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Memon, Ashfaque; Bennet, Louise; Zöller, Bengt; Wang, Xiao; Palmér, Karolina; Dahlbäck, Björn; Sundquist, Jan; Sundquist, Kristina

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Associations between apolipoprotein M and insulin resistance differ according to country of birth

Short title: ApoM according to country of birth

Ashfaque A. Memon[1,], Louise Bennet[2], Bengt Zöller[1], Xiao Wang[1], Karolina Palmér[1], Björn Dahlbäck[3] Jan Sundquist[1,4] and Kristina Sundquist[1,4]*

[1]Center for Primary Health Care Research, Lund University / Region Skåne, Sweden

[2] Department of Clinical Sciences, Family Medicine, Lund University, Malmö, Sweden

[3]Department of Laboratory Medicine, Skåne University Hospital, Lund University, Sweden

[4]Stanford Prevention Research Centre, Stanford University School of Medicine, Stanford, California, USA

Corresponding Author:

Ashfaque A. Memon

Center for Primary Health Care Research

Wallenberg Laboratory, 6th floor

Inga Marie Nilssons gata 53

Skåne University Hospital

S-205 02 Malmö

Sweden

Email: ashfaque.memon@med.lu.se

ABSTRACT

Background and Aims: Risk of type 2 diabetes mellitus (T2DM) differs according to ethnicity. Levels of apolipoprotein M (ApoM) have been shown to be decreased in T2DM. However, its role in different ethnicities is not known. We examined the differences in plasma ApoM levels in Swedish residents born in Iraq (Iraqis) and Sweden (Swedes) in relation to T2DM and insulin resistance (IR).

Methods and Results: Iraqis and Swedes, aged 45 to 65 years residing in Rosengård area of Malmö were randomly selected from census records and underwent an oral glucose tolerance test. Plasma levels of ApoM were quantified in 162 participants (Iraqis, n=91; Swedes, n=71) by a sandwich ELISA method.

Age-, sex-, and body mass index (BMI) adjusted plasma ApoM levels differed by country of birth, with Swedes having 18% higher levels compared to Iraqis (p=0.001). ApoM levels (mean±SD) were significantly decreased in Swedes with T2DM (0.73±0.18) compared to those with normal glucose tolerance (NGT) (0.89±0.24; p=0.03). By contrast, no significant difference in ApoM levels was found between Iraqis with T2DM (0.70±0.17) and those with NGT (0.73±0.13; p=0.41). In multivariate linear regression analysis with an interaction term between IR and country of birth, low ApoM levels remained significantly associated with IR in Swedes (p=0.008), independently of age, sex, BMI, family history of diabetes, HDL, LDL, and triglycerides, but not in Iraqis (p=0.35).

Conclusion: Our results show that ApoM levels differ according to country of birth and are associated with IR and T2DM only in Swedes.

Keywords: Apolipoprotein M; Ethnicity; insulin resistance; Type 2-diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder caused by defects in insulin secretion, insulin action, or both. If poorly controlled, the resulting metabolic imbalance is associated with numerous disabling complications [1]. The prevalence of T2DM is increasing, and it is estimated that by 2025, 15% of the world's population will have T2DM, impaired fasting glucose (IFG), or impaired glucose tolerance (IGT) [2].

In Sweden, the prevalence of T2DM among non-European immigrants is estimated to be 2-3 times higher than among native Swedes [3]. In addition, T2DM develops an average of 10 years earlier in immigrants from the Middle East than in native Swedes, and Middle Eastern immigrants diagnosed with T2DM more frequently have a family history of diabetes [4].

Apolipoprotein M (ApoM), a member of the lipocalin protein family, is a 25 kDa plasma protein [5]. It is the carrier of the biologically active lipid mediator sphingosine-1-phospate (S1P) in HDL, which exerts endothelial protective functions [6]. Studies suggest that ApoM protects against various cardiovascular diseases (CVDs). For example pre-clinical animal studies suggest an anti-atherosclerotic role for ApoM [7]. Lower levels of ApoM have been observed in patients with critical limb ischemia [8] and were associated with higher risk of recurrent venous thromboembolism in men [9]. In the circulation, ApoM is predominantly associated with HDL [10] and affects the cholesterol balance of cells. Low HDL levels and elevated triglyceride levels are important characteristics of the metabolic syndrome. Furthermore, recent studies suggest that HDL particles and apolipoproteins such as ApoA1 directly and indirectly modulate insulin sensitivity through their antioxidant and anti-inflammatory action [11]. However, the role of ApoM in T2DM is not well defined. There is, however, some evidence to suggest the possible involvement of ApoM in the development of T2DM and metabolic syndrome. For example, cellular cholesterol imbalance can modify β-cell function and thereby increase the susceptibility to T2DM [12]. Furthermore, higher HDL levels are strongly associated with lower risk of future

T2DM. Finally, a single-nucleotide polymorphism in the promoter region of ApoM is associated with levels of plasma cholesterol and causes susceptibility to T2DM in Han Chinese people [13]. These findings imply that factors such as ApoM that affect the cellular contents of cholesterol may have a role in the pathogenesis of T2DM. Lipid levels are known to differ across ethnicities [14] and dyslipidemia is a characteristic feature of the metabolic syndrome and T2DM [15]. However, no previous studies have examined the role of ApoM in relation to IR and T2DM in different ethnicities. In this study we investigated the potential association between ApoM and IR and whether that association differs in Swedes and Iraqis.

MATERIAL AND METHODS

Study subjects

Male and female residents in the Rosengård residential area of Malmö, aged 45 to 65 years and born in Sweden (Swedes) or Iraq (Iraqis) were randomly selected from the census register. No participant born in Sweden had a parent born in Iraq. We chose the age range 45 to 65 years since it was the age group of the non-retired population with the highest probability of identifying individuals with prediabetes or diabetes, since the prevalence of diabetes increases with age.

The study was performed according to the Declaration of Helsinki. The ethical committee at Lund University approved the study (approval no. 2009/36) and written informed consent was given by all the participants in the study after full explanation of the purpose and nature of all procedures.

Clinical variable assessment

Abdominal obesity was defined as a waist circumference of ≥102 cm for males and ≥88 cm for females [16]. Body mass index (BMI), systolic and diastolic blood pressure, and leisure time physical activity (LTPA) were measured as described previously [17].

A standard 75 g oral glucose tolerance test (OGTT) was performed and blood glucose was measured in blood samples collected at 0, 30, 60, and 120 minutes using a photometer (HemoCue AB, Ängelholm, Sweden) [18]. Insulin sensitivity index (ISI) was calculated from the OGTT data using Matsuda indexes, as described previously [19]. Participants were asked not to eat after 10 pm the day before sample collection. Normal glucose tolerance (NGT) was defined as a fasting plasma glucose (0 h sample) level of <6.1 mmol/L and a plasma glucose level of <7.8 mmol/L 2 h after having been given 75 g glucose in the OGTT [20]. IFG was defined as a fasting plasma glucose level of ≥6.1 mmol/L and <7.0 mmol/L and a 2 h glucose level of <7.8 mmol/L. IGT was defined as a fasting plasma glucose level of <7.0 mmol/L and a 2 h glucose

level of ≥7.8 mmol/L and < 11.1 mmol/L. T2DM was defined as a fasting plasma glucose level of ≥7.0 mmol/L and/or a 2 h glucose level of ≥11.1 mmol/L [20]. Participants who had prevalent T2DM (n=22, ~14%) did not go for an OGTT. Serum insulin levels were determined using a radioimmunoassay (Access© Ultrasensitive Insulin, Beckman Coulter, USA) [21]. Homeostasis model assessment (HOMA) was used to estimate insulin resistance (HOMA-IR) as described previously [22]. Cholesterol and triglycerides were analyzed using enzymatic methods. HDL-cholesterol was measured after isolation of LDL and VLDL (Boehringer Mannheim GmbH, Germany) and LDL-cholesterol was analyzed using Friedewald's method [23]. C-reactive protein (CRP) was measured using a kit from Roche Diagnostics according to the manufacturer's instructions [17].

Sample preparation and quantification of ApoM

Fasting samples were collected into a 3.5 ml serum separating tube and were allowed to clot for 1 h at room temperature. They were then centrifuged for 10 min at 4 °C and serum was aliquoted and stored at -80 °C for further use. ApoM levels were measured by a sandwich ELISA method described previously [24]. Briefly, 96-well Costar plates (Corning Inc., Lowell, MA, USA) were coated with a catching monoclonal ApoM antibody (mAb 58) and blocked with quenching buffer. The samples were diluted, added to the plate, and incubated overnight. The bound ApoM was detected by using a biotinylated secondary ApoM antibody (mAb 42) in combination with streptavidin-avidin-horseradish peroxidase and 1,2-phenylenediamine dihydrochloride (Dako, Glostrup, Denmark). The absorbance at 490 nm was measured and compared with a plasma standard curve generated using known amounts of ApoM.

Statistical analysis

Differences in sample characteristics between Swedish-born subjects and first-generation immigrants from Iraq were tested using Student's t-test for continuous variables, chi-square tests for dichotomous variables, and the Wilcoxon rank-sum test for the variables with a non-normal

distribution (Table 1). The differences were also adjusted for sex, age, and BMI using linear regression for continuous variables, logistic regression for dichotomous variables, ordered logistic regression for hyperglycemia, and rank ANCOVA for variables with a non-normal distribution. ApoM levels are presented as means and standard deviations and differences between subjects with NGT, IGT, and T2D, stratified by country of birth, were tested using Student's t-test (Figure 1). HOMA-IR, ISI, HDL and triglycerides were also compared (using linear regression for HDL and median regression for HOMA-IR, ISI and triglycerides) between subjects with NGT, IFG/IGT and T2D (Supplementary table 1).

Univariate linear regression was used to examine the associations of ApoM with HDL (A), HOMA-IR (B), ISI (C), and serum insulin (D) (Figure 2). HOMA-IR, serum insulin, and ISI were all log-transformed due to skewed distributions. The univariate associations were stratified by country of birth to examine whether the associations differed between individuals born in Sweden and those born in Iraq. In a multivariate linear regression analysis, the association between ApoM and HOMA-IR was adjusted for sex, age, BMI, family history of diabetes, and levels of triglycerides, LDL, and HDL. In this model, we also added an interaction term between country of birth and HOMA-IR to examine the differences in associations between Iraqis and Swedes. STATA version 12 (StataCorp LP) was used for all statistical analyses.

RESULTS

In total, 296 individuals were invited to participate. Of these, 194 (Iraqis, n=107; Swedes, n=87) agreed to participate and were included in the study between the 1st of February and 31st of March 2010. Nineteen participants dropped out (Iraqis, n=11, Swedes, n=8), and for 13 patients there was not enough plasma for analysis (Iraqis, n=5; Swedes, n=8). We have no information about the reasons for the drop-outs.

In total, 162 individuals were included (Iraqis, n=91; Swedes, n=71) for ApoM analysis.

Table 1 shows the anthropometric and metabolic characteristics of the study participants born in Iraq and Sweden. Consistent with earlier reported data from the same population-based study, compared to Iraqis, Swedes were older (p=0.001) and had lower BMI values (p<0.0001). The prevalence of family history of diabetes was significantly higher in Iraqis compared to Swedes (p=0.001). Furthermore, mean HDL levels were significantly higher in Swedes than in Iraqis (p=0.009), whereas triglyceride levels were higher in Iraqis than in Swedes (p=0.001). However, the difference in triglyceride levels did not remain significant after adjustment for age, sex, and BMI (p=0.06). In the overall population ApoM levels (mean±SD) were found to be 18% lower in Iraqis (0.72±0.15) than in Swedes (0.85±0.23; p<0.0001), and the difference remained significant after adjustment for age, sex, and BMI (p=0.001) (Table 1). In order to investigate whether the differences in ApoM levels in the two populations were due to difference in HDL levels, we adjusted the data for HDL and found that the differences between the two populations in ApoM levels were independent of HDL levels (data not shown).

Stratification of data according to glucose tolerance showed that 56% Iraqis and 61% had NGT, 21% Iraqis and Swedes had IFG/IGT and 23% Iraqis and 18% Swedes had T2DM (Supplementary table 1). HOMA-IR (p=0.002), ISI (P=0.03), HDL (P=0.03) and Triglyceride (P=0.01) levels were significantly different in participants with NGT, IFG/IGT and T2DM in Swedes. However in Iraqis only HOMA-IR levels were found to be different in participants with

NGT, IFG/IGT and T2DM (Supplementary table 1)

Comparison of ApoM levels (mean±SD) between participants with NGT, IFG/IGT, and T2DM showed that Swedish participants with T2DM (0.73±0.18) had significantly lower levels of ApoM compared to those with NGT (0.89±0.24; p=0.03) (Figure 1). However, there was no difference in ApoM levels among Iraqis with NGT, IFG/IGT, and/or T2DM (p>0.05) (Figure 1). Interestingly, Swedes with NGT had a 22% higher level of ApoM compared to Iraqis with NGT (0.89±0.24 vs. 0.73±0.13; p<0.0001), whereas ApoM levels were almost identical in Iraqi and Swedish participants with T2DM (0.70±0.17 vs. 0.73±0.18; p=0.62), (Figure 1).

There were five participants in the Swedish population with particularly high levels of ApoM (Figure 1 and 2); however, they were not different from the rest of the study population regarding BMI or physical activity (data not shown).

ApoM levels were positively associated with HDL levels in both Swedes (β=0.29; p<0.0001; 95% CI=0.19-0.39) and Iraqis (β=0.20; p<0.0001; 95% CI=0.11, 0.30), (Figure 2). By contrast, ApoM levels were negatively associated with HOMA-IR (β=-0.15; p<0.0001; 95% CI=-0.23, -0.08) and serum insulin (β=-0.17; p<0.0001; 95% CI=-0.26, -0.09) and positively associated with ISI (β=0.14; p=0.002; 95% CI=0.05, 0.023) in Swedes. No significant associations were found between ApoM levels and HOMA-IR (β=-0.03; p=0.16; 95% CI=-0.07, 0.01), serum insulin (β=-0.02; p=0.35; 95% CI=-0.08, 0.03), or ISI (β=0.03; p=0.33; 95% CI=-0.03, 0.09) in Iraqis, (Figure 2). In multivariate regression analysis with an interaction term between HOMA-IR and country of birth included in the model (Table 2), HOMA-IR was associated with ApoM in Swedes (β=-0.08; p=0.008; 95% CI=-0.14, -0.02), independent of age, sex, BMI, family history of diabetes, and levels of triglycerides, HDL, and LDL, but not in Iraqis (β=0.02; p=0.35; 95% CI=-0.03, 0.07). Furthermore, levels of ApoM were also significantly different according to country of birth (Iraqis vs. Swedes), Swedes had higher levels of ApoM independent of age, sex, BMI, family history of diabetes, and levels of triglycerides, HDL, and LDL (β=-0.13; p<0.0001;

95% CI=-0.20, -0.06). The significant interaction term in the model (HOMA*country of birth) shows that the association between ApoM and HOMA-IR differs according to country of birth (β=0.10; p=0.003; 95% CI=0.03, 0.17). Levels of triglycerides (β=0.09; p<0.0001; 95% CI=0.06, 0.12), HDL (β=0.32; p<0.0001; 95% CI=0.24-, 0.39), and LDL (β=0.07; p<0.0001; 95% CI=0.04, 0.09) were also significantly associated with ApoM in the model.

DISCUSSION

For the first time we investigated the relationship between ApoM levels and T2DM in Swedish residents born in Iraq and Sweden. Our results show significantly higher levels of ApoM in Swedes compared to Iraqis. ApoM levels were reduced in T2DM compared to NGT only in Swedes. Moreover, lower ApoM levels were significantly associated with IR in Swedes, but not in Iraqis.

A large proportion of the immigrants in Sweden are from the Middle East and Iraq and constitute one of the largest immigrant groups in Sweden and the largest immigrant population living in Malmö [25]. The prevalence of T2DM in the immigrant population in Sweden has been shown to be higher compared to that in native Swedes [3]. Immigrants from the Middle East are known to have a different form of T2DM compared to the Swedish born population [4]. However, the molecular basis for these differences is not well understood.

Lower levels of HDL are related to a higher incidence of T2DM in various ethnic populations [26]. Furthermore, recent evidence suggests that HDL may directly contribute to the pathogenesis of T2DM through its ability to enhance pancreatic β-cell function and glucose uptake in skeletal muscles [27]. HDL levels are known to strongly correlate with ApoM [24] . Preclinical studies show a reduction in ApoM levels in diabetic and hyperglycemic models [28]. Furthermore in a human study on Caucasians, Plomgaard et al. found a 9% decrease in ApoM levels in patients with T2DM compared to control subjects [29]. In agreement with these results, we also found significantly lower levels of ApoM in participants with T2DM compared to participants with NGT; however, the difference was present only in Swedes but not in Iraqis. The mean plasma ApoM concentration in healthy individuals has been shown to be 0.9 μM [24]. In concurrence with these results, we found approximately the same levels of ApoM in Swedish participants with NGT; however, Iraqi participants with NGT had significantly lower levels of ApoM. Interestingly, Iraqi participants with NGT had nearly identical ApoM levels to Swedish

participants with T2DM. Together these results suggest that ApoM levels are associated with T2DM only in Swedes but not in Iraqis and differences in ApoM levels may partly explain the differences in T2DM in these two populations at molecular level.

IR is a key etiological factor for T2DM. In order to investigate the role of ApoM in the pathogenesis of T2DM, we explored the relationship between IR and ApoM. We found a negative association between ApoM and IR in Swedes but not in Iraqis. Furthermore this association was significantly different according to the country of birth independently of differences in demographics, adiposity, and family history of diabetes between the two populations. One possible mechanism for the differences between the two populations could be genetic influence of ApoM on the development of diabetes. A single-nucleotide polymorphism (T-778C) in the promoter region of ApoM was associated with type 1 diabetes in Han Chinese and Swedish populations [30]. However, it is not known whether the T-778C polymorphism has any role to play in T2DM. Another possible mechanism could be the differences in IR in the two populations, which could also influence the difference in ApoM in the two groups. Another major factor, which could explain the difference in ApoM in the two populations, could be differences in HDL levels. However differences in ApoM levels between Iraqis and Swedes were found to be independent of HDL levels in two populations. Nevertheless, based on our results it can be concluded that ApoM levels differ between Swedes and Iraqis independent of age, sex, BMI and HDL levels. However, the mechanism underlying the differences in ApoM between Iraqis and Swedes and its association with insulin resistance needs to be investigated further.

The main limitation of our study is the cross-sectional design of the study, which meant that a temporal relationship between ApoM and IR could not be determined. Another limitation of the study is the lack of genetic information, which could have explained the differences found in two populations. Finally, it should also be noted that this study was performed in a specific, restricted

sample of the Swedish population. A key strength is that this is the first study of its kind in which ApoM levels in relation to T2DM and IR have been examined in two ethnic groups. In conclusion we show that ApoM levels differ according to country of birth and that the lower levels of ApoM are associated with IR and T2DM in Swedes, but not in Iraqis.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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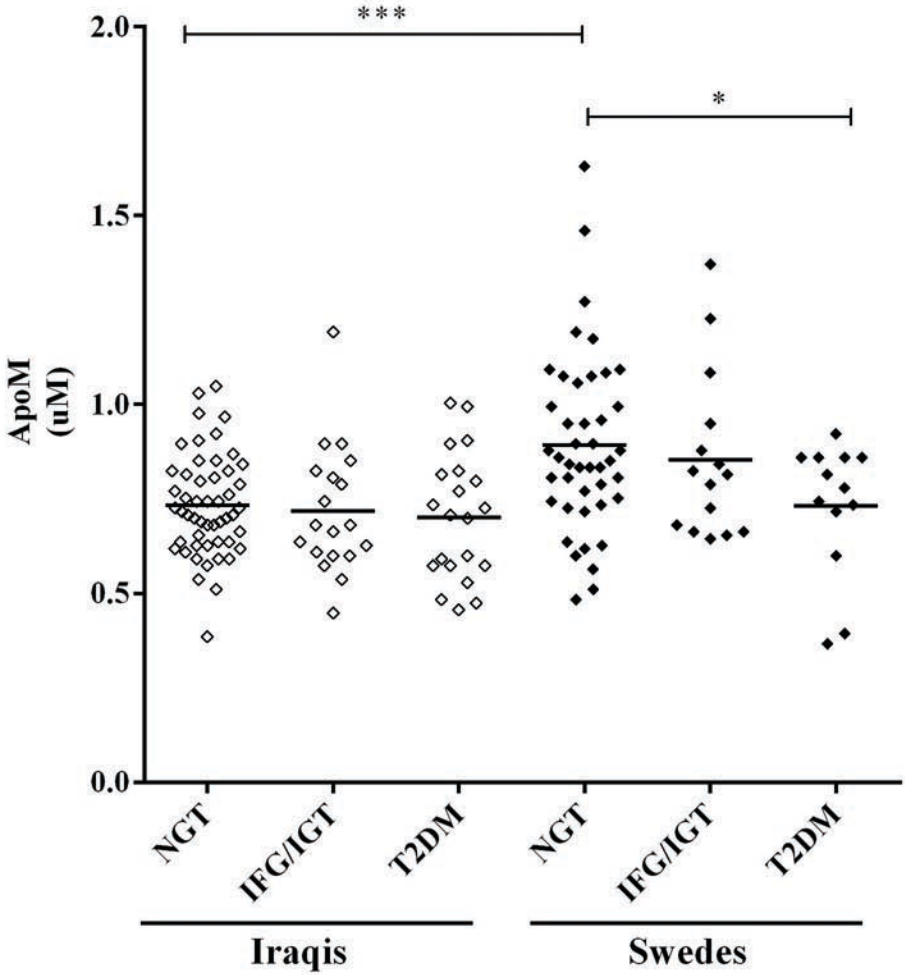
FIGURE LEGENDS

Figure 1. Levels of ApoM in NGT, IFG/IGT and T2DM in Iraqis. No significant difference was found in ApoM levels between NGT, IFG/IGT and T2DM in Iraqi participants ($p>0.05$). Swedish participants with T2DM had significantly lower levels of ApoM compared to those with NGT. No significant difference was found in ApoM levels between NGT and IFG/IGT in Swedes. Iraqis participants with NGT had significantly lower levels of ApoM compared to Swedish participants with NGT ($p<0.0001$). Bars indicate the mean values. *$p<0.05$ and ***$p<0.0001$.

Figure 2. Relationship between ApoM levels and HDL, HOMA-IR and ISI in Swedes (broken line) and Iraqis (solid line). ApoM levels were positively associated with HDL **(A)** in both Swedes, ($p<0.0001$) and Iraqis, ($p<0.0001$). ApoM levels were negatively associated with HOMA-IR **(B), (**$p<0.0001$) and positively associated with ISI **(C)** ($p=0.002$) in Swedes. No significant association was found between ApoM and HOMA-IR and ISI in Iraqis ($p>0.05$). [a]HOMA-IR and [a]ISI, were natural log transformed.

Figure 1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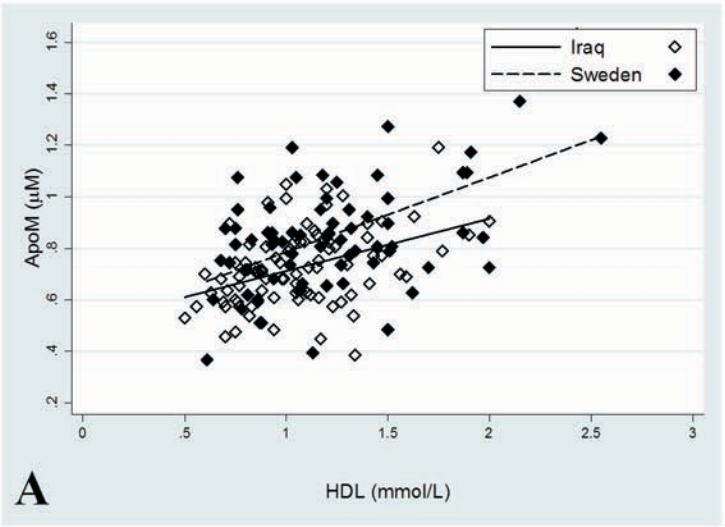
ApoM
(uM)
NGT
IFG/IGT
T2DM
NGT
IFG/IGT
T2DM
Iraqis
Swedes

Figure 2


Iraq
Sweden
ApoM (µM)
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1.5
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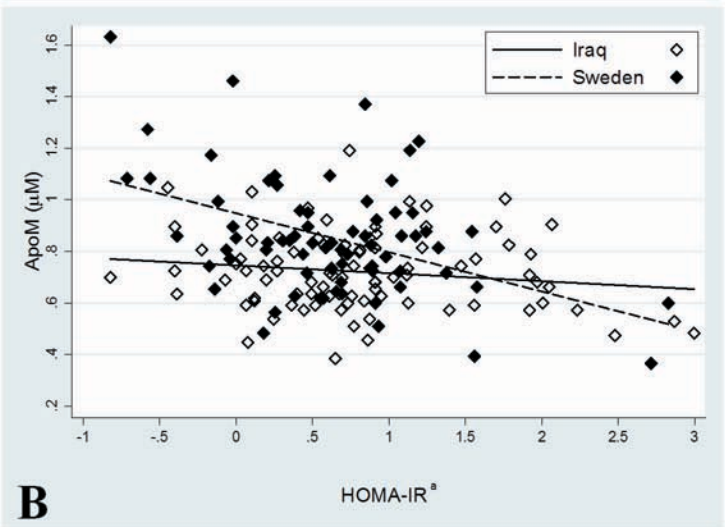
HDL (mmol/L)


Iraq
Sweden
ApoM (µM)
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B
HOMA-IR[a]

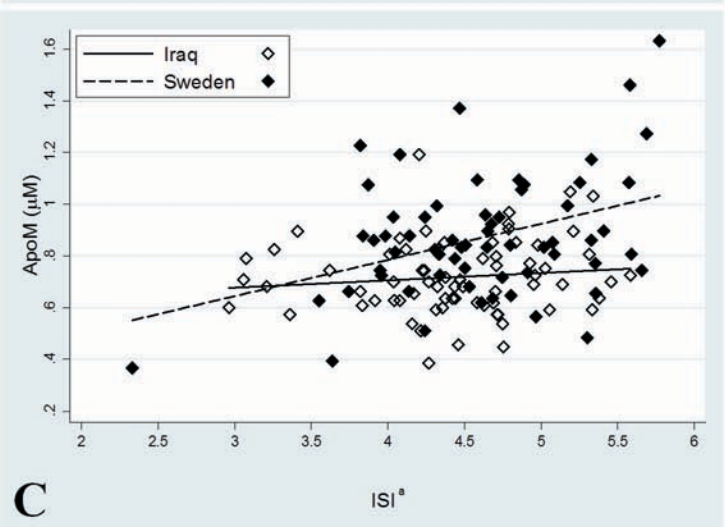
Iraq
Sweden
ApoM (µM)
.2
.4
.6
.8
1
1.2
1.4
1.6
2
2.5
3
3.5
4
4.5
5
5.5
6
C
ISI[a]

Table 1. Characteristics of the study population and ApoM levels according to country of birth

Covariate	Iraq (n=91)	Sweden (n=71)	p-value	Adj. p-value[e]
Sex (female/male)[a, f]	43/48 (47/53)	33/38 (46/54)	0.92	0.81
Age (years)[b, f]	55 (6.0)	57 (5.1)	**0.006**	**0.001**
BMI (kg/m^2)[b, f]	30 (4.5)	27 (5.0)	**0.002**	**< 0.0001**
Waist circumference (cm)[b, e,f]	100 (12.0)	98 (13.3)	0.47	0.25
Sedentary lifestyle (yes/no)[a,e]	57/25 (70/30)	43/27 (61/39)	0.30	0.77
Family history of diabetes (yes/no)[a,e]	47/28 (63/37)	18/50 (26/74)	**< 0.0001**	**0.001**
HOMA-IR[c,e]	2.1 (1.8)	1.9 (1.4)	0.18	0.78
ISI[c,e]	84 (56)	102 (97)	0.16	0.97
Serum insulin (pmol/L)[c,e]	8.0 (5.0)	7.0 (5.0)	0.13	0.73
Fasting glucose (mmol/L)[b,e]	6.3 (2.2)	6.0 (1.3)	0.26	0.40
2 hour glucose (mmol/L)[b,e]	6.9 (2.8)	6.4 (3.0)	0.28	0.34
HbA1c (%)[b, d,e]	5.3 (1.2)	5.1 (0.9)	0.38	0.36
Cholesterol[b,e]	5.2 (1.0)	5.4 (1.1)	0.10	0.23
LDL (mmol/L)[b,e]	3.4 (0.8)	3.6 (1.0)	0.12	0.35
HDL (mmol/L)[b,e]	1.1 (0.3)	1.2 (0.4)	**0.002**	**0.009**
Triglycerides (mmol/L)[c,e]	1.4 (1.1)	1.0 (0.9)	**0.001**	0.06
C-peptide (mmol/L)[c,e]	0.67 (0.28)	0.73 (0.49)	0.26	**0.003**
CRP (mg/L)[b,e]	3.9 (3.1)	3.9 (4.4)	0.98	0.85
ApoM (µM)[b,e]	0.72 (0.15)	0.85 (0.23)	**< 0.0001**	**0.001**

[a]Sex, sedentary lifestyle, and family history of diabetes are presented as numbers and percentages.
[b]Age, BMI (body mass index), waist circumference, fasting glucose, 2 hour glucose, HbA1c, cholesterol, LDL (low-density lipoprotein), HDL (high-density lipoprotein), CRP (C-reactive protein), and ApoM are presented as the mean and standard deviation.
[c]HOMA-IR (homeostasis model assessment-insulin resistance), ISI (insulin sensitivity index), serum insulin, triglycerides, and C-peptide are presented as the median and interquartile range.
[d]HbA1c is presented as the mean in IFCC units (NGSP units).
[e]Adjusted for sex, age, and BMI
[f]Sex was adjusted for age and BMI; age was adjusted for sex and BMI; BMI and waist circumference were adjusted for sex and age.
P-values <0.05 are highlighted in bold.

Table 2. Multivariate linear regression with ApoM (μM) as the outcome and with an interaction term between country of birth and HOMA-IR

Multivariate[a]	**β**	**p-value**[b]	**95% CI**
HOMA-IR[c] (Swedes)	-0.08	0.008	-0.14, -0.02
Country of birth (Iraq vs. Sweden)	-0.13	< 0.0001	-0.20, -0.06
HOMA-IR*Country of birth	0.10	0.003	0.03, 0.17
Family history of diabetes (yes/no)	-0.003	0.89	-0.05, 0.05
p-Triglycerides	0.09	< 0.0001	0.06, 0.12
p-HDL	0.32	< 0.0001	0.24, 0.39
p-LDL	0.07	< 0.0001	0.04, 0.09

[a]Adjusted for age, sex, body mass index
High-density lipoprotein (HDL); low density lipoprotein (LDL)
[b]Test of whether the regression coefficient is equal to zero
[c]Natural log transformed

Supplementary Table 1. Characteristics of the study population according to glucose tolerance and country of birth

Covariate	Iraq (n=91)				Sweden (n=71)			
	NGT n = 51 (56%)	IFG/IGT n = 19 (21%)	T2DM n = 21 (23%)	p[c]	NGT n = 43 (61%)	IFG/IGT n = 15 (21%)	T2DM n = 13 (18%)	p[c]
HOMA-IR[a]	1.7 (1.1)	2.1 (3.9)	4.0 (3.8)	**< 0.0001**	1.5 (1.0)	2.3 (1.4)	3.0 (1.5)	**0.002**
ISI[a]	108 (71)	67 (52)	43 (44)	0.10	118 (107)	68 (39)	51 (45)	**0.03**
HDL (mmol/L)[b]	1.1 (0.3)	1.0 (0.2)	0.9 (0.3)	0.06	1.3 (0.4)	1.4 (0.5)	1.0 (0.2)	**0.03**
Triglycerides (mmol/L)[a]	1.5 (1.1)	1.4 (1.1)	1.3 (1.0)	0.62	0.9 (0.8)	1.1 (0.7)	1.6 (1.1)	**0.01**

[a]HOMA-IR (homeostasis model assessment-insulin resistance), ISI (insulin sensitivity index) and triglycerides are presented as the median and interquartile range.
[b]HDL (high-density lipoprotein) are presented as the mean and standard deviation.
[c]Test of differences in covariate between NGT, IFG/IGT and T2DM.
P-values <0.05 are highlighted in bold.