Renin-Angiotensin System Overactivation in Type 2 Diabetes, a Risk for SARS-CoV-2 Infection?

Abu Saleh Md Moin PhD[1], Ahmed Al-Qaissi MD[2,3], Thozhukat Sathyapalan MD[2], Stephen L. Atkin MD, PhD[4], Alexandra E. Butler MD*[1]

[1]Diabetes Research Center (DRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar

[2]Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, Hull, UK

[3]Leeds Medical School, Leeds, UK

[4]Royal College of Surgeons in Ireland Bahrain, Adliya, Kingdom of Bahrain

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Author emails:
Alexandra E. Butler aeb91011@gmail.com; abutler@hbku.edu.qa
Abu Saleh Md Moin amoin@hbku.edu.qa
Ahmed Al Qaissi ahmedsattar1978@yahoo.com
Thozhukat Sathyapalan Thozhukat.Sathyapalan@hyms.ac.uk
Stephen L Atkin satkin@rcsi.com

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Corresponding author: Alexandra E. Butler, Diabetes Research Center (DRC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), PO Box 34110, Doha, Qatar. aeb91011@gmail.com; abutler@hbku.edu.qa

Phone: +974 66876499

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The novel coronavirus, SARS-CoV-2, binds to target cells via the angiotensin-converting enzyme 2 (ACE2) receptor found in cells in blood vessels, lungs, heart, intestines, and kidneys.

Type 2 diabetes (T2D) is a risk factor for acquiring SARS-Cov-2 infection and is associated with severe disease, acute respiratory distress syndrome and increased mortality (1). Diabetic patients have ACE2 overexpressed in kidney and the circulation; further, ACE2 expression may be increased in other tissues (for example in lungs) as a consequence of angiotensin-receptor blockers (ARBs), widely used in diabetic patients, potentially increasing susceptibility to SARS-Cov-2 infection. Previously, circulatory Renin-Angiotensin System (RAS) activity was described in the setting of sustained hyperglycemia in diabetes (2). Here, we hypothesized that acute normalization of glycemia would modulate RAS overactivation in T2D. Therefore, plasma RAS-related protein levels were determined in T2D versus controls (baseline) and after 1-hour hyperinsulinemic-euglycemic clamp (T2D).

A case-control study of T2D and control subjects, approved by Yorkshire and Humber Research Ethics Committee. The clamp was performed as detailed previously (3); all patients underwent a 10-hour fast prior to the clamp, but ad libitum water ingestion was encouraged. Patients were admitted 1-hour prior to the clamp procedure and remained in a supine position throughout the study. T2D: baseline glucose 7.6±0.4mmol/l (136.8±7.2mg/dl), reduced to 4.5±0.07mmol/l (81±1.2mg/dl) with 1-hour clamp. Controls: maintained at 4.9±0.1mmol/l (88.2±1.8mg/dl). Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement (4) was used to determine RAS-related proteins: renin (REN), angiotensinogen (AGT) and ACE2. Statistical analysis performed using Graphpad Prism 8.0. T2D (n=23) and control (n=23) subjects were

matched for age (p=ns); T2D had higher BMI (p=0.0012); duration of disease 4.5±2.9 years (Table 1). Nine T2D subjects were treated with ACE inhibitor (ACEi) therapy.

In T2D, total basal renin levels were elevated (1730±566 vs 675±72 RFU, T2D vs Control, $p<0.05$) (Figure 1A) whereas angiotensinogen levels were decreased (3786±174 vs 5005±574 RFU, T2D vs Control, $p<0.05$) (Figure 1B). ACE2 levels did not differ between T2D and controls (291±31 vs 281±18 RFU, T2DM vs Control, p=ns) (Figure 1C). Acute normalization of hyperglycemia to euglycemia had no effect on levels of these RAS-related proteins (Figure 1A, B, C).

RAS is overactivated in obesity (5) and the T2D subjects had higher BMI. To elucidate a potential relationship, T2D subjects (and Controls) were stratified into tertiles according to BMI; however, no trends in the protein levels were seen with increasing BMI in either group. Stratification of T2Ds into those treated or not with ACEi revealed no differences in basal RAS-related protein levels or in response to acute normalization of glycemia (ACEi vs non-ACEi: basal AGT 4106±228 vs 3580±235 RFU, normalized AGT: 4324±275 vs 4125±365 RFU, p=ns ; basal REN 1358±258 vs 1969±923 RFU, normalized REN: 1250±207 vs 1903±843 RFU, p=ns; basal ACE2 253±10 vs 317±50 RFU, normalized ACE2: 265±11 vs 299±48 RFU, p=ns) (Figure 1D, E, F).

Of note, plasma glucagon levels were markedly elevated in patients with T2D compared to control (2458.9±291.3 vs 1460.8±135.6 RFU of glucagon, T2D vs Control, $p< 0.01$).

This study showing elevated plasma renin, together with suppressed angiotensinogen and comparable levels of ACE2 protein, suggests RAS overactivation in T2D, independent of obesity, that was not corrected by acute normoglycemia. This suggests that immediate glucose

fluctuations do not modulate RAS and are therefore unlikely to modify SARS-CoV-2 susceptibility.

Renin causes conversion of angiotensinogen to angiotensin I (ANGI); ANGI is further converted by ACE to ANGII. ACE2, by contrast, converts ANGII to ANG-1-7, a peptide beneficial in maintaining normotension. ANGII receptor blockers, such as Losartan, increase ACE2 levels whereas ACE inhibitors do not affect ACE2 levels or activity. In addition, both ACEis and ARBs increased plasma renin activity (PRA), but not the plasma renin concentration, in healthy individuals and patients with hypertension, though the mechanism by which ACEi/ARB enhanced renin activity in humans has not yet been identified. However, glucagon stimulated plasma renin activity has also been reported in humans, and we found very high basal levels of plasma glucagon in patients with T2D compared to controls, suggesting that the impact of ACEi therapy might be counteracted by glucagon in our study.

A limitation of this study is the measurement of plasma proteins that may not reflect tissue level expression. In addition, we report renin concentrations rather than activity that may be discrepant in some circumstances, including an underestimated renin activity in patients with liver cirrhosis and severe cardiac failure, whilst overestimated renin activity is seen with estrogen exposure. Notably, none of these conditions were present in the patients recruited to this study and the women were postmenopausal and not on hormone replacement therapy.

In conclusion, RAS protein levels differed between T2D and controls but were unaffected by glucose normalization, and no differences in plasma ACE2 levels were seen in ACEi-treated T2D subjects. This data supports the concept that discontinuing ACEi therapies in T2D subjects to reduce risk of SARS-CoV-2 infection is not beneficial.

DECLARATIONS

Ethics approval and consent to participate: The study was approved by the Yorkshire and the Humber Research Ethics Committee and all study participants signed an informed consent form prior to participation.

Consent for publication: All authors gave their consent for publication.

Availability of data and materials: All the data for this study will be made available upon reasonable request to the corresponding author.

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Author contributions

ASMM and AEB analyzed the data and wrote the manuscript. AAQ contributed to study design, performed experiments, collected, analyzed, and interpreted data and edited the manuscript. TS supervised clinical studies and edited the manuscript. SLA contributed to study design, data interpretation and the writing of the manuscript. All authors reviewed and approved the final version of the manuscript. Alexandra E Butler is the guarantor of this work.

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Figure legends.

Figure 1. Circulatory levels of renin-angiotensin system (RAS) related proteins in T2D. Plasma total renin (A), angiotensinogen (B) and ACE2 (C) levels in control subjects (open square) and subjects with T2D (black square). The basal level of plasma renin was higher and basal level of plasma angiotensinogen was lower in T2D compared to control. There was no change in the basal level of ACE2 in T2D. Acute normalization of glycemia had no effect on levels of renin, angiotensinogen or ACE2 in T2D (A, B, C). Plasma levels of renin (D), angiotensinogen (E) and ACE2 (F) in T2D subjects who were not taking an ACEi antihypertensive drug (open square) and those who were on ACEi (black square). There was no effect of ACEi on basal levels or acute normalization levels of RAS related proteins in T2D. *$p<0.05$; ns = not significant.