



Metabolic dysfunction in Emirati subjects in Abu Dhabi: Relationship to levels of soluble RAGEs

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ABSTRACT

Background: The United Arab Emirates is experiencing increasing rates of type 2 diabetes (T2D) and its complications. As soluble levels of the receptor for advanced glycation end products, (sRAGE), and endogenous secretory RAGE (esRAGE), the latter an alternatively spliced form of *AGER* (the gene encoding RAGE), have been reported to be associated with T2D and its complications, we tested for potential relationships between these factors and T2D status in Emirati subjects.

Methods: In a case-control study, we recruited Emirati subjects with T2D and controls from the Sheikh Khalifa Medical City in Abu Dhabi. Anthropomorphic characteristics, levels of plasma sRAGE and esRAGE, and routine chemistry variables were measured.

Results: Two hundred and sixteen T2D subjects and 215 control subjects (mean age, 57.4 ± 12.1 vs. 50.7 ± 15.4 years; $P < 0.0001$, respectively) were enrolled. Univariate analyses showed that levels of sRAGE were significantly lower in the T2D vs. control subjects (1033.9 ± 545.3 vs. 1169.2 ± 664.1 pg/ml, respectively; $P = 0.02$). Multivariate analyses adjusting for age, sex, systolic blood pressure, pulse, body mass index, Waist/Hip circumference ratio, fasting blood glucose, HDL, LDL, insulin, triglycerides, Vitamin D and urea levels revealed that the difference in sRAGE levels between T2D and control subjects remained statistically-significant, $P = 0.03$, but not after including estimated glomerular filtration rate in the model, $P = 0.14$. There were no significant differences in levels of esRAGE. Levels of plasma insulin were significantly higher in the control vs. the T2D subjects (133.6 ± 149.9 vs. 107.6 ± 93.3 pg/L, respectively; $P = 0.01$, after adjustment for age and sex).

Conclusion/discussion: Levels of sRAGE, but not esRAGE, were associated with T2D status in Abu Dhabi, but not after correction for eGFR. Elevated levels of plasma insulin in both control and T2D subjects suggests the presence of metabolic dysfunction, even in subjects without diabetes.

Abbreviations: ADAM10, a disintegrin and metalloproteinase domain-containing protein 10; AGEs, advanced glycation endproducts; ARIC, Atherosclerosis Risk in Communities; BMI, body mass index; CARDS, Collaborative Atorvastatin Diabetes Study; CV, coefficient of variation; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; esRAGE, endogenous secretory RAGE; ESRD, end stage renal disease; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL, high density lipoprotein; hsCRP, high sensitivity C-reactive protein; LADA, latent autoimmune diabetes of the adult; LDL, low density lipoprotein; MMP, matrix metalloproteinase; RAGE, receptor for advanced glycation endproducts; SBP, systolic blood pressure; SKMC, Sheikh Khalifa Medical City; sRAGE, soluble RAGE; T2D, type 2 diabetes; TG, triglycerides; UAE, United Arab Emirates; UAEHFS, United Arab Emirates Healthy Futures Study; W/H ratio, Waist/Hip circumference ratio

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Introduction

The United Arab Emirates (UAE) is experiencing a significant rise in the incidence and prevalence of obesity, pre-diabetes and type 2 diabetes (T2D) [1]. In the UAE, between 6% and 14% of affected subjects are undiagnosed for these metabolic disorders [2]. Because of the long-term effects of diabetes on morbidity, mortality and health care delivery systems, efforts have focused on delineating the accompanying comorbidities and the most common diabetic complications. Recently, Jelinek and colleagues enrolled adult patients with T2D from two hospitals in Abu Dhabi and reported that hypertension, obesity and dyslipidemia accompanied the diagnosis of T2D in 83.4%, 90.49% and 93.43% of subjects, respectively [3]. Overall, 83.47% of the patients were affected by more than one complication, including retinopathy (13.26%), coronary artery disease (10.20%) and nephropathy (5.92%). Diabetes duration, kidney function (estimated glomerular filtration rate (eGFR)) and levels of total cholesterol were the most significant predictive risk factors for the development of diabetic complications [3].

Beyond the adult population, increasing numbers of young subjects are developing metabolic syndrome and its associated sequelae in the UAE. Al Dhaheri and colleagues reported that in a cross-sectional study of 555 female Emirati college students, 23.1% were overweight and 10.4% were obese; the overall prevalence of metabolic syndrome was 6.8% and the levels of HbA1c were important predictors of the presentation of metabolic syndrome, as the odds of metabolic syndrome were 22 times higher in subjects with HbA1c $\geq 6.5\%$ [4].

These considerations underscore that the discovery of predictive biomarkers to distinguish diabetic from non-diabetic subjects in the UAE may be of value in identifying subjects most vulnerable to T2D and its complications. In obesity and diabetes, accumulation of the products of nonenzymatic glycation of proteins and lipids, the advanced glycation end products (AGEs), accompanies these disorders [5,6]. The best-characterized cell surface receptor for AGEs is the receptor for advanced glycation end products (RAGE); studies in animal models and human diabetic tissues suggest key roles for RAGE in the pathogenesis of obesity and T2D and its macro- and microvascular complications [7–9]. RAGE is a member of the immunoglobulin superfamily; it contains three extracellular domains, composed of one V-type and two C-type immunoglobulin domains, a single transmembrane spanning domain and a highly-charged cytoplasmic domain that is required for RAGE signal transduction [9]. In addition to cell surface RAGE, RAGE also exists in soluble forms in plasma/serum. Soluble or sRAGE, is produced through the actions of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) and includes the product of an mRNA splice variant of the *AGER* gene, called endogenous secretory (es)RAGE, also known as RAGEv1 [10]. Distinct assay systems detect only esRAGE. Multiple studies have identified associations between the levels of sRAGE or esRAGE and the presence of obesity, diabetes and/or its complications [10–12]. We recently reported findings on the levels of sRAGE and esRAGE in a pilot study of the UAE Healthy Futures Study (UAEHFS), which is a cohort study that is enrolling healthy Emirati subjects (age ≥ 18 years). The pilot UAEHFS showed statistically-significant associations between these markers and body mass index (BMI) and waist/hip (W/H) circumference ratio [13].

Here, we tested the potential association between levels of sRAGE and esRAGE in subjects with established T2D vs. non-diabetic control subjects recruited from the medical clinics of the Sheikh Khalifa Medical City (SKMC) in Abu Dhabi.

Materials and methods

Study group

Participants were recruited from Sheikh Khalifa Medical City, Abu Dhabi. Emirati nationals, aged 18 years and above, that had been

fasting for a minimum of 8 h prior to recruitment were eligible for the study. Pregnant woman and people with end stage renal disease (ESRD) were excluded. Study participants self-reported diabetic status, medication and medical history. Physical measurements and blood samples were collected from participants. All of the 498 consented participants gave blood samples; 8 participants were excluded from analysis due to either low sample volume or poor sample quality. An additional 59 participants that had self-reported as non-diabetes were found to have an HbA1c level of $\geq 6.5\%$ and were therefore excluded from further analysis. The remaining 215 non-diabetic and 216 diabetic subjects were included in the analysis dataset. This study was approved by the Institutional Review Boards (IRB) of Sheikh Khalifa Medical City (SKMC) and New York University Abu Dhabi (NYUAD). All individuals participating in the study read and signed an informed consent.

Physical measurements

Standing height and weight were measured using a stadiometer and a digital column scale, respectively (Seca, Hamburg Deutschland). Waist and hip circumferences were measured using a standard tape (Wessex non-stretchable sprung tape) in order to report the W/H circumference ratio. BMI was calculated according to the following formula: body weight (kg)/height² (meters). Brachial blood pressure (systolic and diastolic (SBP and DBP, respectively)) was recorded on the upper left arm with appropriate cuff size using a semi-automated sphygmomanometer (Omron M10-IT, Omron Corporation, Kyoto, Japan).

Biological samples

Study participants provided blood specimens in 5 ml SST vacutainer and 4 ml plasma EDTA vacutainer. SST vacutainers were subjected to centrifugation (3,500 rpm, 4 °C, 15 min) 30 min post-collection. All samples were refrigerated (4–8 °C) and then transported to the NYU Abu Dhabi (NYUAD) research laboratory in a temperature-controlled cooler. On arrival at the NYUAD research laboratory, the SST samples were aliquoted into 1.0 ml tubes. 1 ml of whole blood was removed from the EDTA vacutainer and stored. The remaining sample was centrifuged at 3500 rpm at 4 °C for 15 min and plasma and red blood cells (RBCs) were aliquoted into 1.0 ml tubes. All aliquots were stored at –80 °C until further testing (see below).

Standard chemistry assays

HbA1c was measured on EDTA-derived whole blood sample and routine clinical chemistry for Urea, Creatinine, Glucose (fasting blood glucose (FBG)), Cholesterol, Triglycerides (TG), LDL, HDL and hsCRP was performed on SST serum. All assays were performed on the Beckman Coulter UniCel DxC 600 Synchron Clinical Systems (Beckman Coulter, USA) according to the manufacturer's instructions. Instrument results were validated against the RIQAS external quality assessment programs for general clinical chemistry and HbA1c. HbA1c is reported in NGSP units. Serum levels of Vitamin D were assayed using the Beckman Coulter Access 2 Immunoassay System (Beckman Coulter, USA) in accordance with the manufacturer's instructions. eGFR was calculated according to the CKD-EPI equations: female with Scr ≤ 0.7 , eGFR = $144 \times (\text{Scr}/0.7)^{-0.329} \times (0.993)^{\text{Age}}$; female with Scr > 0.7 , eGFR = $144 \times (\text{Scr}/0.7)^{-1.209} \times (0.993)^{\text{Age}}$; male with Scr ≤ 0.9 , eGFR = $141 \times (\text{Scr}/0.9)^{-0.411} \times (0.993)^{\text{Age}}$; and male with Scr > 0.9 , eGFR = $141 \times (\text{Scr}/0.9)^{-1.209} \times (0.993)^{\text{Age}}$.

Research assays

Levels of soluble (s) RAGE and esRAGE were determined on plasma obtained from blood in EDTA tubes on samples previously stored at –80 °C using enzyme-linked immunosorbent assay (ELISA) kits in

Table 1
Descriptive table for Controls (n = 215) and T2D Cases (n = 216).

Variable	Controls (n = 215)				T2D Cases (n = 216)				t-test	Logistic Regression ^a	
	Mean	SD	Min	Max	Mean	SD	Min	Max	P value	Odds Ratio	P value
Age (yrs)	50.7	15.4	18.0	86.0	57.4	12.1	21.0	87.0	< 0.0001	–	–
Weight (kg)	75.4	15.5	33.0	147.6	82.3	17.1	52.0	143.0	< 0.0001	1.03	< 0.0001
Height (cm)	162.0	7.6	135.0	185.0	160.4	9.0	137.0	189.5	0.06	0.97	0.07
BMI (kg/m ²)	28.8	5.8	14.3	54.9	32.0	6.0	20.6	54.9	< 0.0001	1.11	< 0.0001
Waist Circumference (cm)	93.6	10.0	67.0	137.0	101.6	12.0	77.0	141.0	< 0.0001	1.07	< 0.0001
Hip Circumference (cm)	102.6	10.8	78.0	160.0	108.8	12.2	78.0	165.0	< 0.0001	1.05	< 0.0001
W/H ratio	0.9	0.1	0.7	1.2	0.9	0.1	0.6	1.3	0.00	163.11	0.01
DBP (mm Hg)	77.4	8.4	53.0	121.0	77.2	10.3	52.0	100.0	0.82	0.99	0.61
SBP (mm Hg)	118.3	11.4	99.0	173.0	126.7	15.5	76.0	180.0	< 0.0001	1.04	< 0.0001
Pulse (beats/min)	75.5	9.0	53.0	97.0	77.7	9.9	51.0	102.0	0.02	1.02	0.03
HbA1c (%)	5.6	0.4	4.2	6.4	7.5	1.6	4.3	13.8	< 0.0001	13.09	< 0.0001
FBG (mg/dl)	97.5	26.0	61.0	309.0	136.8	53.8	59.0	459.0	< 0.0001	1.03	< 0.0001
Insulin (pg/L)	133.6	149.9	7.8	1322.5	107.6	93.3	0.0	652.8	0.03	1	0.01
Urea (mg/dl)	15.4	9.8	1.0	82.0	16.9	8.7	5.0	88.0	0.10	1	0.81
S Creatinine (mg/dl)	0.8	0.7	0.3	5.4	0.8	0.5	0.3	6.5	0.53	0.75	0.13
eGFR (ml/min/1.73 m ²)	99.3	26.0	8.5	153.6	94.7	24.0	5.3	149.1	0.06	1.01	0.24
Total Cholesterol (mg/dl)	182.7	38.7	100.0	317.0	160.8	43.8	72.0	374.0	< 0.0001	0.99	< 0.0001
TG (mg/dl)	105.6	53.4	21.0	280.0	132.4	92.2	31.0	965.0	< 0.001	1.01	< 0.01
LDL (mg/dl)	100.2	29.3	35.3	195.8	87.0	32.5	28.9	220.4	< 0.0001	0.99	< 0.01
HDL (mg/dl)	52.6	13.7	20.6	112.2	46.0	11.3	18.6	79.5	< 0.0001	0.95	< 0.0001
Hs CRP (mg/dl)	0.5	0.7	0.0	4.4	0.6	0.8	0.0	8.0	0.78	1.05	0.71
Vitamin D (ng/ml)	29.3	13.4	6.5	87.7	35.5	17.3	7.0	109.1	< 0.0001	1.02	< 0.01
sRAGE (pg/ml)	1169.2	664.1	339.3	5070.9	1033.9	545.3	282.2	4062.1	0.02	1	0.04
esRAGE (pg/ml)	250.0	260.0	10.0	2380.0	250.0	220.0	10.0	2330.0	0.76	0.7	0.40

P Value < 0.05 is statistically significant are in bold.

* Note: In the logistic regression, age and sex are additionally adjusted.

accordance with the manufacturers' protocols (R&D Systems Quantikine Immunoassay, Minneapolis, MN, and As One International, Santa Clara, CA, respectively). The kit from R&D Systems measures both cell surface-cleaved sRAGE and esRAGE; the kit from As One International measures only esRAGE. Insulin levels were assayed on serum using an ELISA kit following manufacturer's guidelines (Dako, Denmark). Reported results represent the mean of the results from two distinct wells. Interassay variability for the measurement of sRAGE, esRAGE and insulin was CV 8.02%, 7.75% and 6.47%, respectively.

Statistical analyses

Data in the tables are presented with standard deviation (SD) or standard error (SE) values, as indicated. Descriptive analysis and logistic regression with age and sex adjustments on the effect of candidate variables on case-control status are reported in Table 1. In Tables 2 and 3, the multivariate logistic regression models [14] were fitted to survey if levels of sRAGE were associated with case-control status while adjusting covariates with $P < 0.15$ in Table 1. In Table 3, the multivariate logistic regression models with the factors in Table 2 plus eGFR were fitted to survey if levels of sRAGE were associated with case-control status. Levels of total cholesterol and hip circumference were removed in the multivariate analysis because the total cholesterol is highly correlated with levels of HDL ($R = 0.51$, $P < 0.0001$) and levels of LDL ($R = 0.9$, $P < 0.0001$). Hip circumference is correlated with BMI ($R = 0.77$, $P < 0.0001$). Similar analyses were conducted for levels of esRAGE in Tables S1 and S2. The linear regression models are fitted to the log-transformed levels of sRAGE and esRAGE in cases and controls separately, in order to check their association with each of the listed covariates in Table S3, while only age and sex are adjusted. Then, the covariates with $P < 0.15$ in Table S3 were re-fitted into the multivariate linear regression model for log sRAGE/log esRAGE to confirm the association in Tables 4 and 5. Similar methodology was used to assess which covariates are independently associated with eGFR and plasma insulin levels in cases and controls, separately. Specifically, in Tables S5 and S6, we assessed the association of eGFR and plasma insulin, respectively, with the listed covariates in cases and controls,

Table 2

Multivariate logistic regression model fitted for the association between sRAGE and T2D status.

Parameter	Analysis of Maximum Likelihood Estimates			Odds Ratio Estimates		
	Estimate	SE	P value	Estimate	95% Wald Confidence Limits	
Age (yrs)	0.0009	0.0109	0.935	1.00	0.98	1.02
Sex	-0.0683	0.3078	0.824	0.93	0.51	1.71
BMI (kg/m ²)	0.0734	0.0228	0.001	1.08	1.03	1.13
W/H ratio	3.4982	2.3452	0.136	33.06	0.33	> 999.999
SBP (mm Hg)	0.0329	0.0099	0.001	1.03	1.01	1.05
Pulse (beats/min)	0.0156	0.0135	0.248	1.02	0.99	1.04
FBG (mg/dl)	0.0305	0.0050	< 0.0001	1.03	1.02	1.04
Insulin (pg/L)	-0.0045	0.0013	0.001	1.00	0.99	1.00
Urea (mg/dl)	0.0200	0.0150	0.184	1.02	0.99	1.05
TG (mg/dl)	0.0056	0.0024	0.020	1.01	1.00	1.01
LDL (mg/dl)	-0.0063	0.0047	0.179	0.99	0.99	1.00
HDL (mg/dl)	-0.0193	0.0119	0.105	0.98	0.96	1.00
Vitamin D (ng/ml)	0.0306	0.0092	0.001	1.03	1.01	1.05
sRAGE (pg/ml)	-0.0005	0.0002	0.034	1.00	1.00	1.00

P Value < 0.05 is statistically significant are in bold.

separately, while only age and sex are adjusted. The covariates with $P < 0.15$ in Tables S5 and S6 were re-fitted into the multivariate linear regression model for eGFR and plasma insulin to confirm the association in Tables 6 and 7. For the eGFR and insulin level data analyses, levels of serum creatinine and HbA1C were not included, because eGFR and T2D case-control status, respectively, are based on those values.

Results

Univariate analyses – sRAGE and esRAGE levels

To establish if plasma levels of sRAGE and/or esRAGE distinguished

Table 3
Multivariate logistic regression model fitted for the association between RAGE and T2D status with eGFR, additionally adjusted.

Analysis of Maximum Likelihood Estimates			Odds Ratio Estimates		
Parameter	Estimate	SE	P value	Estimate	95% Wald Confidence Limits
Age (yrs)	0.019	0.013	0.1301	1.02	0.99 1.05
Sex	-0.196	0.315	0.5334	0.822	0.44 1.52
BMI (kg/m ²)	0.074	0.023	0.0014	1.076	1.03 1.13
W/H ratio	3.935	2.378	0.098	51.165	0.48 > 999.99
SBP (mm Hg)	0.033	0.010	0.001	1.034	1.01 1.06
Pulse (beats/min)	0.015	0.014	0.2838	1.015	0.99 1.04
FBG (mg/dl)	0.032	0.005	< 0.0001	1.032	1.02 1.04
Insulin (pg/L)	-0.005	0.001	0.0004	0.995	0.99 1.00
Urea (mg/dl)	0.066	0.023	0.0041	1.068	1.02 1.12
eGFR (ml/min/1.73 m ²)	0.027	0.009	0.0035	1.028	1.01 1.05
TG (mg/dl)	0.006	0.002	0.0093	1.006	1.00 1.01
LDL (mg/dl)	-0.008	0.005	0.103	0.992	0.98 1.00
HDL (mg/dl)	-0.021	0.012	0.0927	0.98	0.96 1.00
Vitamin D (ng/ml)	0.033	0.009	0.0005	1.033	1.01 1.05
sRAGE (pg/ml)	0.000	0.000	0.1439	1	1.00 1.00

P Value < 0.05 is statistically significant are in bold.

Emirati subjects with T2D vs. controls in the clinic populations of the SKMC, we enrolled 216 subjects with T2D and 215 control subjects who were free from T2D. Among the control subjects, 72.6% were female and 27.4% were male; in the T2D case subjects, 68.5% were female and 31.5% were male; $P = 0.36$. Table 1 indicates that statistically-significant differences were observed between the T2D case subjects vs. the control subjects with respect to levels of sRAGE, but not esRAGE ($P = 0.02$ and $P = 0.76$, respectively). On account of the significant difference in subject age between the T2D and controls, we corrected these data for age and sex. After this adjustment, levels of sRAGE remained statistically-significantly different between T2D cases vs. control subjects (1033.9 ± 545.3 vs. 1169.2 ± 664.1 pg/ml, respectively; $P = 0.04$). Levels of esRAGE were not statistically-significantly different between the groups ($P = 0.40$) (Table 1, far right column).

Multivariate analyses – sRAGE and esRAGE levels

We next performed multivariate analyses adjusting the covariates with $P < 0.15$ in Table 1. Table 2 reports the multivariate model, including age, sex, SBP, pulse, BMI, W/H ratio and levels of FBG, HDL, LDL, insulin, TG, urea and Vitamin D. The data revealed that levels of sRAGE remained statistically-significantly different between T2D cases

Table 4

The multivariate linear regression models fitted for the association between log sRAGE and covariates in cases and controls, separately. The covariates included in each model are those with p value < 0.15 in the univariate association check reported in Table S4.

Outcome	log_sRAGE						
	Controls Only			T2D Cases Only			
Covariates	Estimate	SE	P value	Covariates	Estimate	SE	P value
Age (yrs)	-0.007	0.003	0.017	Age (yrs)	-0.004	0.003	0.227
Sex (male as reference)	-0.053	0.082	0.514	Sex (male as reference)	-0.012	0.065	0.858
BMI (kg/m ²)	-0.020	0.006	0.001	SBP (mm Hg)	-0.002	0.002	0.238
Insulin (pg/L)	-0.0002	0.0002	0.365	HbA1c (%)	-0.039	0.019	0.046
Urea (mg/dl)	0.010	0.006	0.084	eGFR (ml/min/1.73 m ²)	-0.007	0.002	< 0.0001
eGFR (ml/min/1.73 m ²)	-0.002	0.002	0.476				
Hs CRP (mg/dl)	-0.046	0.051	0.371				
Vitamin D (ng/ml)	0.004	0.003	0.133				

P Value < 0.05 is statistically significant are in bold.

vs. controls, $P = 0.03$ (Table 2). However, after correction for the above factors and, in addition, eGFR, levels of plasma sRAGE were no longer statistically-significantly different between the T2D vs. control subjects, $P = 0.14$ (Table 3).

With respect to plasma levels of esRAGE, Tables S1 and S2 reveal that after multiple covariate adjustment, there were no significant associations between levels of esRAGE and T2D status, irrespective of adjustment, or not, for eGFR. We performed subgroup analyses within male and female subjects separately in T2D cases vs. control subjects and the conclusions regarding significant differences vis-à-vis sRAGE, and the lack of significant difference in levels of esRAGE, were unchanged (data not shown).

Subgroup analyses – sRAGE and esRAGE levels

We performed subgroup analyses in order to determine which of the covariates was/were associated with sRAGE or esRAGE in T2D and control subjects, separately. Fitting the covariates with $P < 0.15$ in Table S3 into the multivariate regression models for levels of sRAGE or esRAGE in T2D and control subjects, we found that in T2D subjects, levels of sRAGE were significantly associated with eGFR and HbA1c; $P < 0.0001$ and $P = 0.046$, respectively. In the control subjects, sRAGE levels were significantly associated with age and BMI; $P = 0.02$ and $P = 0.001$, respectively (Table 4). With respect to esRAGE, in T2D subjects, levels of esRAGE were significantly associated with eGFR and W/H ratio; $P < 0.0001$ and $P = 0.047$, respectively and in the control subjects, levels of esRAGE were significantly associated with BMI, pulse and levels of urea; $P = 0.002$, $P = 0.02$ and $P = 0.02$, respectively (Table 5).

On account of the significant differences in age and that we found no differences in eGFR between the two groups (Table 1), we reasoned that this might be due to differences in subjects' age. In Table S4, we assessed the mean levels of eGFR by age in the subjects according to the following groups: age < 40 years, age ≥ 40 but < 50 years, and age ≥ 50 but < 60 years. Only in the groups ≥ 60 but < 70 years, and ≥ 70 years of age did we observe trends to differences in eGFR between the T2D vs. control subjects ($P = 0.06$ in both cases).

eGFR and covariate assessment

As shown in Table 3, after adjusting for other important variables, we found that the levels of eGFR were significantly higher in cases vs. the controls, which implies that the T2D cases and controls have different kidney function (est = 0.03, $P = 0.004$). To identify the factors significantly associated with eGFR in the T2D cases and controls, separately, we fit the covariates with $P < 0.15$ in Table S5 into the multivariate regression models for eGFR in the T2D and control subjects. We found that in the T2D cases, age and urea were negatively and

Table 5

The multivariate linear regression models fitted for the association between log esRAGE and covariates in cases and controls, separately. The covariates included in each model are those with p value < 0.15 in the univariate association check reported in Table S4.

Outcome	log_esRAGE						
	Controls Only			T2D Cases Only			
Covariates	Estimate	SE	<i>P</i> value	Covariates	Estimate	SE	<i>P</i> value
Age (yrs)	-0.0027	0.0043	0.528	Age (yrs)	0.0038	0.005	0.441
Sex (male as reference)	0.0355	0.1277	0.782	Sex (male as reference)	0.0978	0.0993	0.326
BMI (kg/m ²)	-0.0293	0.0095	0.002	W/H ratio	-1.351	0.6747	0.047
Pulse (beats/min)	0.0131	0.0056	0.021	HbA1c (%)	-0.0448	0.0291	0.125
Insulin (pg/L)	-0.0007	0.0004	0.073	eGFR (ml/min/1.73 m ²)	-0.01	0.0024	< 0.0001
Urea (mg/dl)	0.0204	0.0087	0.02	Vitamin D (ng/ml)	0.0036	0.0028	0.203
eGFR (ml/min/1.73 m ²)	-0.0027	0.0035	0.442				
TG (mg/dl)	0.002	0.001	0.056				
Hs CRP (mg/dl)	-0.0641	0.0808	0.429				

P Value < 0.05 is statistically significant are in bold.

Table 6

The multivariate linear regression models fitted for the association between eGFR and covariates in cases and controls, separately. The covariates included in each model are those with p value < 0.15 in the univariate association check reported in Table S5.

Outcome	eGFR						
	Controls Only			T2D Cases Only			
Covariates	Estimate	SE	<i>P</i> value	Covariates	Estimate	SE	<i>P</i> value
Age (yrs)	-0.74	0.07	< 0.0001	Age (yrs)	-0.76	0.09	< 0.0001
Sex (male as reference)	-4.76	2.40	0.048	Sex (male as reference)	-0.91	2.10	0.665
Urea (mg/dl)	-1.67	0.11	< 0.0001	SBP(mm Hg)	0.04	0.06	0.520
TG (mg/dl)	-0.05	0.02	0.018	Total Cholesterol (mg/dl)	-0.02	0.02	0.315
HDL (mg/dl)	0.03	0.08	0.722	Urea (mg/dl)	-1.60	0.12	< 0.0001

P Value < 0.05 is statistically significant are in bold.

Table 7

The multivariate linear regression models fitted for the association between insulin and covariates with p value < 0.15 in Table S6 in controls and cases, separately.

Outcome	Insulin (pg/L)						
	Controls Only			T2D Cases Only			
Covariates	Estimate	SE	<i>P</i> value	Covariates	Estimate	SE	<i>P</i> value
Age (yrs)	-0.40	0.68	0.557	Age (yrs)	-0.18	0.61	0.767
Sex (male as reference)	-82.67	21.89	< 0.0001	Sex (male as reference)	8.93	14.38	0.535
HbA1c (%)	35.50	22.94	0.123	W/H ratio	-119.57	93.97	0.205
FBG (mg/dl)	1.87	0.39	< 0.0001	DBP (mm Hg)	1.50	0.63	0.017
TG (mg/dl)	0.48	0.18	0.006	FBG (mg/dl)	0.15	0.12	0.233
Hs CRP (mg/dl)	12.53	13.65	0.360	Total Cholesterol (mg/dl)	-0.01	0.41	0.985
Vitamin D (ng/ml)	-0.72	0.71	0.313	TG (mg/dl)	0.10	0.07	0.158

P Value < 0.05 is statistically significant are in bold.

significantly associated with eGFR; $P < 0.0001$ in both cases. In the control subjects, age, TG, and urea were negatively associated with eGFR; $P < 0.0001$, $P = 0.02$, and $P < 0.0001$, respectively. In the control subjects, males have significantly higher levels of eGFR than females; $P = 0.048$ (Table 6).

Insulin and covariate assessment

Finally, our data revealed the unexpected finding that levels of plasma insulin were significantly higher in the control vs. the T2D case subjects, even after correction for age and sex (133.6 ± 149.9 vs. 107.5 ± 93.3 pg/L; $P = 0.01$ (Table 1) or after correction for all of the listed variables in Table 3 (est = -0.005, $P = 0.0004$). As shown in Table 7, in the multivariate analyses, only considering the covariates with $P < 0.15$ in Table S6, in the T2D cases, only DBP was positively and significantly associated with plasma insulin levels; $P = 0.02$ and in

the control subjects, FBG and TG levels were positively and significantly associated with plasma insulin levels; $P < 0.0001$ and $P = 0.006$, respectively. In the control subjects, males have significantly higher levels of plasma insulin than the female subjects; $P < 0.0001$.

Discussion

Our study examined two known detectable forms of soluble RAGE. The first, soluble or sRAGE, is detected using an ELISA for total soluble RAGE forms, which includes both the cell-surface cleaved form of RAGE and the second form of soluble RAGE, known as es (endogenous secretory) or esRAGE, which is derived from alternative spliced forms of *AGER*. The measurement of esRAGE may be directly performed using an ELISA that selectively recognizes this form of soluble RAGE. Although both forms have been studied extensively in metabolic diseases, few studies have examined these levels in the UAE in subjects without vs.

with metabolic disorders. We previously reported on levels of sRAGE and esRAGE in a pilot cohort study of the UAE Healthy Futures Study (UAEHFS). In that work, we reported a reduction in sRAGE and esRAGE levels in normal to pre-diabetic to frankly diabetic subjects. In contrast to the present case-control study, the mean age of the overall subject group in the pilot cohort study was lower (31.78 years) [13].

Here, we sought to test the levels of sRAGE and esRAGE in subjects with known metabolic dysfunction (T2D) vs. apparently healthy controls; the present subjects, however, were older than those studied in the earlier cohort study (Table 1). The observation that levels of sRAGE were significantly lower in T2D vs. control subjects after correction for risk-associated factors, except for eGFR, is largely consistent with previous studies in which levels of sRAGE were reported to be lower in T2D compared to control subjects [12,15]. Further, consistent with the results of our study, others demonstrated the dependence of levels of sRAGE on renal function in other populations. Kankova and colleagues studied 265 subjects with T1D or T2D (or LADA, “latent autoimmune diabetes of the adult”) and found that levels of sRAGE were significantly higher in subjects with diabetic nephropathy vs. those with normoalbuminuria; after multivariate regression modeling, GFR was the only independent variable found to be associated with levels of sRAGE [16]. In other work, increased levels of sRAGE were independently associated with the development or worsening of established kidney disease and mortality over the ensuing 5 years [17]. In the subjects reported here, the values of eGFR were within the normal range and were not significantly different between the two groups. However, since we did not assess any additional measures of renal abnormalities or albuminuria, we are unable to fully comment on the status of renal function and diabetic renal disease. Further, it is acknowledged that a limitation of this work is that estimation equations like CKD-EPI for eGFR are affected by the limitations inherent in the use of serum creatinine. This is particularly true in certain populations, in which serum creatinine levels are less accurate. These include diabetic patients with high GFR [18], specific ethnic groups such as Asians, pregnant women and amputees, as examples. In all of these settings, use of a confirmatory test such as estimated GFR from cystatin C, or creatinine-cystatin GFR estimating equations, collection of a 24-hour clearance of an exogenous filtration marker, would provide a more accurate assessment of GFR than that estimated from creatinine.

Collectively, the present and previous studies underscore that much remains to be learned regarding the direct or indirect effects of extensive renal disease on levels of both sRAGE and esRAGE. Specifically, does renal disease increase cell surface RAGE, thereby facilitating further release of cell surface RAGE; does extensive renal disease significantly modulate the expression and functional activity of the various proteases responsible for cleavage and release of sRAGE; and/or is there a substantial effect of advanced renal disease on the regulation of esRAGE? At this time, the answers to these questions are not known but these considerations suggest that comprehensive assessment of sRAGE levels in Emirati subjects should take into account the state of renal function and that multiple measures of renal function should be tested, where feasible.

In contrast to the present study, other reports have revealed opposing results on the “directionality” of sRAGE levels in non-diabetic vs. diabetic subjects, even those within the same general age range as the Emirati population examined here. In a Japanese population, Nakamura and colleagues found that serum sRAGE levels were significantly higher in T2D vs. non-diabetic subjects (965.3 ± 544.2 vs. 415 ± 150.4 pg/ml) [19]. In a Czech population, Skrha and colleagues showed that sRAGE levels were significantly higher in the T2D vs. non-diabetic subjects (1119 ± 619 vs. 785 ± 314 pg/ml) [20]. The reasons for these apparent differences are unclear. However, multiple confounding variables may underscore these findings, such as obesity; altered renal function; *AGER* single nucleotide polymorphisms; polymorphisms in other genes linked to RAGE ligands, AGEs, such as fructoamine 3-kinase (*FN3K*) and glyoxalase1 (*GLO1*); as well as other

superimposed disorders, such as inflammation or tumors, that might independently impact sRAGE levels [21,22]. Although we did not detect any differences in levels of esRAGE between the two groups, others have reported that esRAGE levels were lower in T2D vs. control subjects [12,23]. In the case of esRAGE, there also appears to be a relationship to kidney disease, as it was reported that in non-diabetic subjects undergoing hemodialysis, levels of esRAGE were significantly lower than those of controls [24].

In addition to metabolic and anthropomorphic factors, racial differences affecting sRAGE levels have been reported. The Atherosclerosis Risk in Communities (ARIC) Study identified that there were racial differences in levels of sRAGE and esRAGE; lower levels of both sRAGE and esRAGE were associated with black race and with genetic variants in *AGER* gene and that this was true even after multiple adjustments [25]. As these observations suggest roles for genetic factors in regulation of sRAGEs, it is notable that in the UAE, there is a high rate of consanguineous marriage, estimated to be approximately 50.5%, with estimated population confidence limits, 49.2–51.8% [26,27]. While such consanguinity has been suggested to be linked to higher occurrence of malignancies, congenital abnormalities and other illnesses, no study has yet tested links between consanguinity and levels of sRAGEs. Thus, it is plausible that yet-to-be-identified genetic and/or environmental vulnerabilities in Emirati subjects may importantly influence the levels of sRAGE and esRAGE.

A limitation of our study was the inability to collect data on subjects' medication usage. Given the age and chronic disease status of many of the subjects enrolled in this case-control study, it is likely that multiple medications were prescribed to these subjects. Levels of sRAGE and esRAGE are mutable and medications have been shown to affect these levels in individuals. Koyama and colleagues reported that treatment of T2D patients with pioglitazone, but not glimepiride, resulted in significant increases in plasma sRAGE and esRAGE levels [28]. In a distinct study, rosiglitazone or sulfonylurea was administered to 64 T2D subjects for 6 months; at the end of that time, in the group treated with rosiglitazone, but not sulfonylureas, increased circulating levels of both sRAGE and esRAGE were observed [29].

Others studied the effects of statins on levels of sRAGE and esRAGE. In a group of T2D Chinese subjects, 6 months treatment with atorvastatin resulted in significantly higher levels of esRAGE but not sRAGE [30]. Colhoun and colleagues studied the effect of administration of atorvastatin to T2D subjects enrolled in CARDS (Collaborative Atorvastatin Diabetes Study) and found that whereas sRAGE and esRAGE were associated with incident coronary heart disease (but not stroke), treatment with atorvastatin (3.9 years) had no effect on levels of sRAGE or esRAGE [31]. In a study in diabetic Sprague Dawley rats, atorvastatin administration resulted in reduced mesangial expansion and microalbuminuria; in parallel, kidney levels of RAGE expression were reduced and serum and renal sRAGE levels increased [32]. However, the renal levels of esRAGE were unchanged, underscoring that regulation of esRAGE levels is complex. Overall, because we were unable to track medication usage in the SKMC population, we cannot exclude that the differences between the findings of sRAGE vs. esRAGE in this population were not due, at least in part, to differences in medication usage.

Finally, the finding that levels of fasting plasma insulin were significantly higher in the control vs. T2D case subjects (133.6 ± 149.9 vs. 107.5 ± 93.3 ; $P = 0.01$, Table 1) was unexpected. Multivariate analyses revealed that in the control subjects, levels of insulin were significantly associated with fasting blood glucose and triglyceride levels; in the T2D case subjects, levels of insulin were significantly associated with diastolic blood pressure. As these factors are associated with metabolic syndrome [33], it is possible that these findings underscore that even in non-diabetic Emirati subjects, insulin resistance and other components of the metabolic syndrome may be present, especially in older individuals.

In conclusion, in this SKMC population of Emirati subjects, levels of sRAGE, but not esRAGE, correlated with T2D case vs. control status, but

not after correction for levels of eGFR. The finding of significantly higher plasma insulin levels in non-diabetic control vs. T2D case subjects may suggest a substantial degree of insulin resistance in this population, even in the absence of hyperglycemia and HbA1c levels diagnostic of T2D. Although the levels of insulin were not significantly related to sRAGE or esRAGE, our collective findings may suggest that there is substantive evidence of metabolic dysfunction in this older Emirati population. More research is needed to highlight the root causes and to pinpoint biomarkers and remediable factors.

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Author contributions

MHH, MMB, MN, AA, RR, CKI, AS, HL and AMS designed the study, conducted data collection, analyzed data and drafted the manuscript. DG, LA, HA, AE, HAS, and RA conducted data collection, analyzed data and reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcte.2019.100192>.

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