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The AGE-RAGE axis in an Arab population: The United Arab Emirates Healthy Futures (UAEHFS) pilot study

Claire K. Inman
NYU Abu Dhabi


Abdullah Aljunaibi
Zayed Military Hospital


Hyunwook Koh
NYU Grossman School of Medicine

Abdishakur Abdulle
NYU Abu Dhabi

Raghib Ali
NYU Abu Dhabi

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Author First name, Last name, Institution

Claire K. Inman, Abdullah Aljunaibi, Hyunwook Koh, Abdishakur Abdulle, Raghib Ali, Abdullah Alnaeemi, Eiman Al Zaabi, Naima Oumeziane, Marina Al Bastaki, Mohammed Al-Houqani, Fatma Al-Maskari, Ayesha Al Dhaheri, Syed M. Shah, Laila Abdel Wareth, Wael Al Mahmeed, Habiba Alsafar, Fatme Al Anouti, Ayesha Al Hosani, Muna Haji, Divya Galani, Matthew J. O'Connor, Jiyoung Ahn, Tomas Kirchhoff, Scott Sherman, Richard B. Hayes, Huilin Li, Ravichandran Ramasamy, and Ann Marie Schmidt



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Original research

The AGE-RAGE axis in an Arab population: The United Arab Emirates Healthy Futures (UAEHFS) pilot study



Claire K. Inman^{a,1}, Abdullah Aljunaibi^{b,1}, Hyunwook Koh^c, Abdishakur Abdulle^a, Raghib Ali^a, Abdullah Alnaeemi^d, Eiman Al Zaabi^e, Naima Oumeziane^f, Marina Al Bastaki^f, Mohammed Al-Houqani^g, Fatma Al-Maskari^h, Ayesha Al Dhaheeriⁱ, Syed M. Shah^h, Laila Abdel Wareth^j, Wael Al Mahmeed^k, Habiba Alsafar^{l,m}, Fatme Al Anoutiⁿ, Ayesha Al Hosani^a, Muna Haji^a, Divya Galani^a, Matthew J. O'Connor^p, Jiyoungh Ahn^c, Tomas Kirchhoff^c, Scott Sherman^c, Richard B. Hayes^c, Huilin Li^c, Ravichandran Ramasamy^{o,2,*}, Ann Marie Schmidt^{o,2,*}

^a Public Health Research Center, New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

^b Department of Pediatrics, Zayed Military Hospital, Abu Dhabi, United Arab Emirates

^c Department of Population Health, New York University School of Medicine, New York, NY, USA

^d Department of Cardiology, Zayed Military Hospital, Abu Dhabi, United Arab Emirates

^e Department of Pathology, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates

^f Abu Dhabi Blood Bank, SEHA, Abu Dhabi, United Arab Emirates

^g Department of Medicine, College of Medicine and Health Sciences, UAE University, Al-Ain, United Arab Emirates

^h Institute of Public Health, College of Medicine and Health Sciences, UAE University, Al-Ain, United Arab Emirates

ⁱ Department of Nutrition, College of Architecture, UAE University, Al-Ain, United Arab Emirates

^j Pathology and Laboratory Medicine Institute, Cleveland Clinic, Abu Dhabi, United Arab Emirates

^k Department of Cardiovascular Medicine, Cleveland Clinic, Abu Dhabi, United Arab Emirates

^l Center for Biotechnology, Khalifa University of Science, Technology & Research, Abu Dhabi, United Arab Emirates

^m Biomedical Engineering Department, Khalifa University of Science and Technology, Abu Dhabi, United Arab Emirates

ⁿ College of Natural and Health Sciences, Zayed University, Abu Dhabi, United Arab Emirates

^o Diabetes Research Program, Division of Endocrinology, Diabetes and Metabolism, Department of Medicine New York University School of Medicine, New York, NY, USA

^p New York University Abu Dhabi, Abu Dhabi, United Arab Emirates

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ABSTRACT

Aims: The transformation of the United Arab Emirates (UAE) from a semi-nomadic to a high income society has been accompanied by increasing rates of obesity and Type 2 diabetes mellitus. We examined if the AGE-RAGE (receptor for advanced glycation endproducts) axis is associated with obesity and diabetes mellitus in the pilot phase of the UAE Healthy Futures Study (UAEHFS).

Methods: 517 Emirati subjects were enrolled and plasma/serum levels of AGE, carboxy methyl lysine (CML)-AGE, soluble (s)RAGE and endogenous secretory (es)RAGE were measured along with weight, height, waist and hip circumference (WC/HC), blood pressure, HbA1c, Vitamin D levels and routine chemistries. The relationship between the AGE-RAGE axis and obesity and diabetes mellitus was tested using proportional odds models and linear regression.

Results: After covariate adjustment, AGE levels were significantly associated with diabetes status. Levels of sRAGE and esRAGE were associated with BMI and levels of sRAGE were associated with WC/HC.

Conclusions: The AGE-RAGE axis is associated with diabetes status and obesity in this Arab population. Prospective serial analysis of this axis may identify predictive biomarkers of obesity and cardiometabolic dysfunction in the UAEHFS.

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* Corresponding authors at: Diabetes Research Program, Division of Endocrinology, Diabetes and Metabolism, New York University School of Medicine, 550 First Avenue, Smilow 904, NY, NY 10016, USA (R. Ramasamy). Diabetes Research Program, Division of Endocrinology, Diabetes and Metabolism, New York University School of Medicine, 550 First Avenue, Smilow 901C, NY, NY 10016, USA (A.M. Schmidt).

E-mail addresses: ravichandran.ramasamy@nyumc.org (R. Ramasamy), annmarie.schmidt@nyumc.org (A.M. Schmidt).

¹ Joint first authors.

² Joint senior authors.

Introduction

The World Health Organization (WHO) reported that non-communicable diseases (NCDs) result in the death of approximately 38 million people each year. According to the WHO report [1], nearly three-quarters of the deaths from NCDs occur in countries with low- to middle income. Cardiovascular diseases (CVDs) account for the greatest proportion of these deaths; important risk factors include tobacco use, unhealthy diets, physical inactivity and harmful consumption of alcohol [1]. The consequences of obesity include insulin resistance and the development of Type 2 diabetes mellitus, which are major causes of morbidity and mortality, on account of accelerated atherosclerosis, heart attacks and strokes [2,3].

The United Arab Emirates (UAE) has rapidly evolved from a semi-nomadic society to a flourishing high income society in which consumption of high energy foods and reduced physical activity have resulted in increasing rates of obesity and its sequelae in adults, adolescents and children [4,5]. Hajat and colleagues reported that in the Abu Dhabi Weqaya study, which screened 50,183 adults ≥ 18 years of age, approximately 57% of the subjects were overweight or had obesity; 18% had diabetes mellitus; and 27% had pre-diabetes [6]. These considerations underscore the need to identify the underlying mediating mechanisms of cardiometabolic disease and biomarkers to track individuals particularly vulnerable to obesity and its side effects.

The receptor for advanced glycation endproducts (RAGE) transduces the signals of a unique repertoire of ligands that accumulate in NCDs, such as in obesity, hyperglycemia and aging [7]. Studies in human subjects illustrated that RAGE is highly expressed in obese adipose tissue, to a greater degree than that observed in the adipose tissue of lean subjects; and in atherosclerotic plaques, particularly in the diabetic state [8,9]. This increased expression of RAGE and its ligands in these tissues was mechanistically linked to disease, as mice devoid of *Ager* (gene encoding RAGE) were protected from diet-induced obesity and from accelerated atherosclerosis in diabetes [10,11].

RAGE ligand AGEs, such as carboxy methyl lysine (CML) AGEs, are generated to accelerated degrees in obesity and diabetes mellitus in human cardiometabolic disorders. Two forms of soluble RAGE have been detected in human serum/plasma. The first form, soluble (s)RAGE, is a cell-surface cleaved form of the receptor that results from the actions of matrix metalloproteinases (MMPs) or a disintegrin and metalloproteinase domain-containing protein 10 or ADAM10 [12,13]. The second form, endogenous secretory or esRAGE results from alternative splicing of the human *AGER* mRNA leading to deletion of part of the RAGE transmembrane domain and the cytoplasmic tail [14]. Multiple published studies, largely cross-sectional in design, have reported on associations between the circulating levels of AGE, CML-AGE, sRAGE and/or esRAGE and the presence of obesity, Type 2 diabetes mellitus and its complications [7,15,16]. Others have shown that therapeutic intervention in cardiometabolic diseases may modulate levels of RAGE ligands and the soluble RAGEs [17].

Here, we measured levels of AGEs, CML-AGE, sRAGE and esRAGE in 517 pilot study subjects of the UAEHFS and tested their potential association with obesity, diabetes status (HbA1c) and other risk factors and biomarkers of cardiometabolic disease.

Subjects

517 Emirati subjects were enrolled into the pilot study of the UAEHFS from January 2015 to April 2015 from the Zayed Military Primary Health Care Clinic (ZMH PHCC) and the Abu Dhabi Blood

Bank (ADBB), both of which are licensed for clinical research by the Health Authority of Abu Dhabi (HAAD). In each location, individuals who visited the clinic either for bi-annual medical screening (at the ZMH PHCC) or to donate blood (at ADBB) were invited to participate in the study. Inclusion criteria included: age ≥ 18 years and UAE national residents in the Abu Dhabi Emirate. Exclusion criteria included: age < 18 years, inability to give informed consent for the study, any acute medical illness (such as acute infection, chest pain or breathlessness, etc.) and pregnancy. No subjects were excluded on the basis of pre-existing chronic medical conditions, such as diabetes, hypertension or ischemic heart disease. All participants in the pilot study read and understood the information brochure and signed informed consent prior to recruitment. Subjects who agreed to participate and provided informed consent underwent a variety of physical measurements and analysis of blood samples. The percent missing data for each study variable is listed in the Table 1 and includes 6.2% missing values for HbA1c. The Institutional Review Boards (IRBs) of the Sheikh Khalifa Medical City (SKMC), Zayed Military Hospital (ZMH), Zayed University (ZU), New York University Abu Dhabi (NYUAD), NYU Langone Medical Center, New York, and United Arab Emirates University (UAEU) approved the protocols of the pilot study.

Materials and methods

Clinical measurements

Sitting and standing height was measured using a stadiometer (Seca, Hamburg Deutschland) and waist circumference (WC) and hip circumference (HC) were measured using a standard tape (Wessex non-stretchable sprung tape) [18]. Body mass was measured using the Tanita TC (Tanita Inc., Tokyo, Japan) and body mass index (BMI) was calculated according to the following formula: body weight (kg)/height² (meters).

Brachial blood pressure (systolic and diastolic) was recorded twice on the upper left arm with appropriate cuff size with two-minute interval between readings using a semi-automated sphygmomanometer (Omron M10-IT, Omron Corporation, Kyoto, Japan).

Biological samples

Study participants provided specimens including blood (8 ml SST vacutainer and 8 ml plasma EDTA vacutainer). SST vacutainers were subjected to centrifugation (3500 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 where the SST samples were aliquoted into 1.0 ml tubes. 2 mls of whole blood were removed from the EDTA vacutainer and stored in 1 ml aliquots. 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 per Table 1 was performed on SST serum. All assays were performed on the Beckman Coulter UniCel Dx C 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 and then converted to IFCC (mmol/mol) according to the following formula: IFCC = $(10.93 \times \text{NGSP}) - 23.5$. Serum levels

Table 1

Descriptive table on the effect of candidate variables, including the markers of AGE-RAGE axis, obesity indices and other potential covariates, on diabetes status (HbA1c levels) for 517 subjects. Note that the data include 6.2% missing values for HbA1c.

Continuous variables	All individuals (n = 517)		Normal (HbA1c < 5.7) (n = 331)		Prediabetic (5.7 ≤ HbA1c < 6.5) (n = 121)		Diabetic (6.5 ≤ HbA1c) (n = 33)		% of missing	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
CML-AGE (pmol/ml)	6853.91	13618.98	6068.87	12182.11	8980.72	17477.03	7192.74	10629.61	7.74	0.095
AGE (Arbitrary Units)	1318.15	1085.66	1417.17	1196.84	1042.72	628	1222.62	1012.27	5.42	0.003
sRAGE (pg/ml)	1093.15	513.41	1151.6	544.22	1002.96	433.74	851.33	363.24	5.61	<0.001
esRAGE (ng/ml)	0.22	0.16	0.23	0.17	0.21	0.12	0.17	0.08	5.61	0.034
BMI (kg/m ²)	28.24	6.24	26.88	5.2	30.57	6.4	34.54	7.89	16.83	<0.001
WC/HC (ratio)	0.88	0.1	0.86	0.09	0.91	0.1	0.95	0.09	21.86	<0.001
Age (years)	31.78	10.48	29.08	8.45	36.41	10.5	43.84	13.03	9.09	<0.001
eGFR (ml/min/1.73m ²)	102.71	26.63	105.15	26.91	97.79	21.5	98.75	35.7	14.31	0.008
Systolic blood pressure (SBP) (mm Hg)	116.86	15.59	113.8	13.86	121.51	16.21	131.29	19.18	17.41	<0.001
Diastolic blood pressure (DBP) (mm Hg)	77.49	9.94	76.01	9.02	79.49	11.23	85.6	9.44	17.41	<0.001
Cholesterol (mg/dL)	196.01	40.8	192.31	37.19	208.85	43.11	188.24	57	5.42	0.009
Triglycerides (mg/dL)	120.58	88.71	108.98	74.73	131.55	83.7	196.85	167.58	5.42	<0.001
HDL (mg/dL)	52.07	12.82	53.32	12.89	50.81	12.63	44.59	10.19	7.16	0.001
HS-CRP (mg/dL)	0.85	0.22	0.84	0.23	0.87	0.21	0.86	0.24	6	0.17
Vitamin D (ng/ml)	22.06	10.55	21.36	10.28	22.37	10.7	26.32	11.14	7.16	0.041
Serum creatinine (mg/dL)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Categorical variables	N	%	N	%	N	%	N	%	% of missing	P-value
Sex	473		304		173		31		8.51	0.142
Male	323	62.48	200	65.79	81	73.64	22	70.97		
Female	150	29.01	104	34.21	29	26.36	9	29.03		

† *Univariate analyses:* The P-values were estimated based on univariate proportional odds models for the effect of each candidate covariate on diabetes status. Diabetes status was coded as 0 for normal (HbA1c < 5.7), 1 for prediabetic (5.7 ≤ HbA1c < 6.5), and 2 for diabetic (6.5 ≤ HbA1c). SD = standard deviation.

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 equation: $eGFR = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if a female subject})$.

Research assays

Soluble RAGE, esRAGE

Soluble (s) RAGE and esRAGE levels were assayed on plasma obtained from blood in EDTA tubes on samples previously stored at −80 °C using enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturers' protocol (R&D Systems Quantikine Immunoassay, Minneapolis, MN, and B-Bridge ELISA, B-Bridge International, Cupertino, CA, respectively). Reported results represent the mean of the results from two distinct wells/sample. Interassay variability for the measurement of sRAGE and esRAGE was CV 8.02 and 7.75, respectively.

Protein-bound CML

Protein-bound CML in serum was quantified using liquid chromatography (LC)-mass spectrometry (MS) as previously published [19,20]. Agilent 6538 Accurate-Mass Quadrupole Time-of-Flight (Q-TOF) LC/MS system was used to measure CML in hydrolyzed serum samples.

AGE detection by relative fluorescence

AGE fluorescence at 440 nm (excitation at 370 nm), was determined in the acid hydrolysates of serum, as previously published [21] using a Fluorescence Microplate reader (BioTek Synergy H1 microplate reader). Relative fluorescence was determined in the hydrolyzed samples after diluting 60 μl of each sample with 2.0 ml of distilled water. A control buffer was used as a blank to subtract background fluorescence levels.

Statistical analysis

Descriptive analysis and the univariate proportional odds models on the effect of candidate variables, including the markers of AGE-RAGE, obesity indices and other potential covariates on diabetes status are reported in Table 1. The normality of each candidate variable was checked by histogram and the Shapiro-Wilk test [22] (Fig. S1). Since none of the variables passed the normality test, the non-parametric correlation method was considered in the following analyses. Pairwise Spearman correlation coefficient estimates [23] among BMI, WC/HC, eGFR, systolic blood pressure (SBP), diastolic blood pressure (DBP), cholesterol, triglycerides, high density lipoprotein (HDL), high sensitivity C-reactive protein (hs-CRP), Vitamin D and serum creatinine, as well as their p-values, are reported in 1. Since DBP is strongly correlated with SBP (correlation = 0.772, p < 0.001) and serum creatinine is strongly correlated with eGFR (correlation = −0.862, p < 0.001), we only fitted SBP and eGFR in the multiple regression models. Proportional odds models [24] were fitted to survey if CML-AGE, AGE, sRAGE, and esRAGE are associated with diabetes status, as defined by HbA1c levels, with covariate adjustments (Table 2). The diabetes status was coded for HbA1c (%) as 0 for normal (HbA1c < 5.7 (37 mmol/mol)), 1 for prediabetes (HbA1c ≥ 5.7 (37 mmol/mol) and < 6.5 (48 mmol/mol)), and 2 for diabetes mellitus (HbA1c ≥ 6.5 (48 mmol/mol)) and treated as ordinal. Pairwise Spearman correlation coefficient estimates among CML-AGE, AGE, sRAGE esRAGE, BMI and WC/HC, as well as their p-values, were reported to determine if they are related to each other (Table 3). Multiple linear regression models were used to survey if CML-AGE, AGE, sRAGE, and esRAGE are associated with obesity in terms of measures of BMI (Table 4) or WC/HC (Table 5), with covariate adjustments.

Results

We determined HbA1c levels and measured BMI and WC/HC in the subjects. Based on HbA1c levels (%), the cohort was divided

Table 2

The proportional odds models were fitted for the association between diabetes status and each of CML-AGE, AGE, sRAGE, and esRAGE. Separate models were fitted for different covariate adjustments. Diabetes status was coded as ordinal: 0 for normal stat (HbA1c < 5.7), 1 for prediabetic state (5.7 ≤ HbA1c < 6.5), and 2 for diabetic state (6.5 ≤ HbA1c). Each continuous predictor was standardized to have mean 0 and standard deviation 1.

Covariate adjustment	CML-AGE			AGE			sRAGE			esRAGE		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
1	0.099	0.103	0.334	−0.302	0.144	0.036	−0.287	0.132	0.029	−0.173	0.118	0.143
2	0.099	0.103	0.333	−0.31	0.145	0.033	−0.284	0.132	0.031	−0.17	0.118	0.15
3	0.073	0.112	0.515	−0.382	0.165	0.021	−0.063	0.138	0.649	−0.03	0.125	0.809
4	0.124	0.127	0.329	−0.308	0.159	0.053	−0.213	0.141	0.13	−0.126	0.126	0.318
5	0.061	0.115	0.597	−0.307	0.155	0.047	−0.235	0.141	0.094	−0.123	0.124	0.322
6	0.104	0.105	0.324	−0.273	0.144	0.058	−0.25	0.134	0.062	−0.15	0.12	0.212
7	0.09	0.103	0.378	−0.301	0.145	0.038	−0.181	0.133	0.173	−0.117	0.12	0.33
8	0.097	0.103	0.347	−0.367	0.154	0.017	−0.258	0.132	0.051	−0.159	0.118	0.178
9	0.098	0.103	0.343	−0.307	0.145	0.034	−0.284	0.132	0.031	−0.171	0.118	0.149
10	0.058	0.117	0.618	−0.404	0.177	0.023	−0.009	0.145	0.95	<0.001	0.131	0.998
11	0.095	0.131	0.465	−0.354	0.172	0.04	−0.087	0.146	0.549	−0.044	0.13	0.734

† Covariate adjustment: 1: Baseline (age + sex); 2: Baseline + eGFR; 3: Baseline + BMI; 4: Baseline + WC/HC; 5: Baseline + SBP 6: Baseline + total cholesterol + total triglycerides + HDL; 7: Baseline + hs-CRP; 8: Baseline + Vitamin D; 9: Baseline + serum creatinine; 10: Baseline + eGFR + BMI + SBP + total cholesterol + total triglycerides + HDL + hs-CRP + Vitamin D; 11: Baseline + eGFR + WC/HC + SBP + total cholesterol + total triglycerides + HDL + hs-CRP + Vitamin D.

* Note: For the covariate adjustment, 10 and 11, the complete estimated models including all covariate estimates for AGE are addressed through Tables S2 and S3. SE: standard error.

Table 3

Pairwise Spearman correlation coefficient estimates and their p-values among CML-AGE, AGE, sRAGE, esRAGE, BMI, and WC/HC. As the normality of each variable was not satisfied visually and by the Shapiro-Wilk test (Fig. S1), the non-parametric method, Spearman's rank test, was used (Table S1).

	CML-AGE	AGE	sRAGE	esRAGE	BMI	WC/HC
CML-AGE	–	−0.516, <0.001	0.043, 0.351	0.076, 0.096	−0.036, 0.466	0.001, 0.986
AGE	−0.516, <0.001	–	−0.08, 0.076	−0.037, 0.415	0.025, 0.609	−0.041, 0.42
sRAGE	0.043, 0.351	−0.08, 0.076	–	0.671, <0.001	−0.322, <0.001	−0.201, <0.001
esRAGE	0.076, 0.096	−0.037, 0.415	0.671, <0.001	–	−0.194, <0.001	−0.093, 0.068
BMI	−0.036, 0.466	0.025, 0.609	−0.322, <0.001	−0.194, <0.001	–	0.476, <0.001
WC/HC	0.001, 0.986	−0.041, 0.42	−0.201, <0.001	−0.093, 0.068	0.476, <0.001	–

Note: In each cell, the first number in the pair is the Spearman correlation coefficient estimate and the second number is the associated p-value based on the Spearman's rank test on whether it is significantly different from 0. For example, with respect to the correlation between sRAGE and BMI, the correlation estimate is −0.322 and its p-value is <0.001.

Table 4

The linear regression models fitted for the association between BMI and each of CML-AGE, AGE, sRAGE, and esRAGE. Separate models were fitted for different covariate adjustments. Each continuous predictor was standardized to have mean 0 and standard deviation 1.

Covariate adjustment	CML-AGE			AGE			sRAGE			esRAGE		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
1	0.054	0.324	0.868	0.057	0.282	0.839	−1.675	0.284	<0.001	−1.182	0.283	<0.001
2	0.073	0.325	0.822	0.023	0.286	0.937	−1.667	0.286	<0.001	−1.169	0.286	<0.001
3	0.054	0.313	0.864	−0.027	0.273	0.922	−1.525	0.28	<0.001	−1.049	0.276	<0.001
4	0.058	0.321	0.856	0.157	0.275	0.567	−1.445	0.282	<0.001	−1.024	0.277	<0.001
5	−0.068	0.302	0.822	0.026	0.263	0.923	−1.315	0.274	<0.001	−0.896	0.268	0.001
6	0.067	0.325	0.836	0.029	0.285	0.919	−1.672	0.288	<0.001	−1.148	0.285	<0.001
7	0.062	0.326	0.849	0.044	0.287	0.877	−1.67	0.286	<0.001	−1.173	0.286	<0.001
8	−0.012	0.295	0.968	0.048	0.257	0.851	−0.979	0.273	<0.001	−0.661	0.261	0.012

*Note: For the covariate adjustment, 8, the complete estimated model including all covariate estimates for sRAGE and esRAGE are addressed through Tables S4 and S5 respectively. SE: standard error.

† Covariate adjustment: 1: Baseline (age + sex); 2: Baseline + eGFR; 3: Baseline + SBP; 4: Baseline + total cholesterol + total triglycerides + HDL; 5: Baseline + hs-CRP; 6: Baseline + Vitamin D; 7: Baseline + serum creatinine; 8: Baseline + eGFR + SBP + total cholesterol + total triglycerides + HDL + hs-CRP + Vitamin D.

into three subgroups: 331 subjects had HbA1c < 5.7 (37 mmol/mol) (normal); 121 subjects had HbA1c ≥ 5.7 (37 mmol/mol) and <6.5 (48 mmol/mol) (prediabetes); and 33 subjects had HbA1c ≥ 6.5 (48 mmol/mol) (diabetes mellitus) (Table 1). Within these subgroups, the mean age ± SD was 29.08 ± 8.45 years, 36.41 ± 10.5 years and 43.84 ± 13.03 years, respectively, and the proportion of male subjects was 65.79%, 73.64% and 70.97%, respectively, and the proportion of female subjects was 34.21%, 26.36%, and 29.03%, respectively (Table 1). The mean BMI ± SD of the subjects in the three subgroups by HbA1c levels was 26.88 ± 5.2, 30.57 ± 6.4 and 34.54 ± 7.89, (in kg/m²), respectively, and the mean WC/HC ± SD in the three subgroups by HbA1c levels

was 0.86 ± 0.09, 0.91 ± 0.1 and 0.95 ± 0.09, respectively. These data demonstrate a step-wise increase in BMI and WC/HC with markers predictive of diabetes status (p < 0.001) (Table 1).

Table 1 reports the univariate analysis and related summary statistics for the relationships between the measures of CML-AGE, AGE, sRAGE and esRAGE, with the first major clinical parameter of this study, diabetes status, based on the HbA1c levels. AGE, sRAGE and esRAGE were significantly associated with diabetes status, but no significant relationship was observed for CML-AGE. Other potential covariates, eGFR, SBP, DBP, total cholesterol, triglyceride, HDL, hs-CRP, Vitamin D and serum creatinine, were also surveyed and are reported in Table 1.

Table 5

The linear regression models fitted for the association between WC/HC and each of CML-AGE, AGE, sRAGE, and esRAGE. Separate models were fitted for different covariate adjustments. Each continuous predictor was standardized to have mean 0 and standard deviation 1.

Covariate adjustment	CML-AGE			AGE			sRAGE			esRAGE		
	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value	Estimate	SE	P-value
1	<0.001	0.005	0.993	−0.005	0.004	0.22	−0.016	0.004	<0.001	−0.009	0.004	0.029
2	<0.001	0.005	0.972	−0.004	0.004	0.282	−0.015	0.004	0.001	−0.008	0.004	0.051
3	0.001	0.005	0.874	−0.005	0.004	0.229	−0.015	0.004	0.001	−0.009	0.004	0.035
4	−0.001	0.005	0.884	−0.004	0.004	0.308	−0.013	0.004	0.003	−0.008	0.004	0.068
5	<0.001	0.005	0.975	−0.005	0.004	0.192	−0.014	0.004	0.001	−0.008	0.004	0.057
6	<0.001	0.005	0.971	−0.005	0.004	0.213	−0.016	0.004	<0.001	−0.009	0.004	0.029
7	<0.001	0.005	0.985	−0.005	0.004	0.24	−0.015	0.004	0.001	−0.008	0.004	0.051
8	−0.001	0.005	0.887	−0.003	0.004	0.486	−0.011	0.004	0.016	−0.006	0.004	0.189

*Note: For the covariate adjustment, 8, the complete estimated model including all covariate estimates for sRAGE are addressed through Table S6. SE: standard error.

† Covariate adjustment: 1: Baseline (age + sex); 2: Baseline + eGFR; 3: Baseline + SBP; 4: Baseline + total cholesterol + total triglycerides + HDL; 5: Baseline + hs-CRP; 6: Baseline + Vitamin D; 7: Baseline + serum creatinine; 8: Baseline + eGFR + SBP + total cholesterol + total triglycerides + HDL + hs-CRP + Vitamin D.

We next performed multivariate analyses to assess if CML-AGE, AGE, sRAGE and esRAGE are associated with diabetes status defined by HbA1c levels, after adjusting for other confounding factors. Here, we report eleven different covariate adjustments, as described in Table 2. We could not find any significant association for CML-AGE with or without covariate adjustments (Tables 1 and 2). The significant univariate association of sRAGE and esRAGE with diabetes status (Table 1) was due to other confounding factors, as their statistical significance was lost after adjusting for the covariates investigated through models 1–11 (Table 2). In contrast, the significant and negative association between AGE and HbA1c levels was retained after covariate adjustment except in Model 4 and Model 6 with p-value = 0.053 and 0.058, respectively (Table 2).

We next surveyed how the markers of the AGE/RAGE axis, CML-AGE, AGE, sRAGE and esRAGE, and BMI and WC/HC within individual subjects may be related to each other. Our analyses revealed (Table 3, Fig. 1) that 1) CML-AGE is significantly correlated with

AGE, but not with sRAGE, esRAGE, BMI or WC/HC; 2) AGE is significantly correlated with CML-AGE, but not with sRAGE, esRAGE, BMI or WC/HC; 3) sRAGE is significantly correlated with esRAGE, BMI and WC/HC, but not with AGE or CML-AGE; 4) esRAGE is significantly correlated with sRAGE and BMI, but not with CML-AGE or AGE; and 6) WC/HC is significantly correlated with sRAGE and BMI, but not with CML-AGE, AGE or esRAGE. Hence, these data suggest an intra-correlation structure between the markers of the AGE-RAGE axis and BMI or WC/HC. We performed the following two analyses to test the association between them.

First, we addressed the association of CML-AGE, AGE, sRAGE and esRAGE with the second major clinical parameter of study, BMI. In the univariate analyses, only sRAGE and esRAGE were statistically significantly and negatively associated with BMI (Table 3). Here, we report eight different covariate adjustments, as described in Table 4, which revealed that the significant and

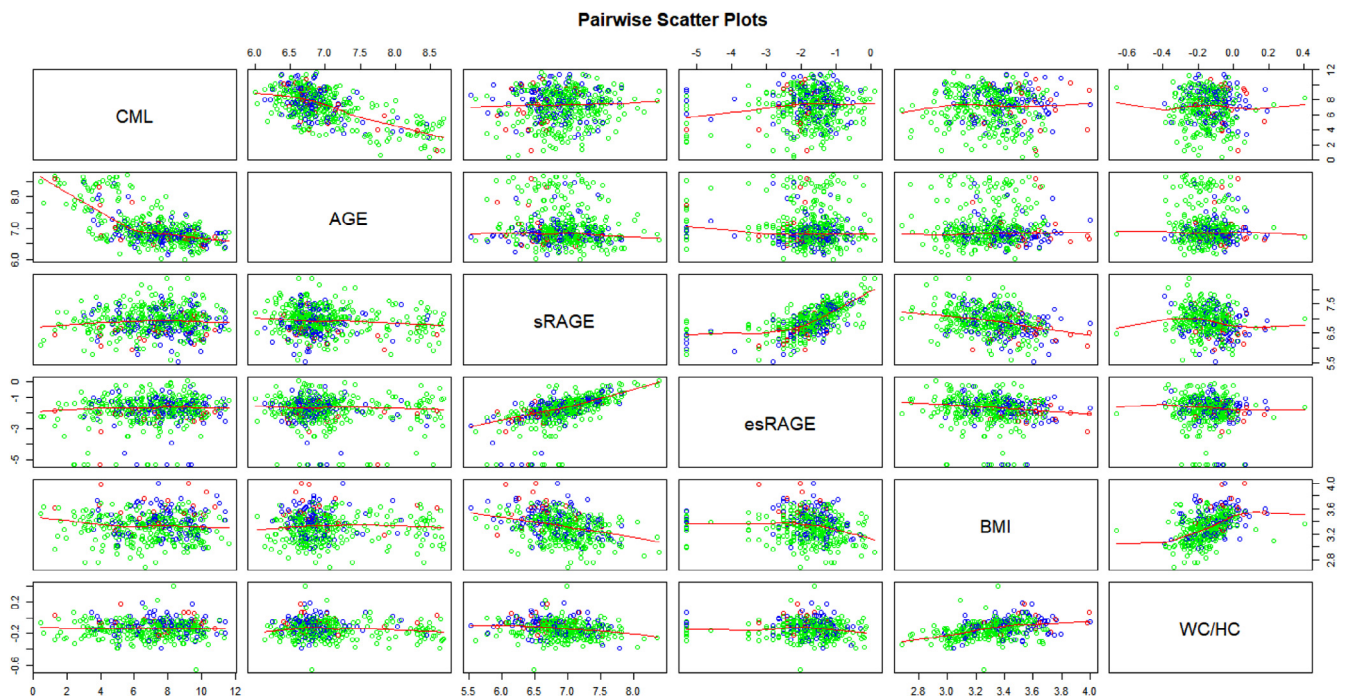


Fig. 1. Pairwise scatter plots among CML-AGE, AGE, sRAGE, esRAGE, BMI, and WC/HC. Green circles are for normal individuals ($HbA1c < 5.7$), blue circles are for prediabetic individuals ($5.7 \leq HbA1c < 6.5$), and red circles are for diabetic individuals ($6.5 \leq HbA1c$). As the normality of each variable was not satisfied by the Shapiro-Wilk test, logarithm was taken to each variable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

negative association for sRAGE and esRAGE with BMI remained after any further covariate adjustment, but no significance was observed for CML-AGE and AGE.

Second, we addressed the association of CML-AGE, AGE, sRAGE and esRAGE with the third major clinical parameter of study, WC/HC. In the univariate analysis, only sRAGE was significantly and negatively associated with WC/HC (Table 3). Table 5, using the covariate adjustments as shown in Table 4, reports that only sRAGE is significantly and negatively associated with WC/HC after any further covariate adjustment. No statistically significant differences were observed for AGE, CML-AGE or esRAGE.

Discussion

In the United Arab Emirates, the rising rates of excessive body mass, metabolic dysfunction and Type 2 diabetes mellitus and their cardiovascular consequences threaten the health and well-being of its citizens. The UAEHFS aims to identify the causes of these common metabolic diseases in a prospective manner and to identify predictive biomarkers. In the context of RAGE, published studies suggested that in subjects with Type 2 diabetes mellitus and obesity with BMI 30–35 undergoing bariatric surgery, higher baseline levels of sRAGE predicted weight loss and remission from Type 2 diabetes mellitus at six months and three years post-surgical intervention [25,26]. Hence, this pilot study served to test the feasibility of this approach with respect to predictors of obesity and metabolic disease in an Arab population.

Although there were no significant associations with levels of sRAGE and diabetes status levels of sRAGE remained significantly associated with BMI and WC/HC after all covariate adjustment. The general directionality observed in this study was that as diabetes status progressed from normal to diabetes and as BMI and WC/HC rose, levels of sRAGE declined. Associations between diabetes mellitus and obesity and sRAGE have been reported in other populations but the results are discordant on whether higher or lower sRAGE levels associated with overall cardiometabolic disease. Sebeková and colleagues reported in young to middle-aged subjects free of diabetes mellitus or medications that levels of sRAGE and RAGE ligands CML-AGE declined prior to the manifestation of metabolic syndrome [27]. These considerations highlight a number of important points: First, Gaens and colleagues showed that in obese individuals, lower levels of RAGE ligand CML-AGE were identified in plasma compared to lean subjects and, thus, the CML-AGEs were believed to be “trapped” in obese adipose tissue, in which higher tissue levels of RAGE were noted [9]. Second, with respect to the discordance between “high” vs. “low” levels of sRAGE and status of metabolic disease, Thomas and colleagues noted that studies of the general population cohorts revealed that lower levels of sRAGE were associated with poor health outcomes [28–30]. This point bears direct relevance to the design of the UAEHFS, a population-based cohort study. Third, genetic variations in the gene encoding *AGER* have been suggested to contribute to the levels of sRAGE [31,32]. Of note, genetic factors are to be examined in the UAEHFS and the findings might provide novel insights into *AGER*-specific variants linked to the AGE-RAGE axis, diabetes mellitus and obesity; no such data exist at this time in this population.

The present findings indicate a number of significant associations (negative) between levels of esRAGE and BMI and WC/HC, even after covariate adjustment, but not with diabetes status. It has been shown that lower levels of esRAGE were associated with obesity and risk of metabolic syndrome [33–35]. Vazzana and colleagues showed that a weight loss program in five women with obesity resulted in increases in levels of esRAGE [33]. Comparable to findings with sRAGE, it has been reported that renal function, genetic polymorphisms in *AGER*, medications and the study of pop-

ulations vs. known diseased subjects may affect esRAGE levels and thus account for these disparate results [36–38]. These considerations underscore the importance of measuring baseline levels and prospective repeat measures of esRAGE in the UAEHFS subjects with respect to associations with obesity and/or diabetes mellitus.

In this study, no significant associations were observed between CML-AGE and BMI, WC/HC or diabetes status, but we did observe significant associations between AGE and diabetes status, but not with BMI or WC/HC. There are multiple possible reasons for these findings, such as (1) the present pilot study was not designed to test specific hypotheses regarding the form of AGE and diabetes or obesity status and hence was insufficiently powered to detect significant differences; (2) factors relevant to local dietary patterns or exercise in the UAE might contribute to distinct effects on AGEs vs. CML-AGE in this population, which might impact the overall detectable AGE or CML-AGE circulating burden in these subjects [39]; (3) heretofore unknown polymorphisms in genes that regulate total AGE or CML-AGE burden might be present in this population, such as variations in the gene encoding glyoxalase 1 (*GLO1*), whose product detoxifies methylglyoxal, a pre-AGE species [40–42] or in association with variations in genes encoding AGE receptors [43]; and (4) the overall low prevalence of diabetes mellitus in this pilot study ($n = 33/517$ or 6.2%) suggests that frank diabetes mellitus, at least in this population, may exert the greater impact on levels of CML-AGE than prediabetes or normal states, with or without obesity. In this context, it is important to note that in obesity, as cited above, circulating AGE levels (such as CML-AGE) might be lower in obese vs. lean subjects due to tissue trapping [9]. In contrast, multiple studies have suggested higher levels of circulating AGEs in subjects with diabetes mellitus and its complications [44]. Irrespective of these caveats, it will be important to prospectively follow levels of the AGE-RAGE axis in this population to determine if levels of AGE or CML-AGE reach significance with respect to diabetes status, BMI or WC/HC, as cohort subjects age and as more subjects are expected to develop Type 2 diabetes mellitus over time.

In conclusion, the results of this pilot of the UAEHFS suggest that levels of AGE, but not CML-AGE, sRAGE or esRAGE were associated with diabetes status, and that levels of sRAGE and esRAGE, but not CML-AGE or AGE were associated with obesity status in the UAE population. Hence, prospective and serial analysis of these endpoints in the UAEHFS subjects may identify predictive biomarkers of obesity and cardiometabolic dysfunction, especially when analyzed with the results of dietary and life-style surveys, genetic analyses, microbiome factors and markers of cardiovascular disease.

Author contributions

CKI, RR and AMS designed the study, conducted data collection, analyzed data and drafted the manuscript. AA and RA designed the study, conducted data collection, analyzed data and reviewed the manuscript. AAJ, AAN, EAZ, NO, MAB, MAH, FAM, AAD, SMS, LAW, WAM, HA, FAA, AAH, MH, DG, and MJOC conducted data collection, analyzed data and reviewed the manuscript. HK and HL analyzed data and drafted the manuscript. JA, TK, SS, and RBH designed the study, conducted data collection, analyzed data and reviewed the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jcte.2017.08.001>.

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