Effect of starvation on SARS CoV2 Viral Propagation

Aim of the study: To determine whether glucose and FBS serum deprivation can affect the replication of the SARS CoV2 virus and the viral propagation *in vitro*

Experimental design:

Standard cell culture conditions: Vero cells are seeded in 24 well culture plates in Dulbecco Minimum Essential Medium (DMEM) (Gibco) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Gibco), 3.7 g/L sodium bicarbonate maintained at 37°C, with 5% CO_2.

Reduced glucose and serum culture conditions: At 70-80% confluence the cells were primed with different concentrations of glucose (low concentration 1g/L, or high concentration of 2g/L, 3g/L or 4.5g/L) along with normal 10% FBS or decreased FBS (0.5%, 0.75% or 1.0%) for 24 hours.

Measurement of viral propagation: After 24 hours, the culture media were removed and the cells were infected with SARS CoV2 virus (A3i clade) in DMEM culture media (without FBS) for 3 hours. Later, the virus containing medium was aspirated and replaced with fresh DMEM medium containing different concentrations of glucose and FBS, as described above. After 72 h incubation, the supernatants were recovered and clarified by centrifugation at 5000 rpm for 10 minutes and subjected to viral RNA quantification by RT-qPCR with SARS CoV2 specific primers.

Controls: Uninfected Vero cells and those infected with viral stock diluted at 1:10 (~MOI, 0.1) is treated as cell control and infection control, respectively.

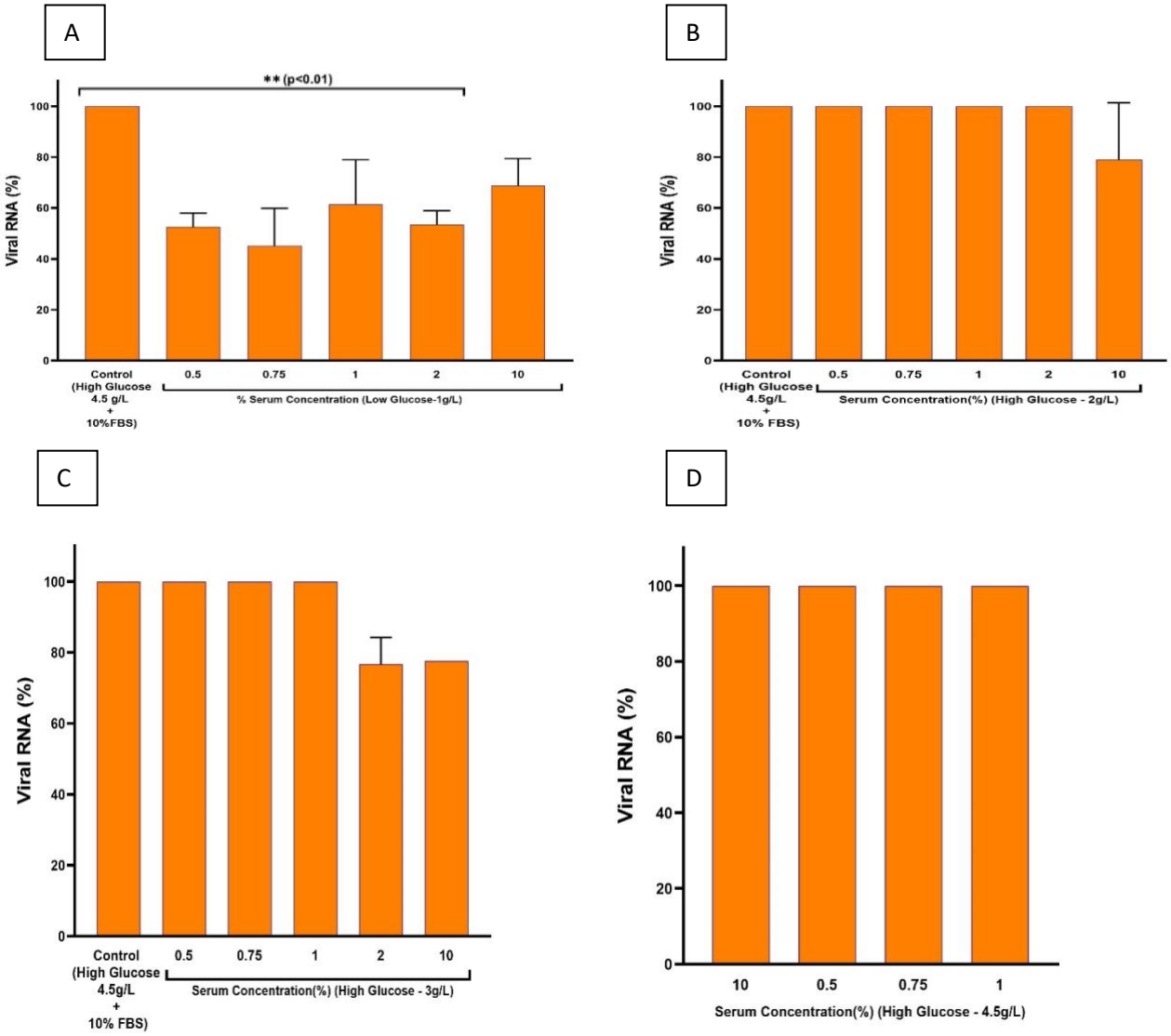
The RT-qPCR data are mean of at least three independent experiments. Data is represented as mean ± SD using GraphPad Prism 8 (Ver 8.4.2 GraphPad Software, LLC.).

The amount of viral RNA measured by RT-qPCR reflects the degree of infection by the SARS-CoV-2 virus into the mammalian cells in this *in vitro* cell culture assay system.

Results:

Panel A: Infecting cells grown in Low glucose (1g/L) conditions leads to a decrease in the SARS-CoV-2 viral RNA measured in all concentrations of FBS serum, compared to control cells grown at 4.5g/L glucose and 10% serum (viral RNA reduced to 45% to 75%)

Panels B,C,D: Not much change is seen in viral RNA measured under higher glucose concentrations, irrespective of the amount of FBS serum in the medium



Interpretation of the results: The results show that the SARS-CoV-2 amount in the cells decreased at all the concentrations of FBS under **low glucose (1%) condition** compared with high 4.5 g/L glucose with 10% FBS (control) (Fig A). Whereas no major change in the viral release was observed in the groups containing 2% or 3% glucose concentrations as quantified by qRT-PCR (Fig B,C).

The viral RNA% detected was not influenced much by changing the FBS amount in the serum (Fig D).

Conclusions: SARS-CoV-2 propagation, as measured by viral RNA %, is found to be decreased in cell culture based assay system upon decreasing the glucose concentration from 4.5 g/L to 1 g/L. This preliminary *in vitro* study shows that there is evidence of a role of glucose rather than serum FBS in influencing the viral propagation, with very high glucose levels contributing to viral propagation in cells.

Further molecular studies are essential to establish the relation between glucose starvation versus high glucose levels and SARS-CoV2 viral propagation, including in humans under physiological *in vivo* conditions. Such studies will better determine the physiological relevance for low glucose intake versus high glucose levels in humans in context of viral replication and infection.

References

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