Investigating the effects of 5.5 mmoL (Physiological) vs 25 mmoL (Supraphysiological) glucose concentration in culture media on LHCN-M2 cell viability, ATP production and differentiation.

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Introduction

- *In vitro* cell culture media composition is important in supporting cellular proliferation and differentiation. Skeletal muscle cell models are important for investigating mechanisms of skeletal muscle metabolism and disease.
- However, culture media for skeletal muscle cells often contain glucose concentrations (**GC**) five times higher than what's considered normal in fasting human plasma, thus is not representative of the *in vivo* metabolic environment and therefore may hinder the translational relevance.
- The human skeletal muscle cell line, LHCN-M2, have been established and characterised as an immortalised myoblast cell line of which can differentiate into multi nucleated myotubes.
- Currently, LHCN-M2 cells are cultured in high concentrations of glucose (25 mmoL), that are not representative of the *in vivo* metabolic environment.

AIM: To determine the impact of media containing GC of 5.5 mmoL (physiological) vs 25 mmoL (supraphysiological) on cell viability, ATP production and differentiation in human LHCN-M2 myoblasts.

In order to create a more physiologically relevant model which will enhance the translational potential of results when investigating cellular mechanisms of physiological and pathophysiological conditions in skeletal muscle.

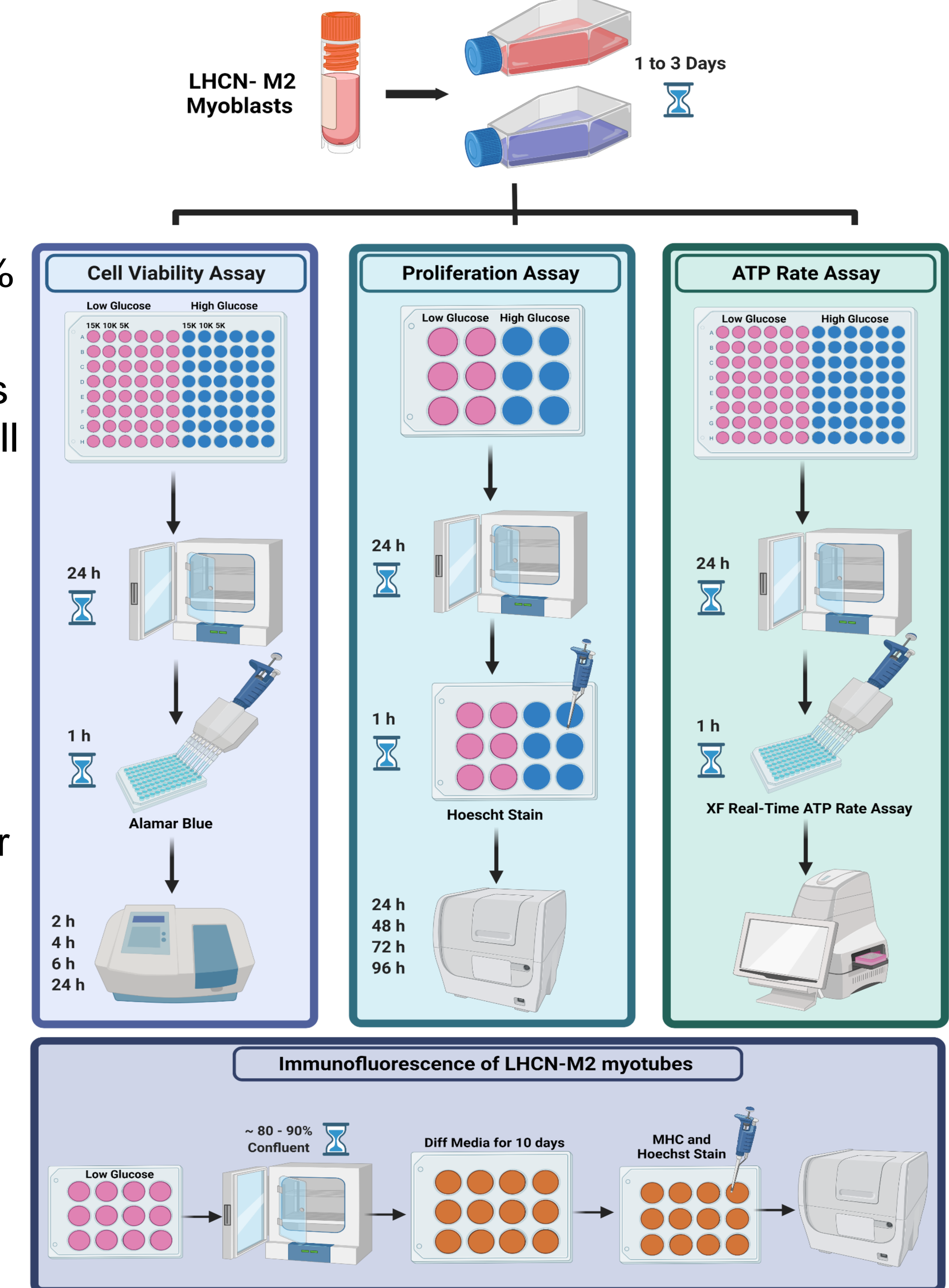
Methods

Cell Culture and Differentiation: LHCN-M2 cells, were cultured in proliferation media containing 5.5 or 25 mmoL GC, differentiation was induced by supplementing cells with 5.5mmoL media containing either 0.5%, 1% or 2% human serum for 10 days.

Cell Viability: Alamar Blue solution was added aseptically at 10% of the final well volume (10µL), the presence of the reduced dye was measured using a spectroscopy at 2, 4, 6 and 24 hours

ATP production rates: ATP rate assay was performed using Agilent Seahorse XFe96 and by injecting 1.5µM Oligomycin and 0.5 µM Rotenone and Antimycin A mix. Cell count was used for the normalisation of all data

Immunofluorescence: Cells were washed and fixed, blocked and incubated overnight with Myosin heavy chain (MF-20) antibody. Secondary antibody (AlexFluor 555) with Hoechst 33342 were used to visualise myotube formation and nuclei respectively



Results

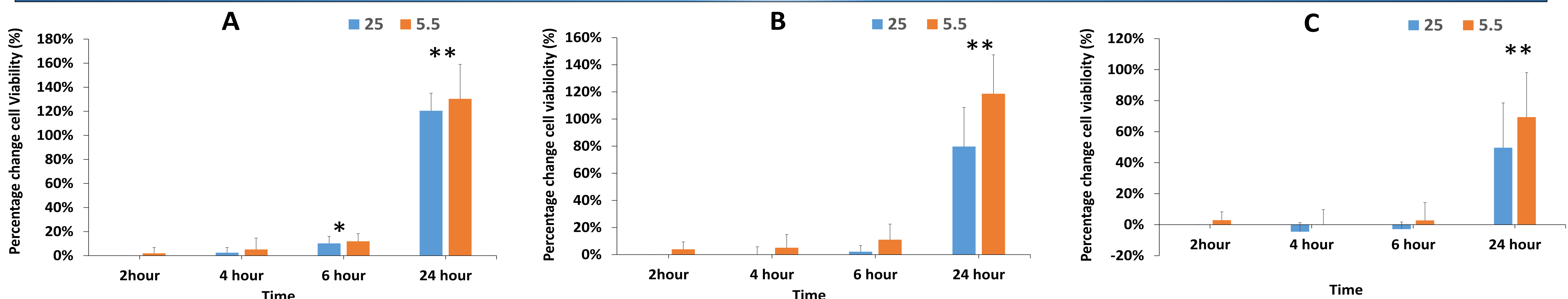


Figure 1: *Percentage change in LHCN-M2 myoblast viability A (15 x 10^{-4} cell/mL), B (10 x 10^{-4} cell/mL) and C (5 x 10^{-4} cell/ml) grown in 25mmoL (blue) or 5.5mmoL (orange) GC. Data is mean ± standard deviation from 5 independent experiments (control 2 hour HG). *both 25 and 5.5 GC at 6 hours significantly different to 25 and 5.5 GC at 2 hours ($P < 0.05$). **both 25 and 5.5 GC 24 hour significantly different to 25 and 5.5 GC at 2, 4 and 6 hours ($P < 0.05$).*

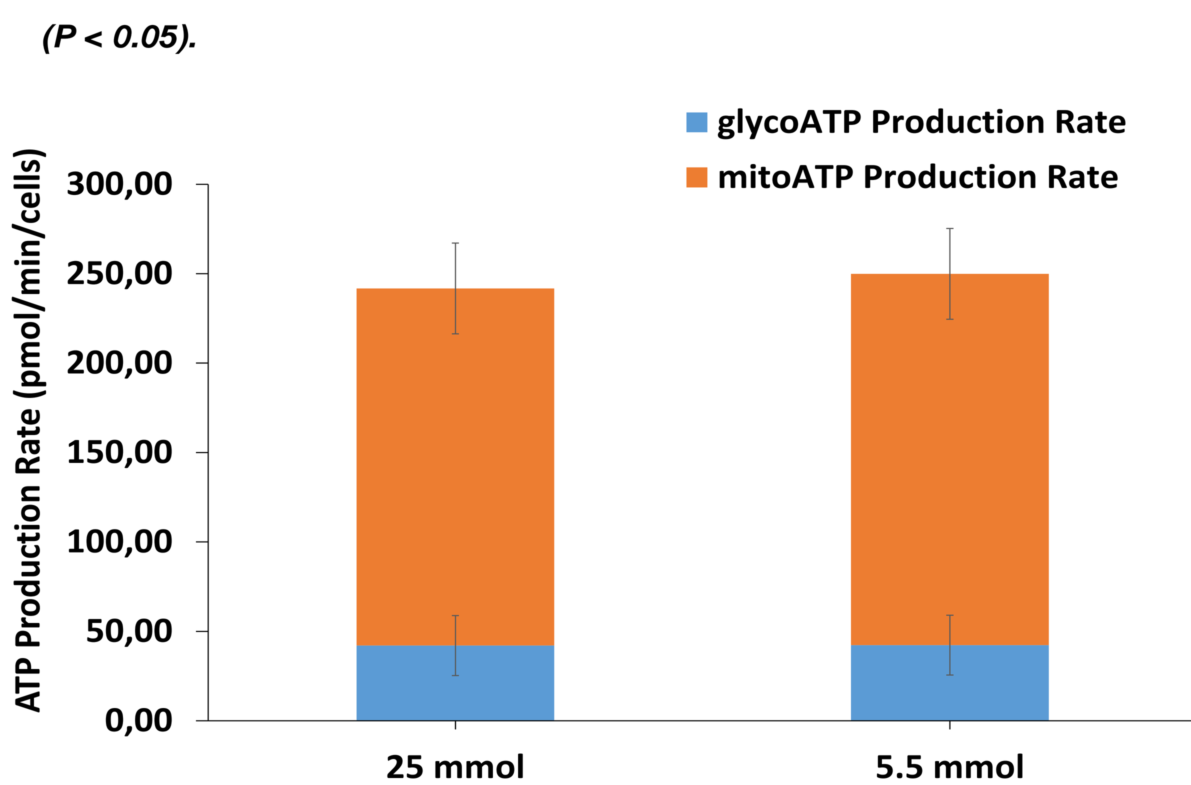


Figure 2: *LHCN-M2 myoblast basal glycolytic, mitochondrial and total ATP production rates in 25mmoL and 5.5mmoL GC. Data is mean ± standard deviation from 4 independent experiments. There was no significant difference in mitochondrial, glycolytic and total ATP production rates ($P > 0.05$).*

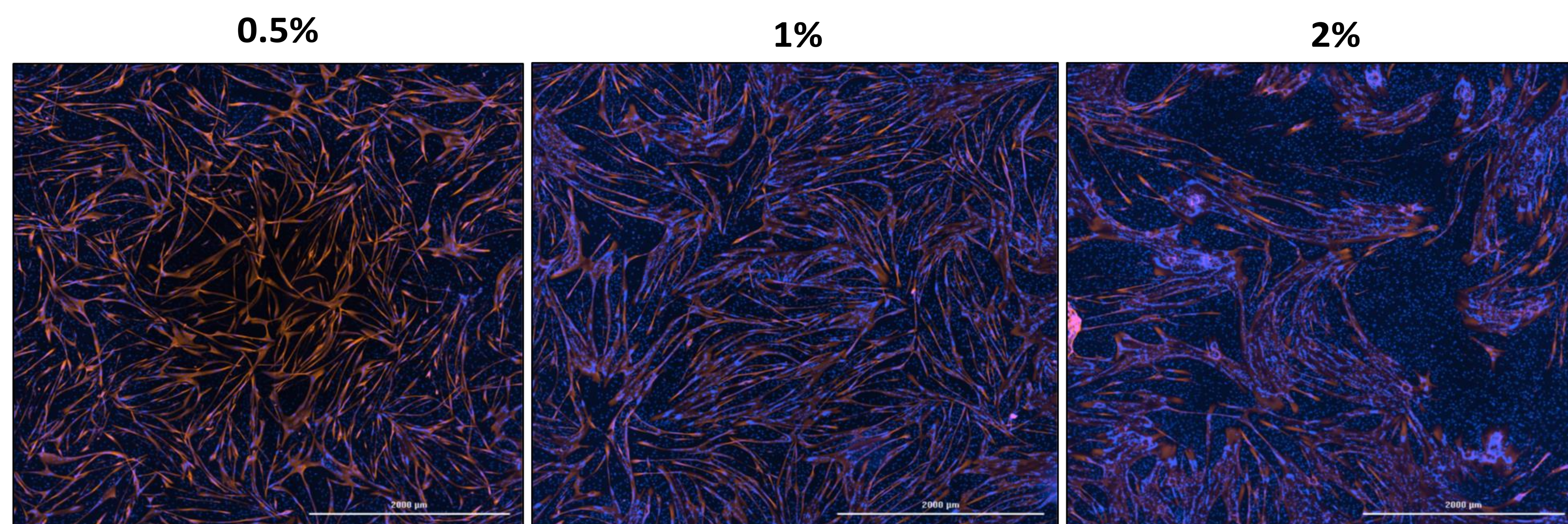


Figure 3: *Representative immunofluorescence images (4x objective) of MHC expression (Sainted Red) and Hoechst Nuclei dye (Stained Blue) in LHCN-M2 Myotubes differentiated in 5.5mmoL GC containing either 0.5%, 1%, or 2% Human Serum.*

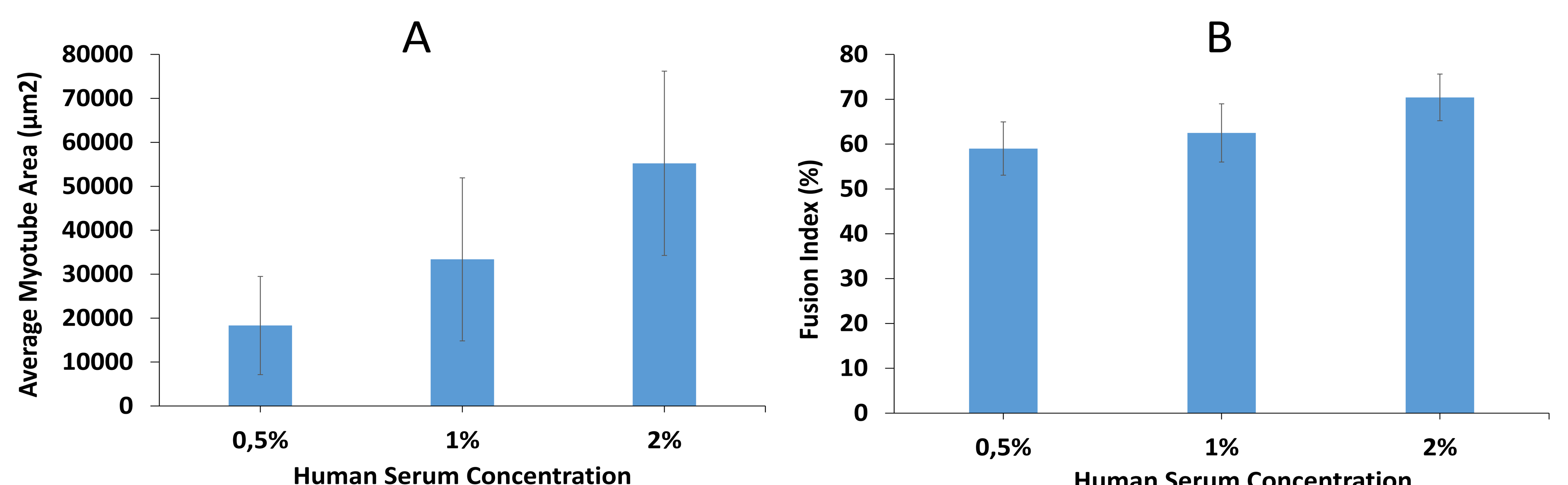


Figure 4: (A) Average LHCN-M2 Myotubes Area (B) *Fusion Index of LHCN-M2 cells, differentiated in 5.5mmoL GC containing either 0.5%, 1%, or 2% human Serum. Data is mean ± standard deviation from 3 independent experiments. There was no significant difference in myotubes area or fusion index between serum concentrations ($P > 0.05$).*

Conclusion

Using media containing 5.5 mmoL GC had no impact on the viability or ATP production rate compared to 25 mmoL GC. This data also shows the ability to differentiate LHCN-M2 cells efficiently in 5.5 mmoL GC with human serum. Therefore, progressing to an *in vitro* model with more physiologically relevant culture conditions compared to what is observed *in vivo*. This model also enables subsequent experiments to be conducted under more relevant GC in assay media, avoiding potential effects of acute substrate restriction.

Poster presented at: