenously into the tail vein. This was followed by uterine exteriorisation to allow serial live imaging at 3–5 min intervals under isofluorane anaesthesia for approximately 1 h post-injection using the IVIS imaging system (wavelengths: Ex 760 nm/Ex 790 nm, IVIS Lumina XRMS system, Perkin Elmer). An additional animal was imaged without dye injection to confirm the absence of non-specific signal. Dams were then humanely killed by cervical dislocation. Fluorescent emission signals in a region encompassing the middle conceptus of each horn were measured in each animal throughout each time course. Background signal was measured in an area adjacent to each mouse and was subtracted from the signal measured over the conceptus.

2.3. Experiment 2 – time course of placental and fetal dye uptake in conscious mice

In order to determine the time course for placental and fetal uptake of IRDye 800CW 2-DG following maternal tail-vein injection, 16–25 week old dams, pregnant at e17.5, were injected with 10 nmol of IRDye 800CW 2-DG in 50 μL PBS (intravenous, tail vein injection). Injected mice were returned to their home cage for 3, 30, 60 or 120 min (n = 2–3/group) before being anaesthetised with Avertin and humanely killed by cervical dislocation. Individual fetuses and placentas were dissected, weighed and imaged using the IVIS imaging system, using settings as described above. Background signal measured in an area adjacent to each tissue was subtracted from tissue fluorescent signals. Immediately after being imaged, all fetuses were snap-frozen in liquid nitrogen, while placentas were alternately snap-frozen or fixed in 4% paraformaldehyde with 2.5% polyvinylpyrrolidone-40 in 70 mM phosphate buffer for 24 h. Samples were subsequently washed in four changes of 1 X PBS over 48 h, incubated in 30% sucrose solution for 2 h before being embedded in Optimum Cutting Temperature compound (OCT, Tissue-Tek, Sakura Finetek Japan Co. Ltd., Tokyo, Japan).

To develop a method to measure fluorescent signals in stored tissues, frozen placentas and fetuses were homogenised in radioimmunoprecipitation assay (RIPA) buffer (20 mM Tris (pH 8.0), 150 mM NaCl, 1% octylphenoxypolyethoxyethanol (IGEPAL), 0.5% sodium deoxycholate, 5 mM Ethylenediaminetetraacetic acid (EDTA)

and 0.1% sodium dodecyl sulfate) using the PowerLyzer 24 (Mo Bio Laboratories, Inc. Carlsbad, CA). The placentas were homogenised for 3 cycles of 15 s duration each, at 3500 rpm with pause/dwell time of 30 s. The fetuses were homogenised using the same settings, except that the cycle number was increased to 10. For each sample, 50 μL of homogenate and RIPA buffer was pipetted in triplicate into an opaque 96-well plate, before being imaged with the Odyssey CLx Infrared Imaging System (LI-COR Biosciences, Lincoln, NE) using the 800 nm channel at 42 μm resolution and a 4.0 mm focus offset. Background values were measured in wells containing only RIPA buffer. Images were analysed using Image Studio™ Lite (LI-COR Biosciences, Lincoln, NE) image processing software. The triplicate fluorescence emission values (minus background values) from each respective fetus and placenta were averaged and expressed as signal intensity per fetus or placenta. Fetal signals were also expressed relative to placental weight. Relationships between signals obtained using the IVIS imaging system on whole tissues and signals obtained using the Odyssey CLx Infrared Imaging System on homogenised tissues were evaluated by Pearson's correlation.

2.4. Experiment 3 – specificity of dye uptake

In order to test the specificity of dye uptake, we compared fetal and placental fluorescent signals after maternal tail vein injection with 0.2 nmol/g body weight of IRDye 800CW 2-DG or IRDye 800CW carboxylate (LI-COR Biosciences, Lincoln, NE) in PBS (total volume 50–100 μL) in mice aged 20–21 weeks at e17.5-e18.5 of pregnancy. IRDye 800CW carboxylate contains the fluorophore conjugated to carboxylate and is provided by the manufacturer as a negative control to the IRDye 800CW 2-DG [22,24]. When injected into tumour-bearing mice, fluorescent signals 18–72 h post-injection were ~3-fold higher for IRDye 800CW 2-DG than for IRDye 800CW carboxylate, and IRDye 800CW carboxylate is completely cleared from non-tumour-bearing mice within 48 h post-injection [24]. Dams were humanely killed at 2 h post-maternal injection (n = 3/group), based on the results of our previous experiments, and at 24 h post-maternal injection (2-DG: n = 6; carboxylate: n = 4), based on data collection times used in previous *in vivo* studies using this probe [24]. Fetal and placental tissues were dissected, weighed and snap-frozen for subsequent homogenisation and tissue from the fetuses with the highest and lowest placental efficiency (FW:PW ratio) of each litter was imaging using the Odyssey CLx Infrared Imaging System as described above. Data from fetuses within a litter were treated as repeated measures of the dam. A repeated measures ANOVA was performed to compare fetal dye uptake (fetal fluorescent signal per gram placenta) between dyes and between the littermates with the highest and lowest placental efficiency of each litter (within litter factor), separately for litters collected at 2 and 24 h.

2.5. Experiment 4 – relationship between fetal dye uptake and fetal blood flow

To assess the relationship between fetal dye uptake and indices of fetal blood supply, CBAF1 dams at 14–18 weeks of age were injected (tail vein) with 0.2 nmol/g body weight of IRDye 800CW 2-DG at e17.5. Approximately 24 h after maternal-dye injection, umbilical artery blood flow velocities were assessed by Doppler ultrasound, using the Vevo 3100 Ultrasound Biomicroscope (FUJIFILM VisualSonics, Toronto, Ontario, Canada) with a 32–55 MHz transducer probe, as described previously [28]. Mice were anaesthetized with isoflurane (5% induction, 1.5% maintenance, in medical air). Doppler ultrasound recordings were taken from the umbilical arteries of at least two, and where possible, four fetuses per dam. Recordings were analysed for peak systolic velocity (PSV), time-averaged velocity (TAV), end diastolic velocity (EDV), and fetal heart rate from 3 consecutive cardiac cycles (Fig. 1). [29]. Resistance index (RI = [PSV − EDV]/PSV) and pulsatility index (PI = [PSV − EDV]/time averaged velocity [TAV]) were calculated for each fetus. Following Doppler imaging, dams were humanely killed by cervical dislocation, and fetuses and placentas dissected and weighed. Fetal size (abdominal circumference, crown to rump length, and head width) was also measured, prior to snap-freezing of fetal and placental tissues for subsequent homogenisation and imaging using the Odyssey CLx Infrared Imaging System as described above. Relationships of fetal dye uptake (fetal signal per gram placenta) with umbilical blood flow parameters and fetal weight and size were assessed by Pearson's Correlation analysis using data for each individual fetus.

3. Results

3.1. Experiment 1 – live imaged time course

In the uterine region of anaesthetised e17.5 pregnant C57Bl6/J mice, fluorescent signal localised rapidly to the placenta after maternal tail vein injection with IRDye 800CW 2-DG (Fig. 2). Fluorescent signals could be readily detected in the conceptus regions of mice injected with 10 or 20 nmol dye and signal intensity remained stable in individual animals for > 1 h (Supplementary Fig. 1).

3.2. Experiment 2 – time course of placental and fetal dye uptake in conscious mice

Signal intensity from whole fetuses by IVIS imaging was initially low and placental signals were high at 3 min and then remained stable between 30 and 120 min following maternal tail vein injection (Fig. 3A and B). Fluorescence emission measured in homogenised fetuses and placentas followed a similar pattern over time (Fig. 3C and D). Fluorescence signals obtained from the same tissues using the two approaches were strongly positively correlated (fetal tissues: $p < 0.001$; $R = 0.662$; placental tissues: $p < 0.001$; $R = 0.813$).

3.3. Experiment 3 – specificity of dye uptake

Fluorescent signals were higher in the highest compared to lowest placental efficiency littermates regardless of dye type at 2 h (Fig. 4A) and 24 h post-maternal injection (Fig. 4B). Signal intensities were similar in fetuses from dams injected with 2-DG- or carboxylate-conjugated IRDye 800CW at each time point (Fig. 4A and B). Differences in signal intensity between lowest and highest placental efficiency littermates did not differ between dye types at either time point (Fig. 4A and B).

3.4. Experiment 4 – relationship between fetal dye uptake and fetal blood flow

Fetal dye signal relative to placental weight correlated positively with fetal weight (Fig. 5A) and FW:PW ratio (Fig. 5B), fetal head width ($p < 0.001$, $R = 0.533$) abdominal circumference ($R = 0.514$, $P < 0.001$) and crown to rump length ($p < 0.001$, $R = 0.582$), and negatively with umbilical pulsatility (Fig. 5C) and umbilical resistance (Fig. 5D) indices at e18.5 of gestation. Additionally, relative fetal dye uptake correlated positively with umbilical end-diastolic velocity ($p = 0.005$, $R = 0.475$) and fetal heart rate ($p = 0.003$, $R = 0.515$), but was not correlated with peak systolic velocity ($p = 0.112$, $R = 0.282$).

4. Discussion

This is the first study to test the use of a fluorescently-labelled dye for measuring placental nutrient transport. As originally aimed, we developed a method to quantify feto-placental uptake of the near-infrared IRDye 800CW 2-DG dye in term mice. Consistent with reported within-litter differences in uptake of radiolabelled glucose in mice [5,20], fetal uptake of IRDye 800CW 2-DG relative to placental weight was greater in littermates with high compared to low placental

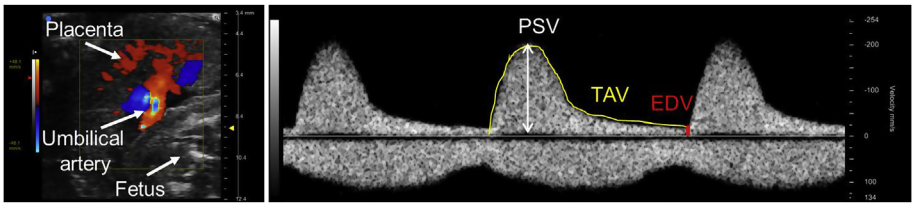

Fig. 1. Umbilical blood flow characteristics at e18.5 in naturally-mated CBAF1 dams were characterised for 3–4 individual fetuses per litter.

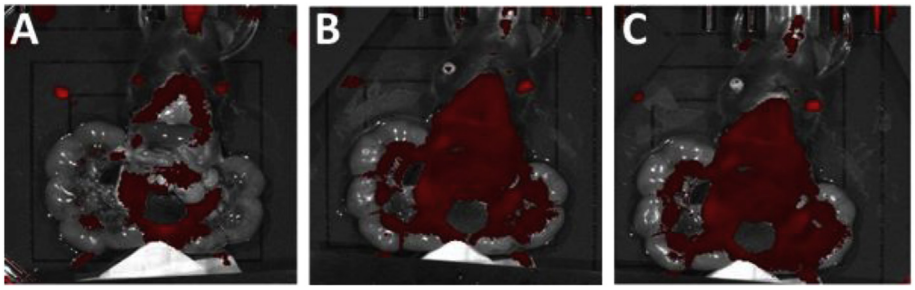

Fig. 2. Fluorescent signal detected by the Lumina IVIS Live Imaging System (ex 760 nm/em 790 nm) in anaesthetised C57Bl6/J mice on day e17.5 of pregnancy at 8 (A), 38 (B) and 68 (C) min after tail vein injection with IR Dye 800CW 2-DG.

efficiency, and correlated positively with fetal size. Fetal uptake of dye relative to placental weight also correlated negatively with markers of impaired placental function, including umbilical blood flow resistance indices. Although these data suggested that uptake of IRDye 800CW 2-DG provides a proxy measure of placental function, signals were similar whether the fluorophore was conjugated with carboxylate (negative control) or glucose, implying that uptake is at least in part via other mechanisms than glucose transporters.

Placental efficiency, commonly defined as the ratio of fetal to placenta weight (FW:PW ratio), acts as a proxy measure of placental function up to that stage of gestation [5,6,18]. The characteristics of a highly efficient placenta include a large surface area for exchange and appropriate expression and activity of nutrient transporting proteins to ensure optimum nutrient delivery to the fetus [2,6,30]. In the present study, we observed differences in fetal uptake of IRDye 800CW 2-DG based on the relative placental efficiency of littermates within near-term mouse litters. Consistent with the results of radioisotope tracer studies [5,20], the uptake of the 2-DG dye was significantly higher in the fetuses with the highest compared to the lowest placental efficiencies within the litter. Furthermore, we found that fetal accumulation of the dye relative to its placental weight, was positively correlated with fetal biometric parameters at birth, including birthweight, head width, abdominal circumference and crown to rump length. Together, these data imply that the uptake of the fluorescent 2-DG dye provides

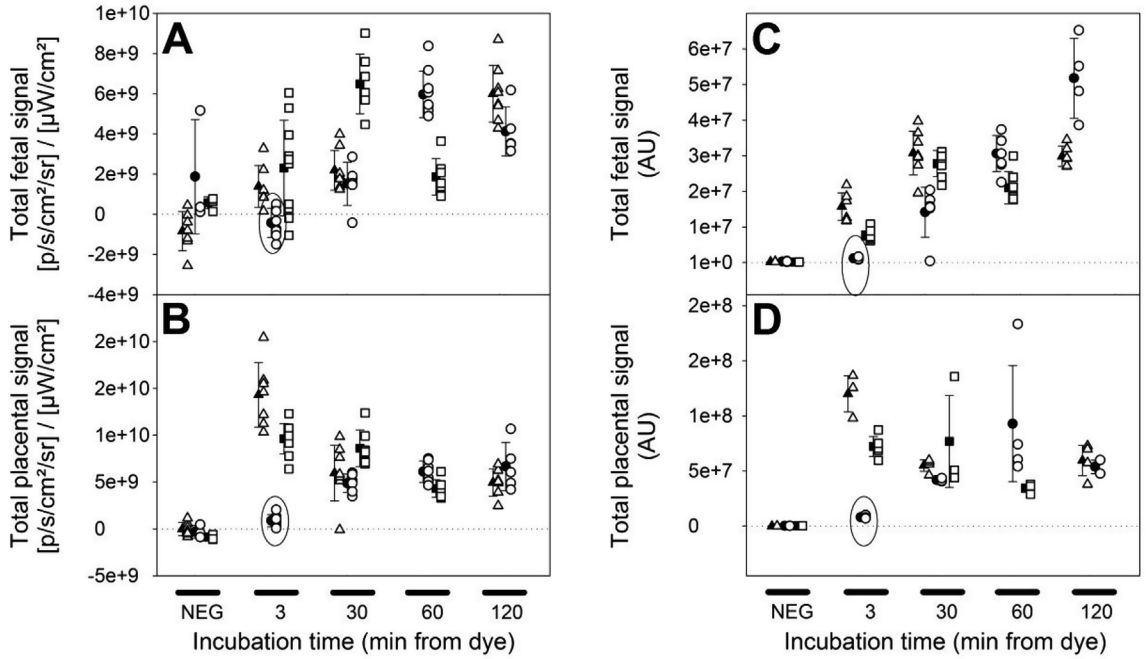

Fig. 3. Fluorescent signal was measured by IVIS in whole fetuses (A) and placentas (B) and in homogenised fetuses (C) and placentas (D) by Odyssey CLx (B). At e17.5 of pregnancy, C57Bl6/J mice were injected with 10 nmol IR Dye 800CW 2-DG via the tail vein and humanely killed at 3–120 min after injection. Each cluster of symbols represents a separate litter. Filled symbols show litter averages, unfilled symbols show individual fetal and placental data within each litter and the black oval indicates a litter where dye was inadvertently injected into tail sheath (n = 2–3 dams and litters for each time point). NEG indicates control animals injected with PBS. Placental fluorescence data from Odyssey measures (D) was only available for frozen placentas from each litter.

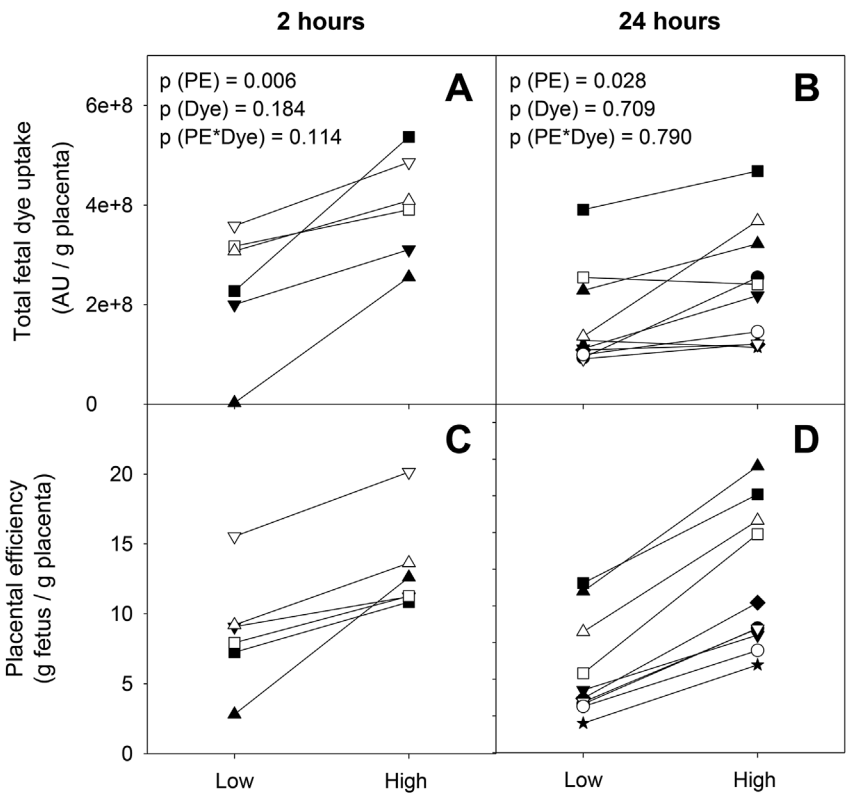

Fig. 4. Fluorescent signals (A, B) measured by Odyssey CLx Infrared Imaging System (800 nm channel), in fetuses (from CBAF1 dams at e17.5–18.5) that had highest and lowest placental efficiency (PE; defined as FW:PW ratio) within the litter. Dams were tail vein-injected with IRDye 800CW 2-DG (black symbols) or IRDye 800CW carboxylate negative control dye (white symbols), and fetuses collected either 2 h (A, C) or 24 h (B, D) post-maternal injection. Panels C and D show corresponding data for placental efficiency – the pairs of littermates on panels A and C and panels B and D share the same symbols. Results are presented as individual data, littermates are linked by solid lines.

an indirect measure of nutrient delivery to the fetus. Ultrasonography is often used in clinical practice to monitor placental function by assessing the resistance to blood flow in the feto-placental unit. In normal pregnancies, low impedance in the umbilical artery allows continuous and stable forward flow throughout the cardiac cycle [31]. Maternal, placental or environmental conditions that negatively influence uterine blood flow are often detected by abnormal umbilical artery Doppler velocimetry, and are associated with pregnancy complications including IUGR and preeclampsia [32,33]. In these complications, fetal delivery of nutrients that cross the placenta by diffusion is often reduced due to low uterine blood flow [34,35]. Hence, as part of the current study, we recorded and analysed fetal umbilical arterial waveforms in late gestation (e18.5) in our final experiment. Even though our investigations focussed on normal mouse pregnancy, and did not include studies of experimental IUGR, we found that lower resistance indices in the feto-placental unit were associated with higher fetal uptake of IRDye 800CW 2-DG per gram of placenta.

Although IRDye 800CW 2-DG uptake was greater in high than low placental efficiency littermates and correlated positively with markers of fetal growth and negatively with umbilical resistance indices, total and differential uptake of the fluorophore were similar regardless of whether it was conjugated to 2-DG or carboxylate. This was unexpected, since in non-pregnant mice, uptake and retention of IRDye 800CW 2-DG 24 h post-injection is ~3–4-fold higher than that of IRDye 800CW carboxylate in kidneys, muscle and liver [24]. Similarly, uptake and retention of these dyes differ in murine cancer. In mice with unilateral brain tumours, fluorescent signals imaged in anaesthetised animals 24 h after injection with IRDye 800CW 2-DG were also ~3-fold greater in the tumour region than in the contralateral brain region, thought to reflect higher glucose metabolic demand within the tumour [22]. Fluorescent signals after injection with IRDye 800CW carboxylate were ~3-fold lower than those in mice injected with the 2-DG-conjugated dye, and were not different in tumour and non-tumour sides of the brain [22]. In our initial live imaging studies, we observed high fluorescence intensity in the bladder area of dams injected with the 2-DG dye (data not shown), suggesting rapid renal clearance. Although the renal threshold for glucose in mice has not been clearly stated in literature, the available data suggest that in rodents, glucose excretion

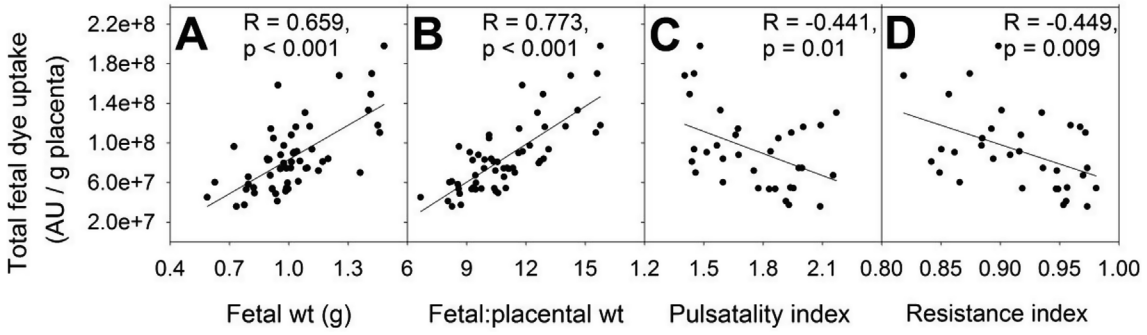

Fig. 5. Correlations between fetal dye signal per gram of placental weight and fetal weight (A), fetal:placental weight ratio (B), and umbilical artery pulsatility (C) and resistance (D) indices. Symbols show data from individual fetuses derived from nine independent litters; fetal and placental weight data was available for all 56 viable fetuses and umbilical artery Doppler ultrasound data was obtained in 33 fetuses.

through urine only occurs at concentrations > 22.2 mmol/L (400 mg/dL) [36]. The maximum amount of the dye injected to dams in this study was 20 nmol, which in a blood volume of approximately 2 mL in a heavily pregnant 35 g mouse should increase circulating glucose by approximately 0.01 mmol/L. Therefore the dye administration itself is not expected to have caused the mice to reach the renal clearance threshold. When measuring the tissue distribution of the IRDye 800CW carboxylate in male and female rats, the highest degree of uptake occurred in the kidneys, regardless of sex [37], also suggesting renal clearance of the fluorophore. In the same study, uptake of the IRDye 800CW carboxylate was markedly higher in the ovaries compared to the testes [37]. SLC2A1 transporter-dependent uptake of IRDye 800CW 2-DG has been previously validated *in vitro*, where it can be inhibited by addition of antibodies raised against SLC2A1, as well as by addition of unlabelled glucose or 2-DG [24]. This is interesting, given that IRDye 800CW 2-DG is estimated to be too large to pass through the predicted three-dimensional model of the SLC2A1 channel [38]. Given these data, and the findings of the current study, it is possible that IRDye 800CW 2-DG is taken up by transporters that are not glucose-specific, and that are co-expressed in the kidneys and the female reproductive tract in rodents. However, further cell assays will need to be performed in order to investigate the mechanisms involved in the cellular uptake of the IRDye, including the placenta. Confirmation that placental IRDye 800CW 2-DG transport is glucose transporter-independent requires inhibition studies using unlabelled glucose or 2-DG or antibodies to glucose transporters in placental cells or cell lines, similar to previous studies in tumour cells [24].

In conclusion, the lack of differential uptake between 2-DG- and carboxylate-conjugated dyes indicate that the IRDye 800CW 2-DG is not able to specifically measure placental glucose uptake via glucose transporters in mice. The use of radio-labelled tracers therefore remains the optimal method to measure placental transport of specific nutrients. However, in term mice, IRDye 800CW 2-DG signals can differentiate between littermates of high and low placental efficiencies, and its relative fetal uptake correlates positively with fetal growth parameters and negatively with markers of impaired placental function. This establishes a fluorescence-based method to indirectly measure overall placental function and nutrient supply to the fetus in murine pregnancy.

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Disclosures

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.01.014.

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