THE RELATIONSHIP BETWEEN APOB AND APOA1 WITH INSULINEMIC STATUS IN PREDIABETIC SUBJECTS

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ABSTRACT

The present study was mainly aimed at exploring the causal association of the atherogenic (ApoB) and antiatherogenic apolipoproteins (ApoA1) and their ratio in the basic defects of pancreatic β cell dysfunction and insulin resistance in type 2 Diabetes Mellitus (T2DM). An intermediate stage towards diabetes, the prediabetic stage, was chosen to explore the association. Following a standardized selection process, 131 subjects were purposefully recruited for the study, including 18 with impaired fasting glucose (IFG), 56 with impaired glucose tolerance (IGT), and 57 with type 2 diabetes (T2DM). Fifty-nine healthy subjects served as controls. Glucose, lipid and insulin were estimated by glucose-oxidase, enzymatic colorimetric assay and enzyme linked immunosorbent assay (ELISA) respectively. Serum ApoB and ApoA1 were estimated by immuno-nephelometric method. Appropriate statistical tools were used to calculate statistical differences using Statistical Package for Social studies (SPSS) for Windows V12. Absolute insulin (mIU) levels were significantly higher in the IGT and T2DM groups compared to controls (p 0.001 and p = 0.001, respectively). HOMA%B (mean±SD) was significantly lower in T2DM groups (p=<0.001) and higher in IGT compared to the controls although it is significantly lower in IFG compared to the controls but mean value is about 90%. HOMA%S was significantly lower in IGT and T2DM group (p=0.001 and 0.002 respectively). ApoA1 levels were significantly higher only in the T2DM group (p = 0.027), whereas ApoB levels were higher in both the IGT and T2DM groups (p = 0.026-0.001).. Neither ApoB nor ApoA1 showed any significant difference in the IFG group as compared to control. ApoB-ApoA1 ratio did not show significant difference among the groups. ApoB showed significant positive correlation with both fasting and postprandial glucose (p=0.006 and 0.040 respectively). In IGT group ApoB was positively correlated with absolute insulin (p=0.025) and HOMA%B (p=0.049) and negatively with HOMA%S (p=0.026). ApoB, but not ApoA1 or the ApoB and ApoA1 ratio, seem to have a causal association with insulin resistance, and elevation of ApoB is also modulated by obesity and atherogenic lipids.

KEYWORDS: Apolipoprotein B, Apolipoprotein A1, ApoB-ApoA1 ratio, insulin, HOMA%B, HOMA%S.

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Introduction

Prediabetic subjects have an increased risk of developing cardiovascular disease (CVD) and all-cause mortality compared to age -adjusted adults with normal glucose tolerance (Decode Study Group, 2001; Coutinho *et al.*, 1999; Liao *et al.*, 2001). About a two- to three fold increased risk for cardiovascular events was observed in younger adults with prediabetes (Zhang *et al.*, 2003; Bjornholt *et al.*, 1999). This level of risk is almost similar for cardiovascular risk among T2DM (Wood *et al.*, 1998; Liao *et al.*, 2001). ApoA1 is the major apo in HDL-c particles and initiates the 'reverse cholesterol transport'. ApoA1 can 'pick up' excess cholesterol from peripheral cells and transfer it back to the liver in the HDL particles. ApoA1 is not contained in the potentially atherogenic ApoB-containing particles, and thus ApoA1 in most cases only reflects the athero-protective part of the metabolism. Apolipoprotein B, a major protein component of circulating plasma lipoproteins, exists in two forms: ApoB-100 and ApoB-48. The first is synthesized exclusively by the small intestine, the second by the liver. In humans, ApoB-100 is found in lipoproteins originating from the liver (VLDL, IDL and LDL). There is one ApoB-100 molecule per hepatic-derived lipoprotein (Marcovina *et al.*, 2006). Hyperinsulinemic and/or insulin resistance were found to be associated with abnormalities of both lipid concentration and lipoprotein composition **(**Peter *et al.*, 1992). IGT is an intermediate stage between normal glucose tolerance and type 2 diabetes. Both insulin resistance and hyperinsulinemia have been suggested to be present in IGT (Haffner *et al.*, 1997). Sierra-Johnson et al. (2007) recently demonstrated that the apoB/apoA-I ratio is an independent predictor of insulin resistance in non-diabetic Americans. As part of series of experiments to explore the etiopathogensis of type 2 diabetes

in Bangladeshi people pancreatic B cell secretory capacity and insulin sensitivity studies in a number of studies involving IFG and IGT subjects (Kabir *et al*., 2008; Rahman *et al*., 2006; Shefin *et al*., 2008). Both IFG and IGT subjects were found to share features of heightened insulin secretion and a tendency to insulin resistance. Although both IFG and IGT shown to confer additional risk in the development of cardiovascular events, so far atherogenic parameters, like ApoB and ApoA1, have not yet been investigated systematically in these subjects.

Methods

Subjects were recruited purposefully for the study. Individuals who were referred to BIRDEM for blood glucose testing and volunteers who agreed to participate in the study underwent a two-sample glucose tolerance test (GTT).Dysregulation in glucose metabolism and/or diabetes mellitus were diagnosed and classified following WHO guidelines (WHO 1999). The study enlisted the participation of a total of 191 subjects Volunteers were recruited every working day in the counseling room at the BMRG, BIRDEM. Nature and method of the study were clearly detailed to each individual and also the close associates. Volunteers reported to the laboratory of BMRG by 9.30 to 10 am. OGTT was done and all the planned information obtained and recorded. Written consent was obtained from the volunteer. An appointment was given to the consenting participants to report to the BMRG laboratory for GTT.

Adult subjects with an age range of 30 to 55 voluntarily agreed to be included in this study by providing consent. Subjects with co-morbid diseases (infection, stroke, myocardial infarction, major surgery, essential hypertension, malabsorption etc.), history of medication, that may significantly affect glucose metabolism (glucocorticoids, oral contraceptives containing levonorgestral or high-dose estrogen, phenytoin, high-dose thiazide diuretics etc.) and pregnant women were excluded. Height, weight, and waist circumference were obtained using standardized techniques and equipment. Glucose was estimated by enzymatic colorimetric (GOD-PAP) method in the Hitachi 704 Automatic Analyzer, Hitachi Ltd., Tokyo, Japan using reagents of RANDOX Laboratories Ltd., UK. Total cholesterol was measured by the enzymatic endpoint method (cholesterol Oxidase/Peroxidase) and , Triglyceride was measured by enzymatic colorimetric (GPO-PAP) method in the auto analyzer, Auto analyzer HITACHI 704, Hitachi Ltd. Tokyo, Japan. High density lipoprotein cholesterol (HDLc) was measured by enzymatic colometric method using reagent of Randox laboratories, UK. The LDL-Cholesterol level in serum was calculated using the Friedewald formula (Friedewald *et al.*, 1972). HOMA-CIGMA software was used to measure B cell secretion (HOMA %B) and insulin sensitivity (HOMA% S). Insulin was measured by enzyme linked immunosorbent assay (ELISA) method using kit from Linco Research Inc., USA. The immunonephelometric method was used to measure Apolipoprotein A-1 and Apolipoprotein B using the Behering Nephelometer 100 Analyzer.

Statistical analysis

Testing for significant differences in mean values of variables between groups were performed by a student's t -test for normally distributed data and by Mann- Whitney U-test for untransformed data.

Results

The absolute insulin (mIU) level was significantly higher in the IGT and T2DM groups compared to controls (p 0.001 and p = 0.001, respectively). HOMA%B (mean±SD) was significantly lower in T2DM groups (p=<0.001) and higher in IGT compared to the controls although it is significantly lower in IFG compared to the controls but mean value is about 90%. HOMA%S was significantly lower in IGT (n=56) and T2DM (n=57) group (p=0.001 and 0.002 respectively). Triglyceride level was significantly higher in all three groups (p=0.044 - <0.001) and total cholesterol only in T2DM group (p=0.001). HDL and LDL cholesterol in all three groups was almost similar. ApoA1 was significantly higher only in the T2DM group and ApoB was higher in IGT and T2DM group (p=0.026-<0.001). Neither ApoB nor ApoA1 showed any significant difference in the IFG group (n=18) as compared to control. ApoB-ApoA1 ratio did not show a significant difference among the groups (Table 1).

Table 1. Characteristics of the study subjects

	Control (n=59)	**IFG** (n=18)	**IGT** (n=56)	**T2DM** (n=57)
Age (yrs)	41.8±6.2	41.6±6.7	42.5±6.8	44.8±7.6*
BMI (kg/m^2)	25.1±3.4	26.2±3.6	25.6±3.4	25.1±3.5
SBP (mmHg)	114±13	116±13	118±14	115±14
DBP (mmHg)	77±9	77±6	77±10	76±9
S Creat (mg/dL)	1.0±0.12	1.1±0.15	1.0±0.1	1.0±0.1
SGPT (U/L)	24.9±14.8	29.9±14.8	28.1±14.6	30.5±19.2

MAC (cm)	28.2±3.0	30.2±1.9*	29.5±2.7*	29.5±2.5*
TSF (mm)	13.9±5.2	18.5±7.9**	15.6±5.8	15.6±5.1
BSF (mm)	8.6±4.1	12.4±0.7***	9.9±4.9	11.5±4.4*
SSF (mm)	22.9±7.8	26.4±10.1	26.2±7.7*	25.2±6.8
WHR	0.91±0.07	0.94±0.07	0.92±0.06	0.95±0.05**
BFM (%)	28.22±5.8	33.4±6.8**	30.6±6.8*	30.2±5.1
F Insulin (µU/ml)	10.6±3.9	14.5±8.8	14.6±6.6***	14.9±8.3**
HOMA%B	118±29	89±37**	139±51**	79±52***
HOMA%S	88±49	70±39	62±28**	62±39**
TG (mg/dl)	133±63	187±96*	159±68*	178±62***
T CHOL (mg/dl)	200±35	211±42	202±40	223±37**
HDL-c (mg/dl)	34.5±9.8	36.5±5.8	34.2±9.3	32.9±10.4
LDL-c (mg/dl)	141±39	135±31	137±40	146±37
ApoA-1 (g/L)	0.97±0.25	1.05±0.27	1.05±0.25	1.08±0.26*
ApoB (g/L)	0.82±0.19	0.91±0.34	0.92±0.26*	1.04±0.29***
ApoB/ApoA1	0.89±0.29	0.83±0.29	0.89±0.27	0.99±0.29

***p<0.001, **p<0.01,*p<0.05

Serum ApoB showed a significant positive correlation with the total cholesterol (p<0.001), LDL-c (p<0.001) and a significant negative correlation with postprandial glucose (p=0.030) (Table 2) in control group; positive correlation with triglyceride (p=0.007), total cholesterol (p=0.038), HDL-c (p=0.038) in IFG group (Table 2), positive correlation with ApoA1 (p=0.037), fasting insulin (p=0.025), HOMA%B (p=0.049), triglyceride (p=0.002), total cholesterol (p<0.001), LDL-c (p<0.001), waist to hip ratio (p=0.004) and negative correlation with HOMA%S (p=0.026) (Table 2) in IGT group. In T2DM group ApoB showed positive correlation with ApoA1 (p=0.004), fasting glucose (p=0.006), postprandial glucose (p=0.040), total cholesterol (p<0.001), LDL-c (p=0.008), and negative correlation with HOMA%B (p=0.017) (Table 2).

Table 2. Spearman's correlation of serum ApoB with different parameters among the study groups

Variables	**Control**		**IFG**		**IGT**		**T2DM**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Age	0.138	0.330	-0.161	0.537	0.076	0.626	-0.009	0.947
BMI	-0.023	0.866	-0.448	0.072	0.145	0.324	-0.020	0.881
WHR	0.156	0.248	0.192	0.445	0.401**	**0.004**	0.119	0.392
ApoA1	0.048	0.720	0.210	0.404	0.293	0.037	0.374*	**0.004**
FSG	-0.054	0.684	0.319	0.198	-0.020	0.891	0.360	**0.006**
PPSG	-0.283	0.030	-0.111	0.662	0.129	0.368	0.276	**0.040**
F Insulin	0.045	0.739	0.068	0.803	0.314	0.025	-0.138	0.312

HOMA%B	0.161	0.228	0.018	0.948	0.277	**0.049**	-0.318	**0.017**
HOMA%S	-0.061	0.649	-0.081	0.766	-0.312	**0.026**	0.067	0.623
Triglyceride	0.245	0.066	0.624	0.007	0.436	**0.002**	0.122	0.383
T Chol	0.533	**<0.001**	0.491	0.038	0.669	**<0.001**	0.565	**<0.001**
LDL-c	0.494	**<0.001**	0.266	0.302	0.556	**<0.001**	0.368	**0.008**
HDL-c	-0.195	0.179	0.492	0.038	-0.041	0.789	-0.114	0.413

***p<0.001, **p<0.01,*p<0.05

Serum ApoA1 showed significant positive correlation with age (p=0.001) and negative correlation with triglyceride (p=0.026) (Table3) in control group (n=59), positive correlation with ApoB (Table 3) both in IGT (p=0.037) and T2DM (p=0.003) group (Table 3). No correlation was found with the testing variables in IFG group (Table 3).

Table 3. Spearman's correlation of serum ApoA1 with different parameters among the study groups

Variables	**Control**		**IFG**		**IGT**		**T2DM**	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Age	0.463	0.001	0.022	0.932	-0.120	0.438	-0.136	0.318
BMI	-0.253	0.060	-0.281	0.275	0.158	0.282	0.196	0.144
WHR	-0.087	0.521	0.044	0.861	-0.002	0.990	0.169	0.223
ApoB	0.048	0.720	0.210	0.404	0.293	0.037	0.374	**0.004**
FSG	-0.150	0.257	-0.056	0.825	0.026	0.859	0.048	0.722
PPSG	0.017	0.897	-0.092	0.716	0.150	0.293	-0.012	0.931
F Insulin	-0.110	0.412	0.079	0.770	0.039	0.787	0.095	0.487
HOMA%B	-0.126	0.344	0.097	0.721	-0.028	0.844	0.049	0.720
HOMA%S	0.157	0.238	-0.074	0.787	-0.034	0.815	-0.113	0.407
Triglyceride	-0.295	0.026	0.331	0.195	-0.074	0.616	0.028	0.842
T Chol	0.038	0.776	0.036	0.887	0.177	0.230	0.149	0.268
LDL-c	0.040	0.785	-0.103	0.694	0.144	0.346	0.028	0.844
HDL-c	0.231	0.111	-0.300	0.226	0.186	0.216	0.193	0.162

***p<0.001, **p<0.01,*p<0.05

Serum ApoB/ApoA1 ratio showed positive correlation with HOMA%B (p=0.027), triglyceride (p=0.002), total cholesterol (p=0.005), LDL-c (p=0.048) and negative correlation with postprandial glucose (p=0.040). In control group, positive correlation with total cholesterol (p=0.017) LDL-c (p=0.037), HDL-c (p=0.005) in IFG group was found (Table 4). Again, positive correlation with waist to hip ratio (p=0.008), total cholesterol (p<0.001), LDL-c (p=0.001) in IGT group was found (Table 4). Moreover, positive correlation with fasting serum glucose (p=0.020), postprandial glucose (p=0.024), total cholesterol (p=0.015), LDL-c (p=0.039) and negative correlation with HOMA%B (p=0.007) was found in T2DM group (Table 4).

Table 4. Spearman's correlation of serum ApoB to ApoA1 ratio with different parameters among the study groups

Variables	Control		IFG		IGT		T2DM	
	r	p	r	p	r	p	r	p
Age	-0.190	0.178	-0.072	0.792	0.121	0.440	-0.007	0.959
BMI	0.261	0.052	-0.452	0.079	0.009	0.951	-0.199	0.138
WHR	0.166	0.218	0.172	0.510	0.379	**0.008**	-0.003	0.985
FSG	0.024	0.858	0.456	0.066	-0.038	0.795	0.307	**0.020**
PPSG	-0.268	**0.040**	-0.033	0.899	0.165	0.251	0.301	**0.024**
F Insulin	0.169	0.205	<0.001	1.000	0.194	0.176	-0.219	0.105
HOMA%B	0.291	**0.027**	-0.054	0.850	0.216	0.131	-0.357	**0.007**
HOMA%S	-0.206	0.122	-0.021	0.940	-0.193	0.179	0.172	0.205
Triglyceride	0.409	**0.002**	0.359	0.172	0.256	0.079	0.016	0.910
T Chol	0.364	**0.005**	0.569	**0.017**	0.504	**<0.001**	0.321	0.015
LDL-c	0.287	**0.048**	0.524	**0.037**	0.487	**0.001**	0.287	0.039
HDL-c	-0.244	0.091	0.645	**0.005**	-0.116	0.447	-0.266	0.052

***p<0.001, **p<0.01,*p<0.05

Discussion

Diabetes has become a major health problem all over the world. The pathogenesis of diabetes, particularly the type 2 variety which constitutes the bulk, still needs to be clearly understood. Both pancreatic β cell secretory defect and/or insulin resistance are implicated in its pathogenesis, though confusion still exists regarding the primacy of the defects. Impaired glucose tolerance and/or impaired fasting glucose are thought to be the intermediate state in the pathogenesis of diabetes mellitus. Insulin resistance also has been suggested to be present both in IGT (Festa *et al.*, 2004; Davies *et al.*, 2000; Schianca *et al.*, 2003) and IFG (Hanefeld 2003; Tripathy *et al.*, 2000) state. Whatever is the predominant defect, it is now known that both insulin secretory dysfunction as well as some degree of insulin resistance is present in full blown clinical diabetes (Weyer *et al.*, 1999). Thus it is important to investigate the covariates of both the defects which can give new insight on the interplay of genetic and environmental risk factors in the development of the diabetic state and its complications. These factors may either be causes or consequence of pancreatic B cell dysfunction or insulin resistance and those should be studied in individual populations due to their substantial variation depending on racial and lifestyle or environmental determinants.

Cardiovascular risk factors are among the most important to be studied in various diabetic populations as these variables can increase the risk of macrovascular disorders up to 4 times and these can also increase the risk of microvascular disorders

in a substantial degree. This phenomenon is characterized by the interaction of sociodemographic, cultural, anthropometric, and biochemical risk factors. Among the biochemical risk factors hyperglycemia, dyslipidemia and insulin resistance may play a central role. In recent years the nature of the atherogenic lipids have become more clear and the involvement of Apo B and Apo A1 has been claimed (Sierra-Jhonson *et al.,* 2007) to be associated with insulin resistance both as cause and effect. However, due to the cross-sectional nature of the studies, the causal relationship between these abnormalities remains unknown.. Only long-term prospective studies can settle the issue more conclusively. Being an intermediate stage to DM the prediabetic states give an opportunity to investigate the possible causal relationship between the atherogenic and antiatherogenic lipoproteins and basic defects of diabetes. The present study followed the approach with healthy subjects (n==59) as negative control and T2DM subjects (n==57) as positive controls.

To our knowledge, this is the first time a systematic measurement of Apo B and Apo A1 has been done on any Bangladeshi population. The data on control subjects showed lower values than those found in other populations (Kim *et al.,* 2005; Zunic *et al.,* 1992) with comparable age and BMI. T2DM subjects showed both elevated ApoA1 and ApoB compared to control. The ApoB data is consistent with the earlier reported data in other population (Chan *et al.,* 2004); however, the elevation of Apo A1 is in contrast with others who found a lower value (Stewart *et al.,* 1998). There was strong tendency of Apo B-Apo A1 ratio to be higher in T2DM group, but the difference was outside the range of statistical significance (p=0.087). Some authors (Sung and Hwang, 2005; Sierra-Johnson *et al*., 2007) claim that the ApoB-ApoA1 ratio is a more sensitive risk factor compared to the single apolipoproteins; the present data, however, do not reflect these claims.

In contrast to the findings in T2DM subjects, the prediabetic groups did not show significantly higher Apo A1 compared to control although there was strong trend in the IGT variety of prediabetes (p=0.094). Regarding ApoB the difference was significant in the IGT group but there was not even a tendency in the IFG group. In case of ApoB-ApoA1 there was neither significant difference nor a tendency in IFG or IGT group compared to control. The data thus show that Apo B seems to be increased in potential cases of diabetes, but the abnormality is limited to only IGT class. At the same time, when investigating this atherosclerosis risk marker, individual ApoB appeared to be more sensitive than the ApoB-ApoA1 ratio. The above findings immediately lead to certain postulates regarding the association of ApoB and ApoA1 with the basic defects of DM. In line with the evidence of Weyer *et al*., (1999) the T2DM cases in the present study show both an insulin secretory defect and insulin resistance. ApoB and ApoA1 in these subjects as compared to control may be related to either or both defects. IGT subjects have elevated insulin secretion increased HOMA% B and decreased sensitivity as compared to control. Thus higher ApoB in this group seems to be associated with insulin resistance. Insulin resistance in Bangladeshi IGT subjects have been reported before (Shefin *etal.,* 2008).

In contrast to theT2DM and IGT groups, IFG subjects show an insulin secretory defect but no insulin resistance which is again consistent previous with studies on the same population (Rahman *et al.,* 2006). It seems that insulin secretory defect alone does not affect the serum levels of ApoB, ApoA1 or their ratios. Careful analysis of these data thus reveals that only insulin resistance may have a causal relationship with ApoB but not with ApoA1. A significant correlation between ApoB and HOMA%S supports this (Table 2).

Although the association of insulin resistance with apolipoprotein B seems plausible, the confounding roles of other factors related to insulin resistance can not be excluded in the present study. Combining analysis from the group difference as well as Spearman correlation, it seems that ApoB is strongly associated with some of the anthropometric (e.g., waist- to- hip ratio - a marker of central obesity) and biochemical (e.g., TG, total cholesterol, and LDL-cholesterol) variables in the IGT group. Some of the correlations are lost in the T2DM group probably due to the major confounding effect of hyperglycemia ($p < 0.006$), but the conspicuous absence of such correlations in the IFG group again points towards the central role of insulin resistance in the genesis of elevated ApoB (Table 2).

In conclusion, ApoB but not ApoA1 or ApoB-ApoA1 ratio seem to have a causal association with insulin resistance, and elevation of ApoB is also modulated by central obesity and atherogenic lipids. Since insulin resistance is a substantial modifiable fact through lifestyle adjustment, the association of the atherogenic apolipoproteins with this defect may have a great public health consequence.

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