LAMINAR SHEAR STRESS REDUCES HYALURONAN MEDIATED MOTILITY RECEPTOR (HMMR) EXPRESSION IN GLOMERULAR ENDOTHELIAL CELLS WITH IMPLICATIONS FOR CELL MOTILITY

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INTRODUCTION

Laminar shear stress (LSS)

is the dragging frictional force created by blood flow, acts primarily on the endothelial cells (EnC).
Estimated mean laminar shear stress inside the kidney glomerulus is 10-20 dyn/cm^2.
LSS is considered to be a factor of vascular health.

Glomerular endothelial glycocalyx

The glomerulus is the filtration unit of the kidney where blood is filtered through the glomerular filtration barrier (GFB) ***Fig 1***.
The glycocalyx covers the endothelial cell surface and is composed of core proteins (proteoglycans PG and glycoproteins) and attached carbohydrate side chains known as glycosaminoglycan (GAG) ***Fig 2***.
There several types of proteoglycans and GAG ***Table 3***.

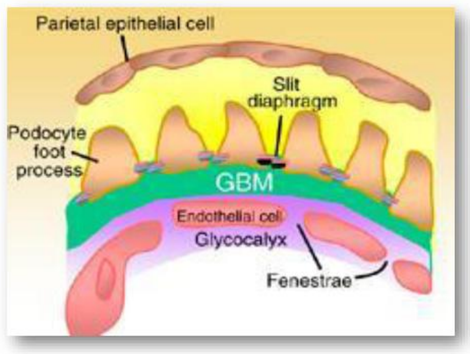
Functions of glycocalyx:

Basically the glycocalyx acts as the glomerular endothelial cell gate keeper.
It acts as:

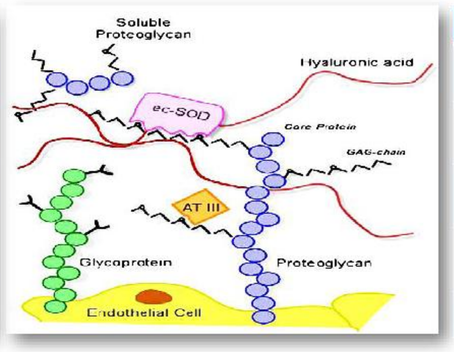
- Mechanotransducer (Glycocalyx translates shear forces into biochemical signals inside the cell)
- Permeability modifier

HMMR:

The hyaluronan motility mediated receptor is a HA receptor present in the glomerular endothelial cell surface. It promotes cell motility and invasion through interactions with HA on the cell surface.



- ***Figure 1:*** *The glomerular filtration barrier (GFB) consists of 3 layers; podocytes, glomerular basement membrane (GBM) and fenestrated glomerular endothelial cells (GEnC) which are covered with glycocalyx.{1}*



- ***Figure 2:*** *The glycocalyx comprises surface-anchored proteoglycans and glycoproteins. Glycosaminoglycan (GAG) side chains attached to these core proteins form a mesh like structure. It entraps soluble proteoglycans and other proteins e.g. albumin.{2}*

Core proteins		GAGs
Syndecans	CD44	Heparan sulfate HS
Glypicans	HMMR	Chondroitin sulfate CS
ESM1	Thrombomodulin	Hyaluronan HA
Versican		Dermatan sulfate DS
Perlecan		Keratan sulfate KS

- ***Table 3:*** *Some components of glycocalyx and their associated GAG side chains. (HMMR) Hyaluronan mediated motility receptor, (TM) Thrombomodulin, (HS) Haparan Sulphate , (CS) Chondroitin Sulphate and (HA)Hyaluronic acid. {3}*

Hypothesis

- Physiologically relevant LSS regulates HMMR expression and modifies glomerular endothelial cell motility ***in vitro***.

Goals

- Validate the effect of LSS on several glycocalyx related genes.
- Investigate HA production and regulation changes with LSS by radiolabeling.
- Investigate the effect of LSS on Endothelial cells motility through HMMR expression changes with or with out HA fragments activation.

RESULTS

Custom-designed taqman qPCR gene arrays:
Demonstrated the effect of LSS on several glycocalyx genes; most prominent changes were a reduction in HMMR expression and increase in versican expressions ***Fig 5.***

Data validation:
We have validated some data obtained from Custom-designed taqman qPCR gene arrays with western blotting and quantitative PCR;

Thrombomodulin expression ***Fig 6***.

Versican expression ***Fig 7***.

CD44 (hyaluronan receptor) expression ***Fig 8***.

Hyaluronic acid mediated motility receptor (HMMR) expression ***Fig 9***.
HMMR recovery ***Fig 10***.

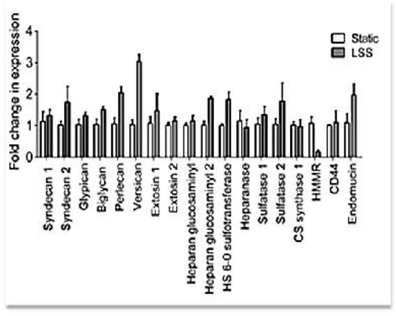


Figure 5: *Glycocalyx array data demonstrating significant changes of some glycocalyx components in response to LSS*

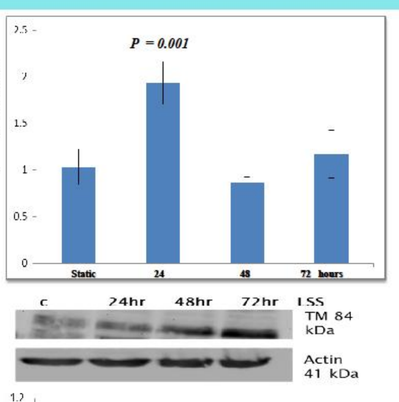


Figure 6: *Showing (above) qPCR analysis of effect of chronic LSS on thrombomodulin, there were an increase within 24 hours of LSS (P value = 0.001, n = 3). (below) WB image showing the effect of chronic LSS on thrombomodulin with actin as a loading control.*

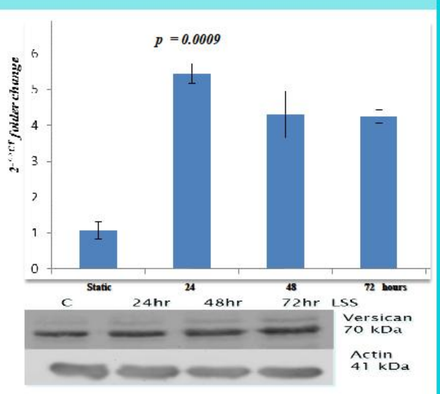


Figure 7: *Showing (above) qPCR analysis of effect of chronic LSS on versican, there were an increase within 24 hours of LSS (P value = 0.0009, n = 3). (below) WB image showing the effect of chronic LSS on versican with actin as dose loading control, there were no significant changes with LSS.*

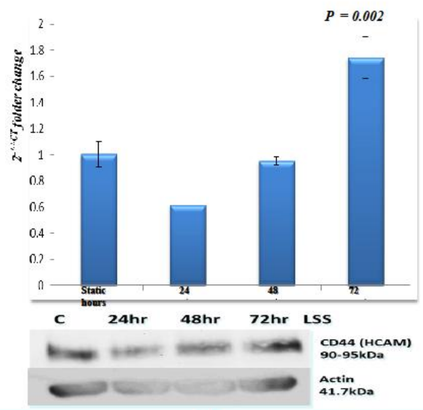


Figure 8; *Showing (above) qPCR analysis of effect of chronic LSS on CD44, there were a trend to increase after 48 hours of LSS (P value = 0.002, n = 3). (below) WB image showing the effect of chronic LSS on CD44 with actin as dose loading control, there was a gradual increase at the protein level, peaking at 72 hours of LSS.*

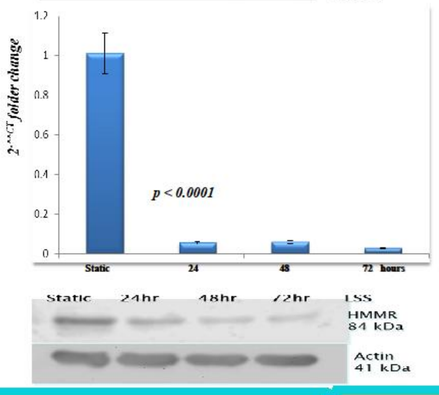


Figure 9: *Showing (above) qPCR analysis of effect of chronic LSS on HMMR, there was a significant decrease within the first 24hours of LSS (n = 3, p value = 0.0000). (below) WB image showing HMMR, there was a significant decrease at the protein level within 24 hours of LSS.*

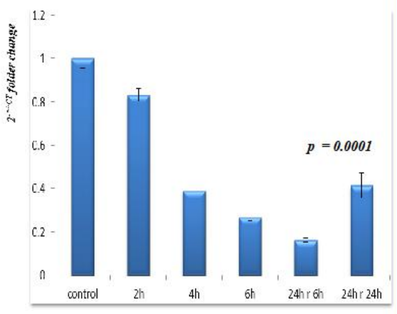


Figure 10: *Showing qPCR analysis of effect of LSS on HMMR with short duration of time (2, 4, 6 and 24 hours) and a recovery phase (24 hours post shear static condition for 6 and 24 hours) where control is static condition, there was a gradual significant decrease of HMMR expression until 24 hours of LSS and a significant gradual rise with cessation of LSS (n = 3, p value = 0.0001).*

Immunofluorescence images:
Human glomerular endothelial cells stained with anti-HMMR green and anti VE-cadherin red, there was a reduction in HMMR expression after 24 hours of LSS ***Fig 11 and 12***. confocal images in Z axis of same slides, ***Fig 13 and 14.***

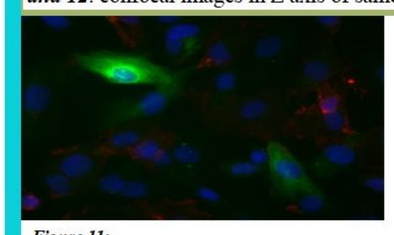


Figure 11: *IF image showing DAPI (blue), HMMR (green) and VE-cadherin (red) in static condition.*

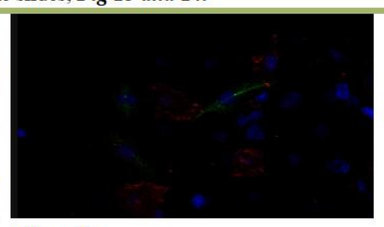


Figure 12: *IF image showing DAPI (blue), HMMR (green) and VE-cadherin (red) post 24 hours LSS.*

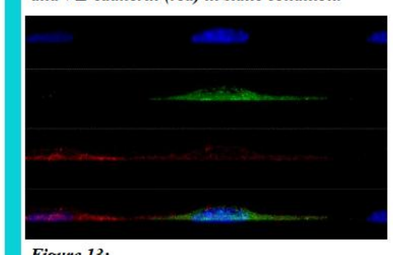


Figure 13: *IF image in z axis showing DAPI (blue), HMMR (green) and VE-cadherin (red) in static condition.*

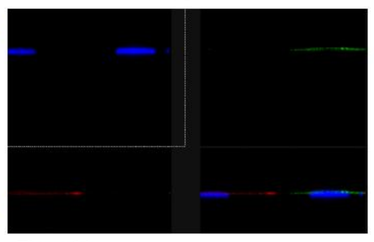


Figure 14: *IF image in z axis showing DAPI (blue), HMMR (green) and VE-cadherin (red) post 24h LSS.*

Radiolabeling HA:
Human glomerular endothelial cells treated with ^{3}H glucosamine isotope and HA quantification is done after High-performance liquid chromatography for separation of GAG.
There are increased production in HA and most of it is released in the media ***Fig 15***. GAGs fractions collected after High-performance liquid chromatography F3 and 4 represent HA, F 5 – 12 represent more sulphated GAGs ***Fig 16***.

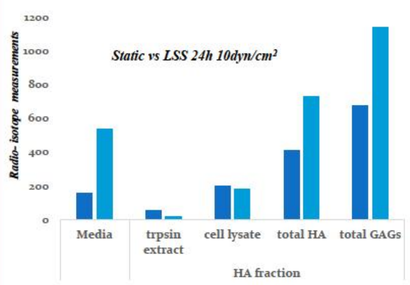


Figure 15: ***HA radiolabeling quantifications:*** ***HA shedding in the media is increased with LSS, total HA is increased with LSS associated with an increased in total GAGs***

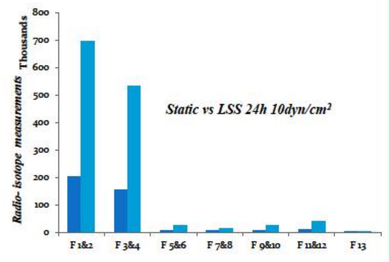


Figure 16: *HLPC fractions collected in static and 24h LSS. Most production increased with LSS in first two fractions F 3 and 4 represent HA.*

Scratch assays:
Human glomerular endothelial cells treated with LSS 10 dyn/cm^2 for 24h vs static condition. Scratches were made at same distance from the center of the dish. There was a reduction in cells migration with LSS compared to static ***Fig 17***.
HA fragments at 200ug/ml concentration induced cells migration in static condition, but this induction is reduced when cells were treated with LSS for 24h ***Fig 18.***

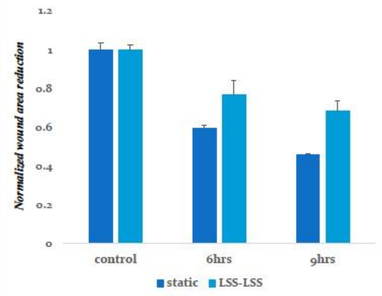


Figure 17: *Scratch assay there was a reduction in migration and motility in cells treated with LSS compared to static cells.*

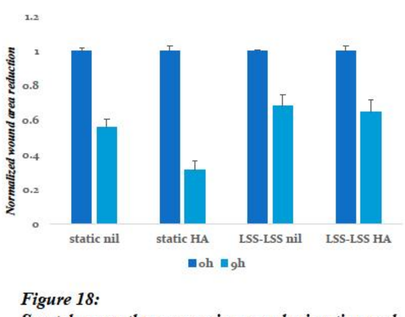


Figure 18: *Scratch assay there was a increased migration and motility in cells treated with HA fragments compared nil cells in static condition, but this difference disappeared when both cells were treated with LSS.*

METHODS

LSS introduction

Human conditioned immortalized glomerular endothelial cells (HciGEnC) grown in (100mm in diameter) petri dishes were exposed to physiologically relevant laminar shear stress of 10 dyn/cm^2 using an orbital shaker (SSM1 Stuart, Staffordshire, UK) ***Fig 4***.

Laminar shear stress can be calculated using this formula;

$$\tau w = \alpha\sqrt{[\rho\eta(2\pi f)^3]}$$

τw = *shear stress* α = *radius of rotation (10 cm)*

ρ = *density of liquid (1.005 g/l)*

η = *fluid viscosity (0.0075 dyn/cm^2 at 37° C)*

f = *rotations per second.*

shear stress of 10 dyn/cm^2 is at frequency of 75 rpm.

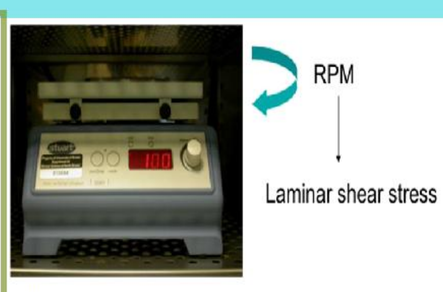


Figure 4: ***SSM1 Stuart Staffordshire orbital shaker; 75 rpm produce 10dyn/cm^2 of laminar shear stress at the edge of petri-dish used.*** ***Equation used***

$$\tau w = \alpha\sqrt{[\rho\eta(2\pi f)^3]}$$

Quantitative PCR

A custom-designed taqman qPCR gene arrays is produced to show the effect of 48 hours physiological relevant laminar shear stress on several glycocalyx related genes. qPCR performed with real time quantitative PCR system (one step plus). Relative quantification analysis was used to calculate the relative fold change (^^CT) between static condition and shear conditions.

Western blotting

Protein samples were extracted from (HciGEnC) using whole cell lysis buffer. Western blotting was performed to detect changes at the protein level with LSS.

Immunofluorescence staining

Images were taken with confocal microscope in imaging facility in medical school university of Bristol.

Radiolabeling HA

^{3}H glucosamine isotope was used to label glycosaminoglycans in glycocalyx, then samples were undergo *Anion exchange Liquid Chromatography (HLPC)* High-performance liquid chromatography and HA was quantified after 24h LSS treatment.

Scratch assays

To analyse cell migration and motility (ciHGEnC) in absence and presence of LSS with and with out HA fragments.

CONCLUSIONS

- Laminar shear stress modulates expression of glycocalyx components.
- HMMR in particular is exquisitely shear-sensitive. And HMMR may be a marker for LSS exposure in endothelial cells.
- HMMR regulate cell motility and migration in presence of HA and HA fragments.
- Ongoing work will determine the biological significance of altered HMMR expression.
- HA production is increased with LSS and most of it is released in Media.

Acknowledgements

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- Academic renal unit members
- Libyan Higher education ministry represented by the Libyan cultural affairs in London for funding my work

References 1. Picture adapted fromJA Jafferson. et al , kidney international (2008) 74;22-36. 2. Picture adapted from Reitsma and al, Eur J Physiol (2007) 454:345–359; 3. Table adapted from Reitsma and al, Eur J Physiol (2007) 454:345–359

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B) Cell signalling. Cell biology. Hormones.

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