

# Cell culture under perfusion conditions reduces cellular metabolic stress and mimics the *in vivo* physiological environment in pancreatic cancer.

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Abstract ID: C42

## Introduction

Tumour responsiveness to chemotherapy in pancreatic cancer varies significantly across patients. This reinforces the need to establish an accurate model that preserves the tumour microenvironment *ex vivo*, allowing a personalized drug screen for patients to identify optimal regime of therapy. The current standard of cell culture is that the media is exchanged at defined time points (i.e. static culture). However, this is not a physiologically representative model as cells are maintained in either a nutrient and substrate 'hyperreplete' or deplete environment, rather than an epistatic supply. We evaluated the role of perfusion culture to establish whether this technique reduced metabolic stress and creates a physiologically representative model of the *in vivo* tumour environment.

## Methods

The human pancreatic cancer cell line PSN-1, was cultured in low glucose media (1 g/L) corresponding to normoglycaemia in healthy subjects. Two culture conditions were chosen: (i) under conventional static culture in a 6-well plate and (ii) a perfusion system using the Alvetex perfusion plate, in which cells were perfused with medium at a rate of 10  $\mu$ L/min. Medium was extracted at multiple predefined time points in order to metabolically profile cells and to quantify the availability of metabolic substrates. Western blotting was performed to compare cellular biological processes under the two culture conditions.

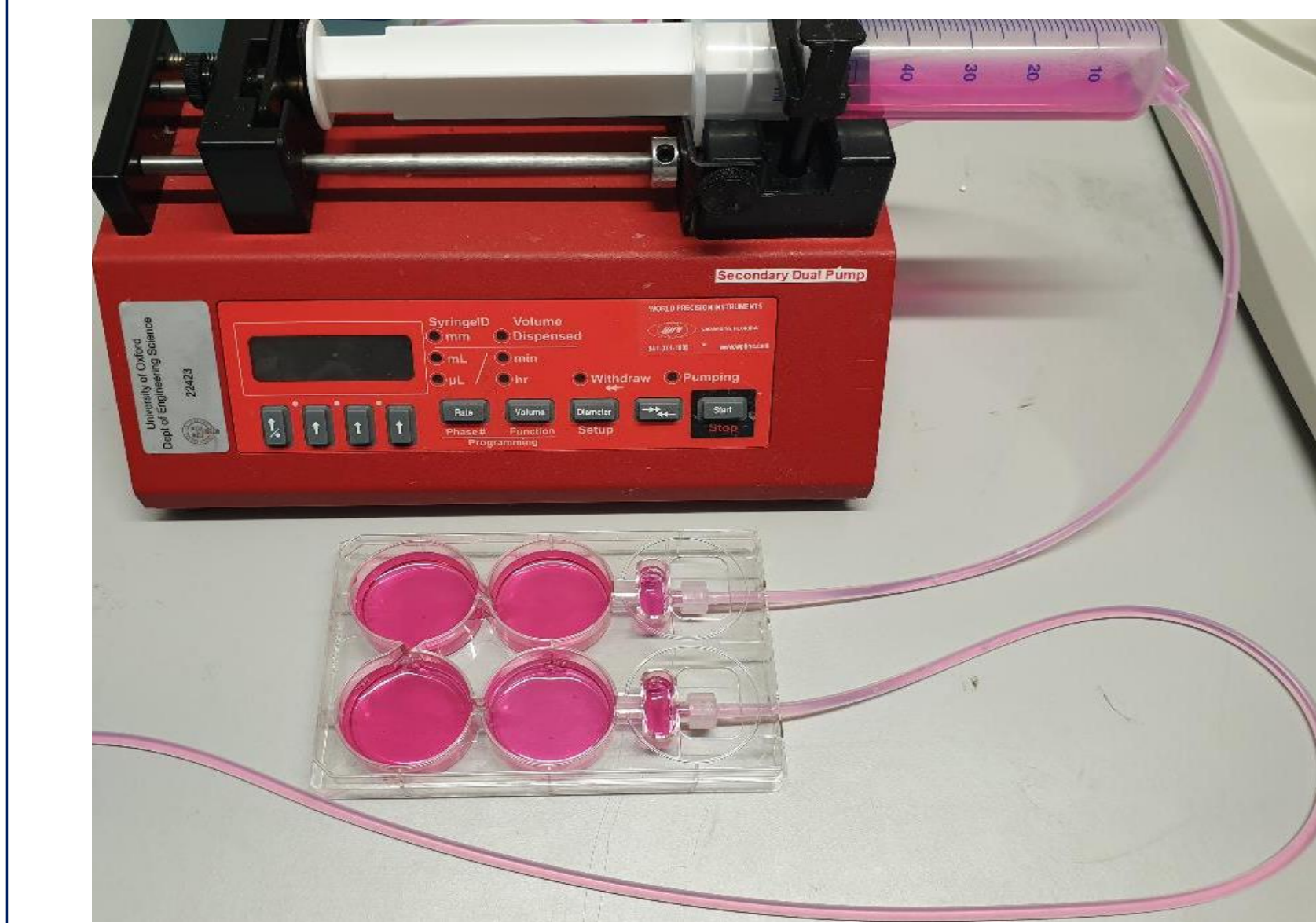
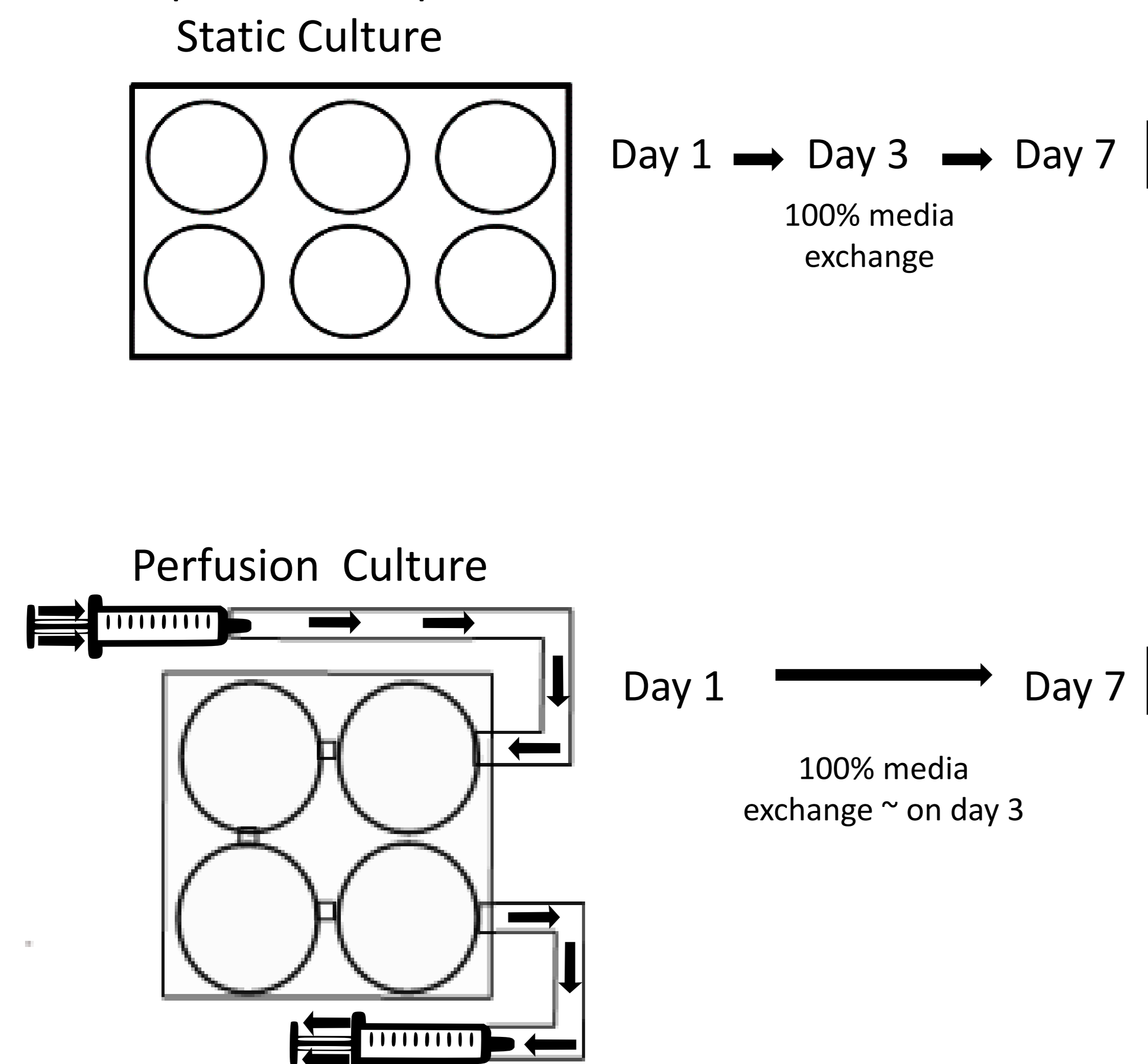


Figure 1 – Illustration of the perfusion system. Inflow to the plate is supplied via pump delivery. Active outflow is achieved with a second pump

Workflow of static vs perfusion experiments:

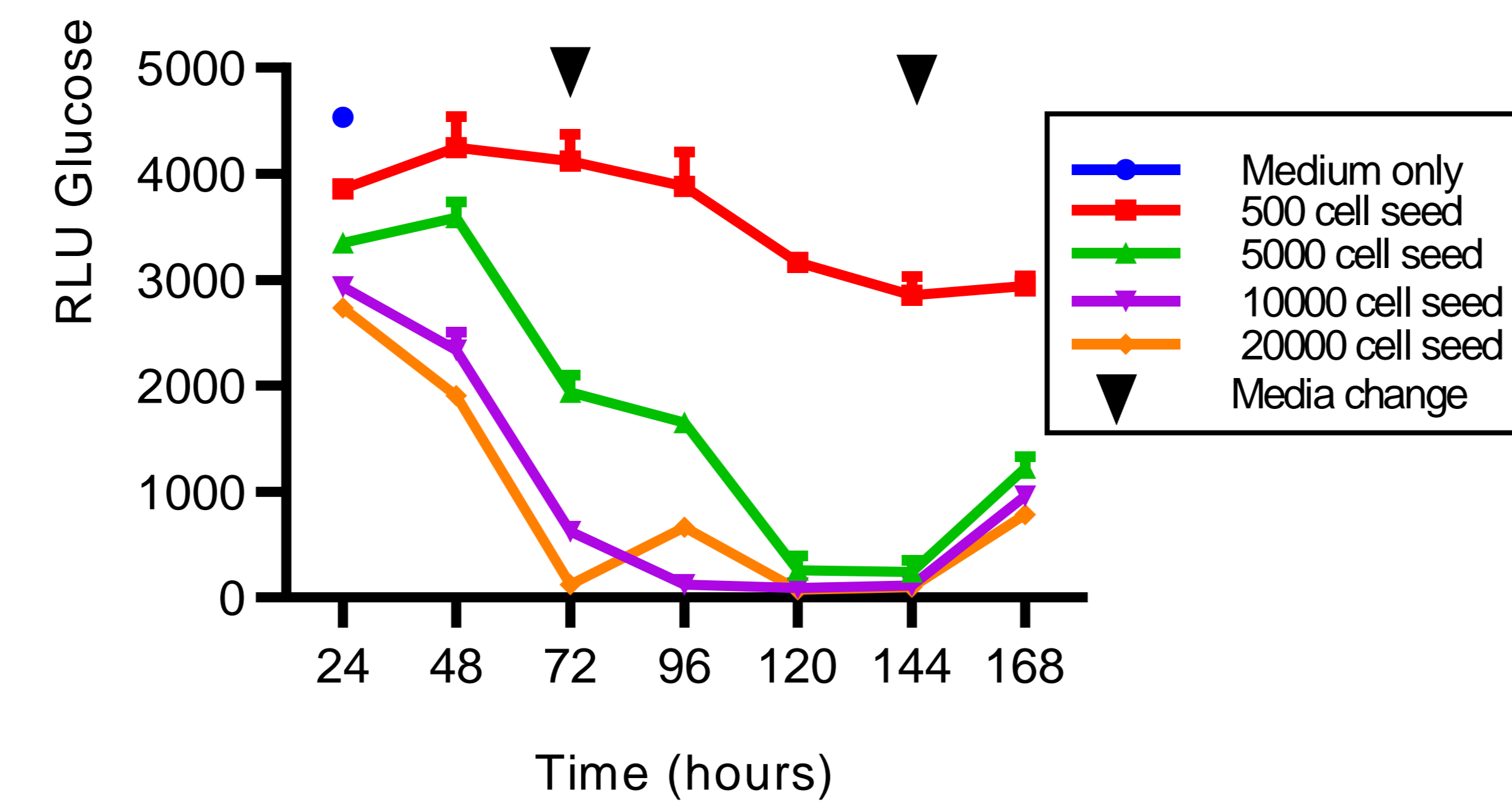


## Results

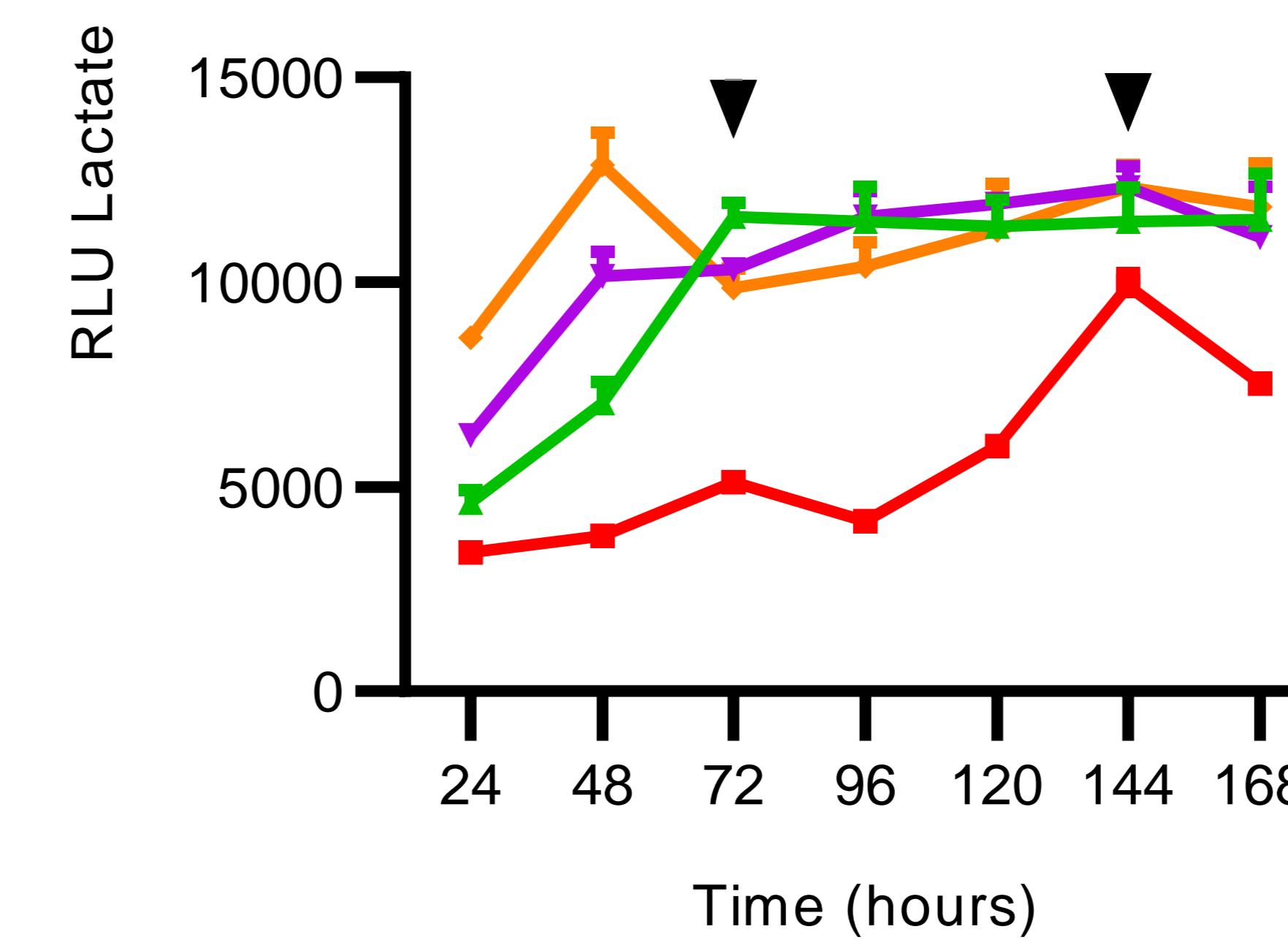
Conventional static culture exposes cells to a substrate deplete environment over time. Renewing media fails to replenish substrate concentration to its baseline value, thus suggesting progressive metabolic stress within the cultured cells. This adversely impacts on cellular synthetic function. The use of perfusion culture allows a constant delivery of metabolic substrates and ensures strict homeostatic control of glucose concentration. Cellular synthetic function is preserved over time.

### 1) Static culture exposes cells to hypoglycaemic conditions over time and anaerobic metabolism rate increases

Glucose concentration in static culture over time

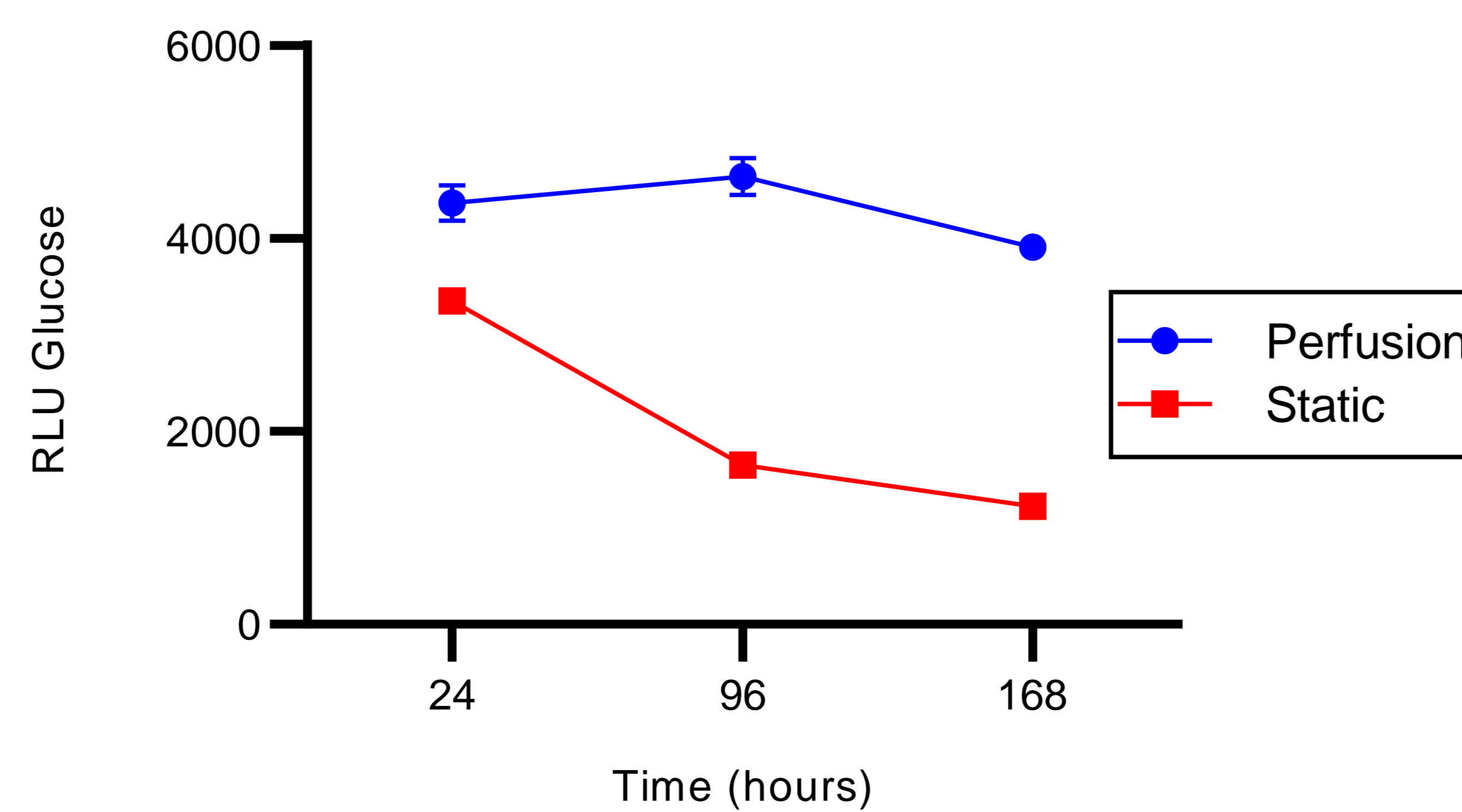


Cellular Lactate secretion whilst in static culture

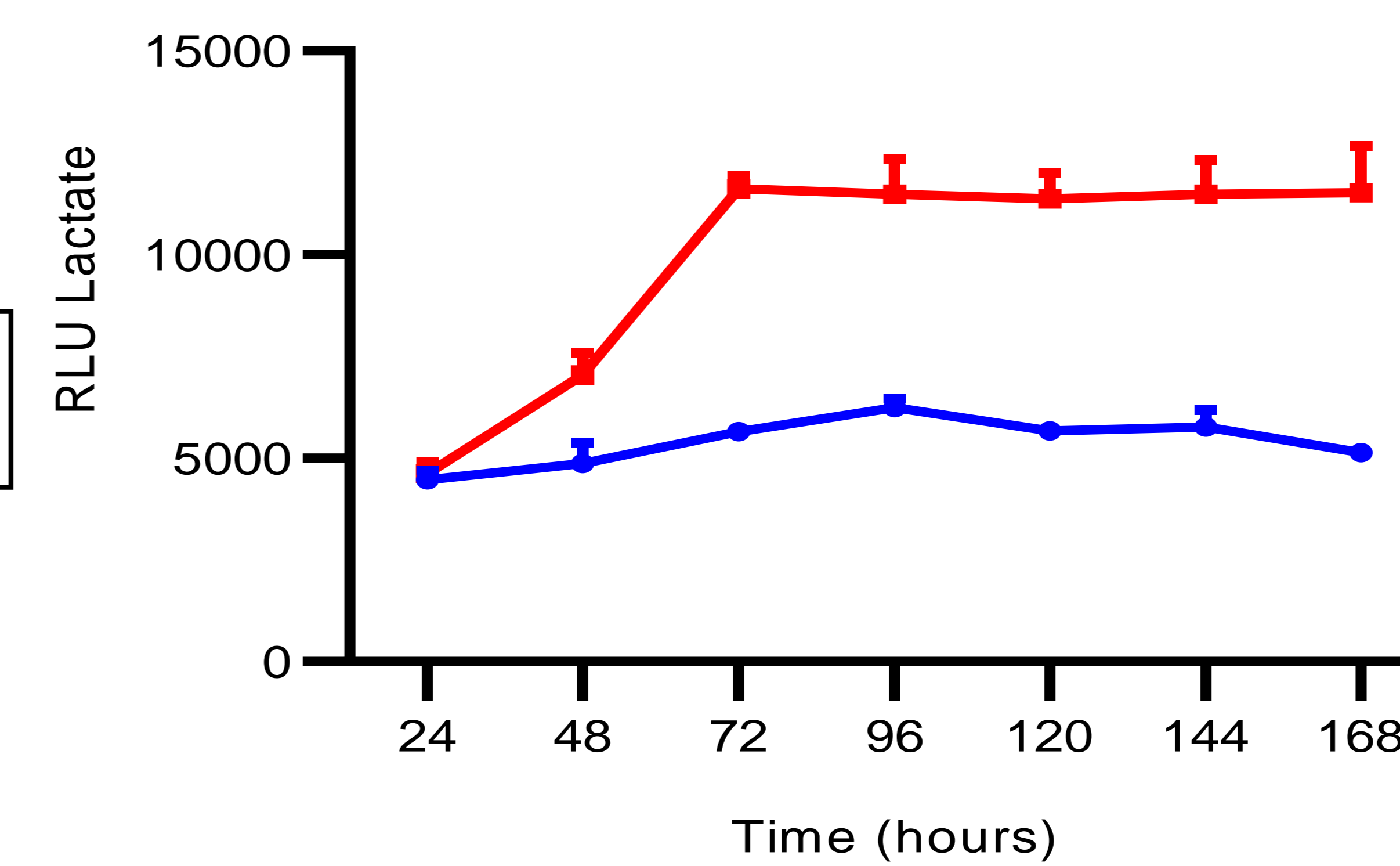


### 2) Perfusion culture ensures constant delivery of glucose to cells over time and is associated with a reduction in lactate production

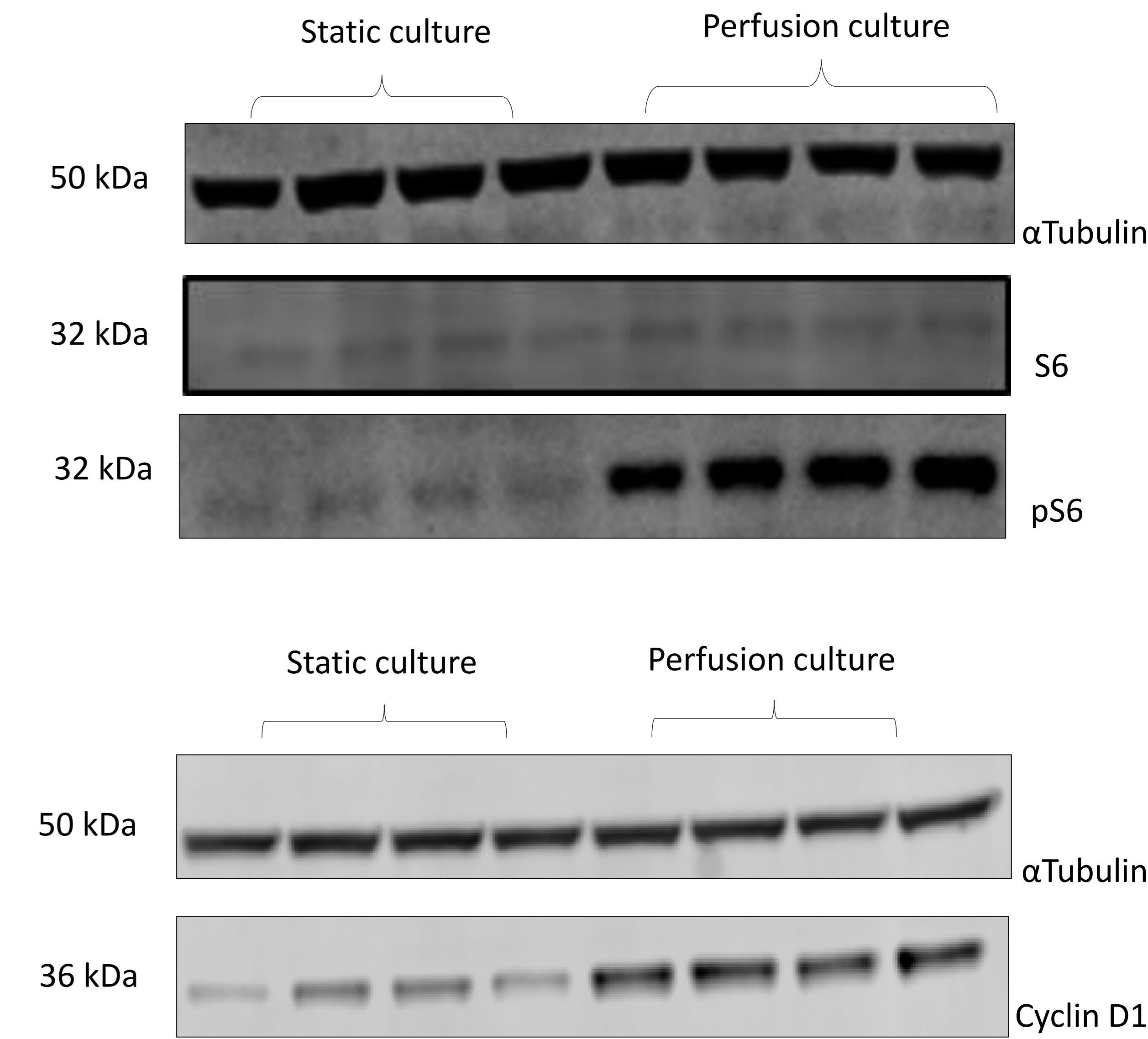
Glucose concentration in static and perfusion culture over time



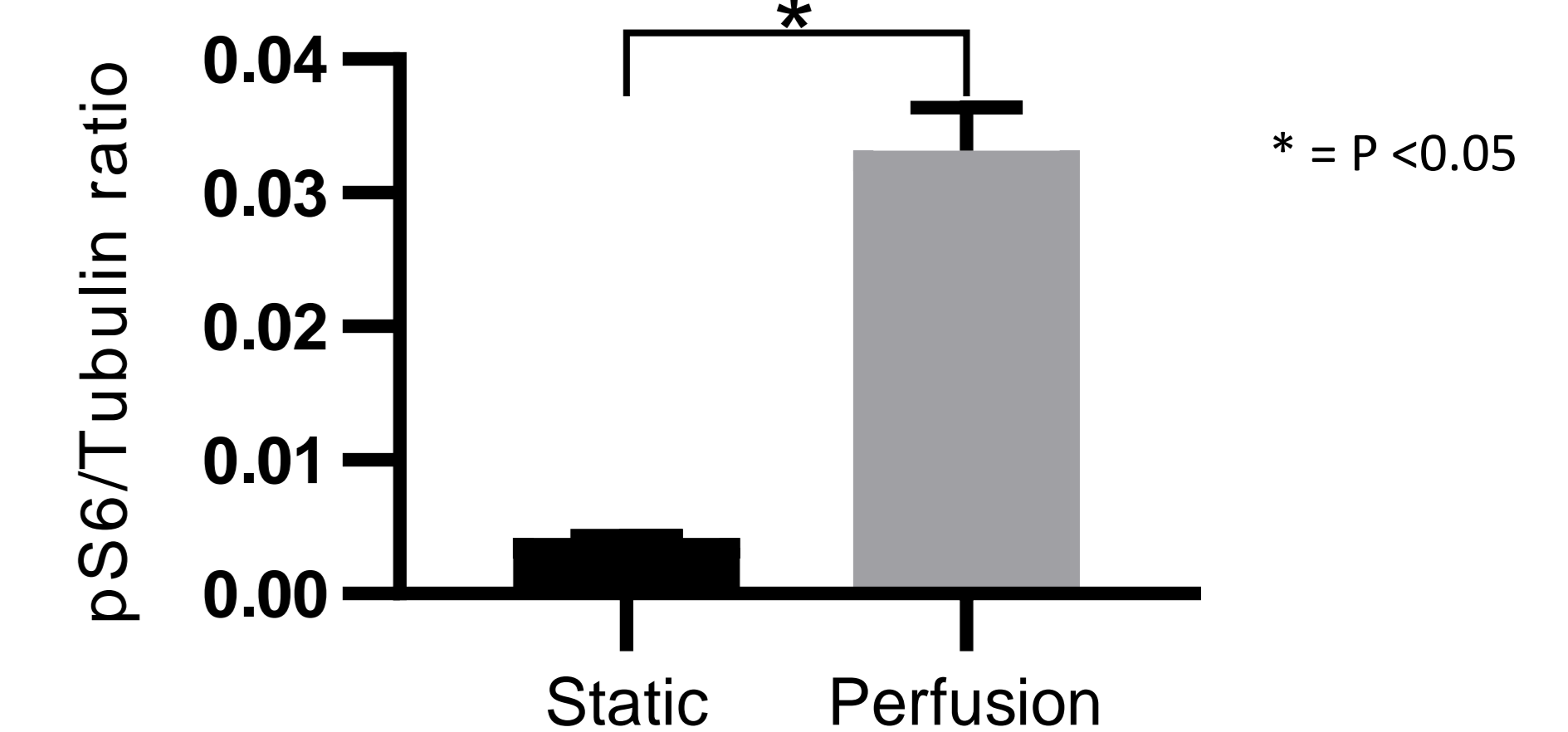
Cellular lactate secretion in static and perfusion culture



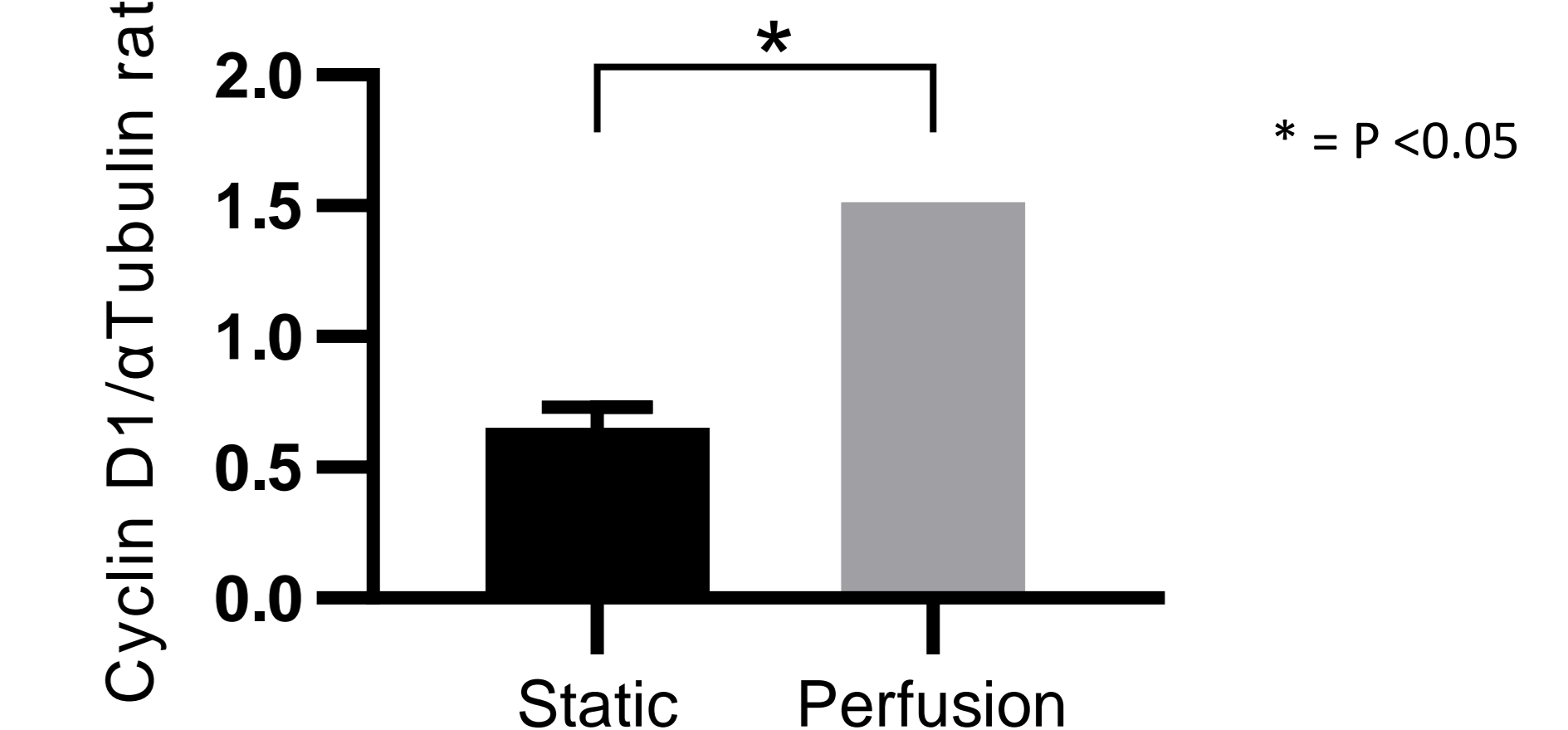
### 3) Protein synthesis via mTOR activity is preserved during perfusion culture and suppressed during static culture



Overall pS6 expression during static and perfusion culture



Overall expression of Cyclin D1 in static and perfusion culture



## Conclusions and future work

Conventional static culture exposes cells to cyclical episodes of metabolic substrate deprivation and subsequent excess. This promotes anaerobic metabolism within the cells and adversely impacts on cellular protein synthesis. Perfusion culture allows constant delivery of metabolic substrates to cells over time, thus ensuring homeostatic regularity and a more physiologically representative technique of culturing cells. This system will be further evaluated as a platform for drug delivery and *ex vivo* tissue culture

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