**Placental perfusion and mathematical modelling**

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**Abstract**

The isolated perfused placental cotyledon technique has led to numerous advances in placental biology. Combining placental perfusion with mathematical modelling provides an additional level of insight into placental function. Mathematical modelling of perfusion data provides a quantitative framework to test the understanding of the underlying biology and to explore how different processes work together within the placenta as part of an integrated system. The perfusion technique provides a high degree of control over the experimental conditions as well as regular measurements of functional parameters such as pressure, solute concentrations and pH over time. This level of control is ideal for modelling as it allows placental function to be studied across a wide range of different conditions which permits robust testing of mathematical models. By placing quantitative values on different processes (e.g. transport, metabolism, blood flow), their relative contribution to the system can be estimated and those most likely to become rate-limiting identified. Using a combined placental perfusion and modelling approach, placental metabolism was shown to be a more important determinant of amino acid and fatty acid transfer. In contrast, metabolism was a less important determinant of placental cortisol transfer than initially thought. Identifying the rate-limiting factors in the system allows future work to be focused on the factors that are most likely to underlie placental dysfunction. A combined experimental and modelling approach using placental perfusions promotes an integrated view of placental physiology that can more effectively identify the processes leading to placental pathologies.

**Introduction**

The placental perfusion system is an indispensable experimental model of placental function. Placental perfusions provide data from an intact system, and the data collected represents the integrated product of multiple aspects of placental function, including blood flow, membrane transport and metabolism. This integration makes the data collected from the perfused placenta biologically relevant in a way that many model systems are not [1]. Understanding how these different processes interact is difficult, and it can be challenging to determine the relative contribution of different processes or whether all the essential processes have been identified. Mathematical modelling of placental function provides a quantitative framework in which to explore how well our understanding of the placenta explains its behaviour and to predict which processes are the most likely to impair placental function in a way that affects fetal growth.

The placenta provides the fetus with nutrients, removes fetal waste products, protects the fetus from harmful compounds in the maternal blood and mediates adaptation of maternal physiology to support the pregnancy. Fetal development is entirely dependent on placental function, and impairment in placental function may have health implications that extend beyond pregnancy and across the life course [2]. In order to identify, prevent and treat placental dysfunction, an integrated understanding of the factors that determine placental function is required and combining mathematical modelling and placental perfusion provides an effective way to achieve this.

The factors that determine placental function are complex and interdependent. For instance, placental metabolism depends on the delivery of substrates by blood flow and membrane transport. Mathematical modelling provides a structured approach to study these relationships and test the biological understanding of the system as represented within the model [3]. Models that can effectively predict biological behaviours suggest that the underlying biological understanding on which they were constructed is correct, or at least that it includes the essential factors. Models that cannot predict biological behaviours suggest there may be a misunderstanding about the underlying biology, but also provide a mechanism to explore what this might be. For instance, initial models of amino acid and lipid transport did not work effectively as the role of placental metabolism had been underestimated [4, 5].

This review will address how the placental perfusion system has been combined with mathematical modelling to improve the understanding of placental function and how this approach could be developed in the future.

**The placental perfusion technique**

Placental perfusion is now widely used to study multiple aspects of placental function. Most laboratories using the placental perfusion technique today base their approach on the work of Professor Henning Schneider whose work demonstrated the potential of this model [6-8]. In the placental perfusion system, an intact cotyledon is identified in a freshly delivered placenta, and the fetal artery and vein supplying the lobule are catheterised and perfused with physiological buffer. Where fetal recovery is sufficient to indicate the system is not leaky, catheters are inserted into the maternal surface of the placenta to represent the maternal spiral arteries and again perfused with physiological buffer. Perfusions can be performed for many hours while maintaining constant metabolic parameters [9]. Depending on the needs of the experiment, the circulation can be established on the maternal and fetal sides with open (not recirculated) or a closed (recircuited) configuration, and this can be changed during the experiment [5].

The maternal and fetal circulations are typically perfused independently, so the flow rate, gas mixture, and the composition of the buffer can be varied independently in each circulation and also with time. The perfusion system allows for multiple measurements to be made across the course of the experiment and from different locations (e.g. maternal and fetal artery or vein). The biological parameters that can be measured in the perfusion system depend on the equipment and assays available (Table 1). Samples of perfusion buffer can be taken from, or sensor probes can be placed in the arterial and venous sides of the maternal, and fetal circulations. Sensor probes (e.g. for oxygen) can also be inserted into the intervillous space. Arterial perfusion pressures can be recorded as well as flow rates. Tissue can also be sampled and analysed at the end of the experiment. Additionally, experimental conditions can be further adjusted by adding transporter or metabolic inhibitors or vasoactive compounds.

As an example, different concentrations of glucose could be added to the buffer flowing into the maternal artery and glucose and its metabolic product lactate measured in the maternal and fetal veins to assess the uptake, metabolism and transfer of glucose [10]. In this glucose experiment, an oxygen electrode could be placed in the intervillous space or the fetal vein catheter to determine if arterial glucose supply affected oxygen consumption.

The flexibility provided by the perfusion technique provides a large amount of control and data to support mathematical modelling. The ability to vary the conditions and to measure multiple parameters across time is ideal for mathematical modelling as measurements collected under multiple input conditions provide the best validation for the model.

***Mathematical models of placental function***

There are many different types of mathematical models. The type of models discussed here are physiologically based mechanistic models which seek to represent the underlying biology. A model of placental function will typically contain several more focused models which represent different aspects of the system. For instance, a compartmental model (where the mother, placenta and fetus are represented as simple well-mixed compartments (Figure 1a)) might need to model blood flow, membrane transport and metabolism separately and combine these to produce the model of the overall system [11]. Where more detailed anatomical structures are available, a more complex geometric model could be produced representing the structure of the placenta and incorporating more sophisticated blood flow modelling than used in compartmental models (Figure 1b) [12].

Before making complex composite models, it is important to test the simpler models of individual processes to ensure they are producing meaningful outputs. For instance, models of membrane transport proteins need to be tested to ensure they can accurately represent biological behaviour [13]. In doing this, modelling can reveal novel information about the function of specific transporters such as the demonstration that the amino acid exchanger LAT2 is not an obligate exchanger [14]. When obligate exchangers transport a substrate across the membrane in one direction then another molecule must be transported in the other direction. but modelling LAT2 activity showed that this is not always the case for this transporter.

Many placental functions involve many different processes (flow, transport and/or metabolism) and modelling can be used to predict which factors are likely to be rate limiting for transfer. Sensitivity analysis can be performed within an experimentally validated model in which the different parameters are altered and the overall effect of each parameter on transfer is estimated (Figure 2). This approach can help identify the factors that are most likely to lead to placental dysfunction.

To test the models experimentally it is important to vary the experimental conditions as a good model will be one that can correctly predict behaviours across a wide range of experimental conditions (Table 1). Typically, the parameters should be tested within the physiological range, but extending these beyond the normal range can help test the models ability to represent the biological system. For example, the use of sub-physiological tracer concentrations of substrate has still allowed meaningful data to be derived about physiological function.

Another approach to modelling placental function is to use phenomenological models which seek to represent biological behaviour but are not based on underlying biological mechanisms. For instance, a phenomenological model might be based on an observation that the clearance of a drug by the placenta was 15%, and so predict that transfer would be 15% of the maternal plasma concentration. While this phenomenological approach is practical and requires less input information, it has limited predictive power, especially where any other parameters have changed (e.g. blood flow, transporter activity, transporter interactions). Another limitation of these phenomenological models is that they will not contribute to an understanding of underlying biology. When looking at models of placental transfer, especially those of drug transfer, it is important to be aware of what sort of model is being used [15, 16].

**Modelling placental transfer of amino acids**

The placental perfusion model has been the focus of considerable work into placental amino acid transport and metabolism [9, 17, 18]. A key contribution of the perfusion model was that it was used to demonstrate how net amino acid transfer to the fetus was mediated by basal membrane facilitated transporters [19]. Placental transfer of amino acids involved complex interactions between different classes of transporter on the microvillous membrane and basal membrane as reviewed previously [1]. Placental uptake of maternal amino acids is mediated by the combined activity of accumulative transporters and exchangers and transfer to the fetus is mediated by the combined activity of facilitated transporters and exchangers [1]. It is important to note that amino acid transfer on both the microvillous membrane and basal membrane does not simply involve the transporters working in parallel, with some amino acids taken up by one class of transporter and the remaining amino acids being transported by the other class of transporter. Mathematical modelling has been used to demonstrate that the system proposed can explain net placental amino acid transfer to the fetus [20].

The perfusion model has been used to study amino acid metabolism and interconversion within the placenta. Perfusion studies using 13C-labelled amino acids demonstrated that the placenta consumes glutamate to make glutamine as well as a range of other amino acids including aspartate, alanine and proline [9]. There is also a high rate of exchange of amino nitrogen within the branched-chain amino acid pool and between this pool and glutamate. This placental amino acid metabolism means that the composition of the maternal amino acid pool does not determine that which is available to be transported to the fetus.

Modelling of phenylalanine transfer across the placenta has identified protein synthesis as another important determinant of amino acid transfer [4]. When phenylalanine transfer was modelled, initial iterations of the model did not work as they predicted too much transfer to the fetal side and that this transfer should increase with time, which was not the case. When the model was investigated, it became clear that the problem was that placental uptake was greater than placental transfer leading to an accumulation of phenylalanine within the placenta. In the model, but not in perfused placentas, this accumulation was driving phenylalanine transfer to the fetus. When free phenylalanine concentrations were determined, they were not accumulating as the model suggested, but large amounts of Phenylalanine were found in protein. When the model was amended to include protein synthesis, it worked well, and its sensitivity analysis suggested that both placental protein synthesis and basal membrane transport were important determinants of amino acid transfer to the fetus [4].

Modelling has also been used to study the role of blood flow in determining placental amino acid transfer. In these experiments, the data initially appeared to show that low maternal blood flow did reduce reduced placental amino acid uptake [4]. However, modelling the results suggested that the uptake flow relationship was only observed because the experiment was conducted with tracer concentrations of phenylalanine that were well below physiological concentrations. Where amino acid concentrations were already very low, initial placental uptake reduced their concentrations further to a level below that at which the transporters could effectively take them up. Physiologically, it is hard to imagine a situation where maternal amino acids could be as low as the tracer concentrations in the experimental model. However, the computational modelling can enable us to use this data to predict uptake under a wider range of conditions. This combined experimental and modelling study demonstrates how modelling can improve our interpretation of the data from perfusion studies.

Combining modelling with placental perfusion has supported a more integrated understanding of placental amino acid transport including membrane transport, blood flow and metabolism. Future perfusion and modelling studies of placental amino acid transfer need to quantify how placental protein synthesis and amino acid interconversion affect the transfer of different amino acids [4, 9].

***Modelling placental transfer of fatty acids***

Fatty acids transfer across the human has been studied in the perfusion model with early studies suggesting the placenta preferentially transfers long chain polyunsaturated fatty acids [21]. Placental fatty acid transfer to the fetus has been thought to be mediated by diffusion of fatty acids across the placenta, down a maternal to fetal concertation gradient. The extent to which transfer of fatty acids across membranes is mediated by transporters, simple diffusion or a mixture of the two is not yet clearly established [22].

If fatty acids simply diffuse down a gradient to the fetus then as maternal fatty acid levels fell, then transfer to the fetus should also fall. However, data from the perfusion model show that delivery of 13C-fatty acids to the fetus remained constant despite decreasing maternal fatty acid concentrations [5]. One explanation for this discrepancy may be that while maternal fatty acid levels are higher than in the fetus, there is not a continuous gradient because placental metabolism may maintain free fatty acid concentrations in tissue at a lower level than in either the maternal or fetal circulation [22]. However, the processes determining fatty acid transfer are not yet clear.

The effect of placental fatty acid metabolism on placental transfer of fatty acids has been highlighted by modelling data from perfusion studies [5]. When fatty acids are taken up into cells, they are rapidly converted to acyl-CoA and incorporated into triglyceride, phospholipid and cholesteryl esters. Fatty acids can also be released form these lipid pools and become available for transfer to the fetus or back to the mother. Initial mathematical models that did not model incorporation and release of fatty acids into lipid pools could not replicate data obtained from the perfusion model for either uptake and transfer of 13C-labelled fatty acids or release of endogenous fatty acids [5]. However, as with amino acids, when metabolism was included in the mathematical model, it was able to replicate the experimental data. A sensitivity analysis suggested that it is metabolism, which controls the rate of transfer across the placenta and not membrane transport as had initially been hypothesised.

One factor that may also control fatty acid transfer is plasma binding protein capacity. Albumin concentrations are reported to have a biologically significant effect on placental fatty acid transfer [23] and future studies modelling this the effects of albumin on fatty acid transfer could provide additional insights into the physiological importance of binding proteins for fatty acid transfer. Albumin is believed to act as an acceptor molecule allowing hydrophobic fatty acids to be taken up from the placenta and carried in the plasma.

A limitation of these studies is that *in vivo* many fatty acids taken up from the placenta may come from lipoproteins, however as these fatty acids are thought to be released by lipases and enter and leave the placenta as free fatty acids the mechanisms will be the same. Comparing the results of perfusion studies with modelling of *in vivo* transfer should lead to a fuller understanding of placental fatty acid transfer [24].

***Modelling placental transfer of cortisol***

The placenta acts as a barrier to protect the fetus from maternal cortisol as inappropriate exposure to early in gestation could adversely affect fetal development and have implications for the subsequent health of the offspring [25]. The barrier to placental cortisol transfer was believed to rely almost entirely on enzymatic degradation of cortisol by the enzyme 11ß-HSD2 in the syncytiotrophoblast. This enzymatic barrier was necessary as cortisol was believed to diffuse freely across the placental membranes and the placenta itself [26, 27]. However, modelling of placental cortisol transfer in the perfused placenta has demonstrated that the barrier to cortisol transfer is not simply metabolic [11]. In the perfusion system, placental 11ß-HSD2 activity could be completely inhibited with carbenoxolone (as demonstrated by inhibition of the conversion of D3-cortisol to cortisone). When 11ß-HSD2 was inhibited cortisol transfer across the placenta increased by 60%. However, the model demonstrates that if cortisol were diffusing without any restriction across the placenta that its concentrations in the fetus should have been 4 times greater. This study demonstrates that although 11ß-HSD2 is an important barrier to placental cortisol transfer, it is not the primary barrier, and there is another diffusive barrier to cortisol transfer that is not yet fully understood. While cortisol should be able to diffuse across cell membranes relatively easily it may find diffusion through waterfilled spaces more difficult where no appropriate binding proteins are present. Interestingly, the release of all metabolites into the maternal circulation was greater than the fetal circulation. We speculate that the water-filled villous stroma may provide a barrier diffusion of hydrophobic solutes, but the additional barrier provided by the fetal capillary endothelium may also be a factor [22].

As cortisol is transported in plasma bound to binding proteins, the effect of these on placental cortisol transfer could be explored in the perfusion system. This could be achieved by altering the albumin concentration in perfusate and investigating human albumin vs bovine albumin effects. Placental fatty acid transfer is reported to be highly dependent on albumin concentrations, and this may also be the case for cortisol [23].

The finding that there may be a physical barrier to cortisol transfer is important as it may also affect the transfer of other steroids across the placenta, including estrogen and estrogen-like compounds. It may also influence the effectiveness of betamethasone treatment given to mature the lungs of babies likely to be born prematurely.

**Placental transfer of oxygen**

Modelling of placental oxygen transfer has focused on image-based modelling [28]. The perfusion system could be used to validate and extend the results of these studies. Placental oxygen transfer is complex as, in addition to diffusion across the placenta villi, it is dependent on the rates of maternal and fetal blood flow, the mixing of blood within the intervillous space and placental metabolism. Oxygen can be measured in the intervillous space and arterial and venous circulations using oxygen sensor probes [29]. In the perfusion model the number and position of maternal canulae may allow experimental modulation of flow and oxygenation within the intervillous space.

Oxygen is likely to have a heterogeneous concentration across all regions of the cotyledon and if this within the perfusion system this could be measured and experientially altered to determine how the effects of this heterogeneity affected ATP dependent transport and metabolism and placental function [30, 31]. As models explaining core processes including transport and metabolism become established the next step is to integrate these within multiscale models which may be able to identify and quantify determinants of placental oxygen transfer.

**Placental transfer of pharmaceuticals**

The perfusion model is recognised as a good model for drug transport across the placenta [32]. Modelling of drug transport tends to be based on pharmacokinetic models which will provide estimates of transfer but do not represent the underlying mechanisms which can be complicated. Drug transport across the syncytiotrophoblast, from the fetus to the mother, is mediated by organic anion and cation transporters predominantly located to the basal membrane and efflux transporters from the ABC and Multi-Drug Resistance families predominantly located on the MVM [33]. This system with efflux transporters on the microvillous membrane pumping drugs back to the mother is primarily set up to remove organic waste products, and related molecules such as drugs, from the fetal circulation. The route by which hydrophilic drugs cross the placenta to reach the fetus is not clear, but this may occur by the poorly understood paracellular route [34].

Combining mathematical modelling with perfusions may provide a mechanism by which to address the interactions between microvillous and basal membrane transporters and help determine which transporters are most influential in removing specific drugs from the fetal circulation. While mathematical models may require too much data to use routinely in assessing placental drug transfer, they may provide valuable insight into the conditions where pharmacokinetic models are likely to become most unreliable. The perfusion model allows factors such as binding to plasma binding proteins to be addressed [35].

Transfer of biopharmaceuticals, such as therapeutic antibodies, across the placenta could also be modelled using perfusion data. This would require adapting the models to represent receptor mediated transcytosis rather than membrane transporters or diffusion.

**Relative permeabilities of the maternal and fetal sides of the placenta**

The relative permeability of the maternal and fetal sides of the placenta can be hard to determine experimentally. One advantage of modelling is that it allows quantitative estimates for variables that cannot be directly measured. Interestingly, modelling of data for fatty acids and corticosteroids suggests that placental permeability for placental to maternal transfer is greater than for placental to fetal transfer [5, 11]. For amino acid transfer to the fetus facilitated transporters on the basal membrane were found to be more limiting than MVM transports that mediated uptake [4]. The lower apparent permeability on the fetal side of the placenta may reflect the fact that transfer must occur across the basal membrane, the stroma and the endothelium. For hydrophobic solutes such as fatty acids and cortisol, we have speculated that transfer of lipid-soluble substrates across the water-filled stroma may be a particular barrier to their transfer [22]. The ability of the model to generate quantitative estimates of the permeability of different sides of the placenta demonstrates how modelling can enhance the data obtained from the perfusion model. This data also focused attention on the role of the stroma and fetal capillary endothelium in determining placental transfer.

There is an asymmetry in the maternal to fetal or fetal to maternal transfer of some proteins which was not seen for smaller molecules such as creatinine [36]. Bulk flow down a fetal to maternal pressure gradient could be one explanation for this observation. It is not clear why this would affect large but not small molecules although if diffusion is sufficiently fast, for small molecules it may dominate and mask any effects of bulk flow. Modelling these processes could help distinguish the driving forces and pathways mediating paracellular diffusion and bulk flow across the placenta.

**Future directions**

While modelling placental perfusion data has only applied to transfer of a limited range of solutes, the approach has already demonstrated its potential to provide insight into the mechanisms underlying placental function. Future work using this combined modelling and experimental approach needs to focus on extending the range of substrates but also on creating more sophisticated and integrated models that will have greater predictive power.

The development of three-dimensional image-based modelling of placental blood flow and transfer provides the opportunity to create better models by incorporating realistic spatial geometries [28, 31]. In the perfused placenta, this could be facilitated using techniques such as microCT which could allow the villous structure of the entire cotyledon to be determined following the perfusion experiment [37].

The incorporation of realistic spatial geometries will also allow the development of more granular models. Currently, the compartmental models treat the cotyledon as a homogenous entity, but this is not likely to be the case. In a real cotyledon, there will be gradients of oxygen and nutrients between the artery and the vein that will be determined by blood flow between the villi and uptake into the villi. This heterogeneity may mean that different regions of the cotyledon function differently. Models that can reflect this will be better able to reflect the variations that are experienced *in vivo* and in pathological placentas.

Current models are based on steady-state conditions, but in reality, over time feedback mechanisms will be regulating many processes within the placenta. Once the mechanistic models are established, modelling intrinsic and extrinsic regulation is the next step in developing a fully representative model of the placenta.

Novel therapeutic approaches are being developed, and we believe modelling can be used to identify the best targets for those interventions, the mechanisms that are most likely to be rate-limiting for fetal growth [38, 39]. The potential for models to help predict the best targets for interventions and future studies will only increase as the models become more sophisticated. As modelling approaches do become more sophisticated, the perfusion system will continue to provide much of the experimental data required to support the development of a virtual placenta.

**Conclusion**

Placental function is complex and dependent on many processes, including blood flow, membrane transport and metabolism. The perfusion system is the best model available that can represent all these factors together. When the perfusion system is combined with mathematical modelling, which allows the contributions of the different factors to be understood. Modelling data from the placental perfusion system have provided novel and unexpected insight into placental function. In particular, it has highlighted the role of metabolism in the transfer of nutrients, the different apparent permeabilities of the maternal and fetal sides of the placenta and the how resistance to diffusion can be a significant determinant of transfer for substrates thought to diffuse freely across the placenta like cortisol. By enabling models of specific aspects of placental function to be rigorously tested placental perfusions support the development of a virtual placenta.

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**Table 1**, How perfusions allow you to change experimental parameters to support modelling

|  |  |  |  |
| --- | --- | --- | --- |
|  | **maternal** | **placental** | **fetal** |
| Blood Flow | Controllable | NA | controllable |
| Arterial pressure | Measurable, can be controlled with effects on flow  |  | Measurable, can be controlled with effects on flow |
| Arterial substrate concertation | Controllable and measurable | Initial tissue concentrations can be measured in adjected tissue at the beginning of the experiential and in perfused region at the end | Controllable and measurable |
| Arterial Oxygen concertation | Experimentally determined | NA | Experimentally determined |
| Arterial pH | Experimentally determined |  | Experimentally determined |
| Blood flow distribution  | number and position of maternal canulae | NA | NA |
| metabolism | Metabolites can be measured in venous outflow | Can be manipulated with toxins etcTissue metabolites can be measured at end of experiment | Metabolites can be measured in venous outflow |
| Diffusion |  | Can’t be controlled but can be measured |  |
| Transporters | Uptake and release can be measured | Can be inhibited by  | Uptake and release can be measured |



**Figure 1**, compartmental models vs. geometric models A, a generic compartmental model showing uptake and metabolism of a substrate. Compartmental models can represent diffusion and transport across the barriers as well as metabolism but do not fully represent the regional heterogeneity in blood flow and substrate concentrations. B, A 3D geometric model which incorporates blood flow within the intervillous space and diffusive transfer to account for regional heterogeneity.



**Figure 2**, Modelling allows quantitative measures of multiple parameters and estimation of their relative importance. A, An illustration of the factors which may affect solute transfer across the placenta. B, a sensitivity analysis for cortisol transfer to the fetus as a function of variations in the model parameters. The reported changes in placental transfer predicted by the model were based on the steady-state results at the highest maternal input concentration. Abbreviations: D, effective diffusive permeability constant; metabolic parameters kM and Vmax, Michaelis-Menten constant; Qf, fetal flow rate; Qm, maternal flow rate.