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The roles of apo E genotype, gender and adipokines in blood plasma lipids in Caucasians with well-controlled type 2 diabetes

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Abstract
Cape Breton Island has among the highest levels of type 2 diabetes in Canada and therefore the risk of associated coronary atherosclerosis-induced myocardial infarction is also very high. Fasting blood serum lipid concentrations are important measures of coronary atherosclerosis disease risk in type 2 diabetics. Apolipoprotein E alleles and genotypes, as well as the adipocytokines, leptin and adiponectin modify fasting blood serum lipid levels and thus the degree of atherosclerosis which may be assessed in part by c-reactive protein (CRP). Control of blood lipid levels is critical to reducing the risk of myocardial infarction. It was hypothesised that there would be no gender differences in myocardial infarction risk including CRP levels and at least some of the lipid levels including their modulating levels of apolipoprotein alleles and genotypes, leptin and adiponectin. The purpose of this study was to assess this hypothesis. Females had significantly higher levels of high density lipoprotein cholesterol (HDL-c) and its atherogenic subfraction HDL_2-c while at the same time having higher levels of HDL_3-c. Serum free fatty acids levels were significantly higher in females as was the leptin level. There were no gender differences in elevated total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), small dense (sd) LDL-c, triglycerides, lipoprotein(a), adiponectin and CRP and the ratios of HDL-c: TC and HDL-c: LDL-c. By trend or significantly, Apo E genotypes and allele presence correlated variously by gender with some lipid levels. Thus despite the higher degree of antiatherogenic HDL-c and HDL_2-c in females, this was not manifested in lesser atherosclerotic severity as assessed by CRP. This may be due to the fact that HDL-c and HDL_2-c were low as was ratio of HDL-c: TC and HDL-c: LDL-c. Thus it is concluded that apo E genotype, lipid levels and the ratios of HDL-c: TC and HDL-c: LDL-c equally favour atherosclerosis in both males and female type 2 diabetics in Cape Breton and more aggressive intervention is required to ameliorate the substantial risk of atherosclerosis induced myocardial infarction presented by these lipid profiles. However, this was only a small study and a larger study would more definitively address the risk of myocardial infarction in both males and females on Cape Breton.

Keywords: *Human, fasting serum lipids, leptin, adiponectin, clinical targets, type 2 diabetes*

Introduction

Cape Breton Island in the province of Nova Scotia, Canada suffers from among the highest rates of type 2 diabetes in Canada, the consequences of which are seen in the overall economy and in the competition for healthcare dollars with other health issues. Consequently it is important to control this disease as much as possible so as to reduce its economic and social impact. There are no reports to date regarding the gender equity of management of the features of dislipidemia, such information being of clear importance for the medical, economic and social impacts of this disease.

Dyslipidemia is a feature of type 2 diabetes that contributes significantly to the major cause of death in these patients, atherosclerosis-induced myocardial infarction.[1,2] All references to dyslipidemia herein refer to blood plasma or serum concentrations in fasted patients. Dyslipidemia features elevated triglyceride concentrations, small dense LDL-c, and in some patients elevated total cholesterol and low density lipoprotein-cholesterol (LDL-c).[2-6] Elevated high density $lipoprotein_3$-cholesterol (HDL_3-c) concentrations may also feature. As well there are decreased fasting blood plasma concentrations of high density lipoprotein-cholesterol (HDL-c), high density $lipoprotein_2$ -cholesterol (HDL_2-c).[3] As triglycerides rise, HDL-c and HDL_2-c fall while small dense LDL-c also rises. This profile is pro-atherogenic and thus a promoter of plaque formation.[3] Free fatty acid concentrations also rise in type 2 diabetes contributing to increased blood plasma glucose concentrations[7] further exacerbating the dyslipidemia. As small dense LDL-c and LDL-c rise there is a greater influx of cholesterol into the arterial wall.[3] Low blood serum fasting levels of adiponectin and elevated leptin contribute to dyslipidemia.[8-19] However, such relationships are in dispute.[20] C-reactive protein (CRP) is a measure of the extent of atherosclerosis and therefore to some degree the risk of myocardial infarction.[21-22]

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The opportunity for cholesterol efflux via HDL-c and more specifically HDL_2-c is lessened with the decrease in concentration of HDL-c and HDL_2-c.[3] Lipoprotein (a) has been suggested to contribute to the atherosclerotic process[23] in type 2 diabetics; one could suggest this in terms of atheromatous plaque formation. The increase in LDL-c and in particular the very aggressive pro-atheromatous sd LDL-c give rise to increased cholesterol influx into the arterial wall.[3] Thus the atheromatous plaque grows resulting in partial or complete occlusion of artery and if the plaque ruptures the opportunity for thrombus or embolus formation is increased.[24] Thrombus or embolus formation can also result in arterial occlusion.[24] Regardless of the cause of occlusion, myocardial infarction will occur.

Apolipoprotein E genotype is known to modify various lipid levels in human type 2 diabetics. There appears to be no work published on lipid levels differentiated on both apo E (alleles or genotype) and gender. However, Oh and Barrett-Connor[25] reported that the presence of E3 and 4 alleles conferred a higher TC and LDL-c compared to the E2 allele in both non-diabetic men and women without a family history of diabetes. Reznik and associates [26] reported a higher HDL-c in men and women type 2 diabetics combined in those with apo E2 allele.

Target blood plasma fasting lipid and lipoprotein levels have been set for type 2 diabetics. These levels are not gender based. It was hypothesised that there would be no gender differences in myocardial infarction risk including CRP levels and at least some of the lipid levels including their modulating factors of apo E allele and genotype, leptin and adiponectin among Caucasian males and females with type 2 diabetes on Cape Breton. The purpose of this study was to test that hypothesis.

Methods

Subjects (n =20 male, 20 female) were randomly chosen from among 84 Caucasians responding in approximately equal sex numbers to a Sydney, Nova Scotia newspaper advertisement and two area physicians. This study received approval from the Cape Breton University Human Ethics Review Committee. Subjects came for visit 1 and 3 months later for visit 2. On both visits, body weight and BMI were determined.

Parameters were measured by following the kit manufacturer's instructions or published method (published method or kit and company in brackets) - triglycerides, (L-Type-TG H, Wako, Richmond, Virginia), total cholesterol (Cholesterol E method, Wako, Richmond VA, USA), HDL and HDL_3 isolated by precipitation (Quantolip, Technoclone, Vienna, Austria) and their respective cholesterols (Cholesterol E method, Wako, Richmond VA, USA), HDL_2-c (calculated as the difference between HDL-c and HDL_3-c), LDL-c (LDL-direct, Cholesterol E method, Wako, Richmond VA, USA), sd LDL-c [27)], Lp(a) by ELISA (Trinity Biotech, Jamestown, NY,USA), free fatty acids (half micro enzymatic method, Roche, Mannheim, Germany), leptin by ELISA (Linco, St. Charles, MO, USA), adiponectin by ELISA (Linco, St. Charles, MO, USA), and CRP by ELISA (Alpha Diagnostic, San Antonio, Texas, USA).

DNA was extracted by the method of Lahiri and Nurnberger[28] and genotyped for apo E via the method of Hixson and associates.[29]

Statistical analyses

The data in tables 1 and 2 was assessed by paired t-test comparing each gender to itself going from visit 1 to visit 2 and via an unpaired t-test comparing males to females in terms of the averages of visits 1 and 2. The level of significance was set at $p < 0.05$ to declare a difference in a given parameter between visits 1 and 2 for a given gender and between the averages of visits 1 and 2 to assess whether there was a gender difference. Pearson correlations were run between the lipid parameters and each of apo E allele presence and genotype.

Results

Subject characteristics are contained in table 1. There were no significant differences in age or BMI between visits 1 and 2 for males or females nor was there any difference between males and females in age or BMI for the means of visit 1 and 2. Levels of fasting blood serum lipids, leptin, adiponectin and CRP are found in table 2 as are the correlations (trends and statistically significant) with Apo E alleles and genotype. There were no significant differences for a given gender in any parameter going from visit 1 to visit 2. When the averages of visits 1 and 2 were compared, females had significantly higher levels, compared to males, of high density lipoprotein cholesterol (HDL-c) and its atherogenic subfraction HDL_2-c while at the same time having higher levels of HDL_3-c. Serum free fatty acids levels were significantly higher in females as was the leptin level. There were no gender differences in total cholesterol (TC), low density lipoprotein-cholesterol (LDL-c), small dense (sd) LDL-c, triglycerides, adiponectin and CRP levels and the ratios of HDL-c: TC and HDL-c: LDL-c. However, a trend toward higher triglycerides and lower HDL_2-c, HDL-c, the HDL:c:TC ratio in males carrying the apo E 3/4 genotype was observed. The apo 4/4 genotype conferred a tendency toward lower HDLc:LDL-c ratio in males. In females, apo E 4/4 gave significantly higher triglycerides while apo E2/4 generated a trend toward a higher HDLc: TC ratio.

Discussion

The parameters measured were all stable (showed no statistically significant difference) for visits one and two for each gender. Thus data showing either a gender difference or similarity for the means of visits one and two is validated.

The patients presented a strongly pro-atherosclerotic fasting blood serum lipid profile despite the higher levels of anti-atherosclerotic HDL-c and HDL_2-c in females compared to males. Still these levels were low and as well HDL_3-c was

Table 1: Pre-treatment characteristics of subjects (all Caucasian). Data (N = 32) is reported as mean ± standard error of the mean (S.E.M.).

	Males visit 1	Males visit 2	Females visit 1	Females visit 2	Males: mean of visits 1 and 2	Females: mean of visits 1 and 2
N	18	18	14	14	18	14
Age (years)	59.5 ± 1.7	60.7 ± 2.9	60.7 ± 2.9	60.7 ± 2.9	59.5 ± 1.7	60.7 ± 2.9
Body mass index (BMI) kg/m^2	30.3 ± 0.7	30.3 ± 0.8	33.7 ± 1.6	33.4 ± 1.7	30.3 ± 0.8	33.6 ± 1.8

Table 2: Blood serum lipids, apo E genotype correlations, adiponectin, leptin and CRP. Data (N = 32) is reported as mean ± standard error of the mean (S.E.M.). Statistically significant differences are marked with a superscript letters with different letters representing a statistically significant difference across a row.

	Males visit 1	Males visit 2	Females visit 1	Females visit 2	Males –mean of visits 1 and 2	Females-mean of visits 1 and 2
N	18	18	14	14	18	14
Triglycerides (mg/dl)	231.8 ± 75.7	250.1 ± 91.8	190.0 ± 22.0	168.3 ± 12.4	241.0 ± 58.6 Apo E 3/4; r = 0.445 p = 0.084	179.1 ± 12.6 Apo E 4/4; r = 0.766 p = 0.001 presence of E4 allele r = 0.455; p = 0.102
Total cholesterol (mg/dl)	232.3 ± 35.9	218.8 ± 20.0	219.1 ± 8.2	202.3 ±11.8	225.1 ± 14.1	210.2 ± 8.3
HDL-c (mg/dl)	39.2 ± 1.2	43.2 ± 2.3	45.5 ± 3.9	48.6 ± 3.9	40.3 ± 1.2 Apo E 3/4 r = - 0.405; p = 0.119	47.3 ± 2.6 [a]
HDL_2-c (mg/dl)	9.3 ± 0.4	10.1 ± 0.6	11.1 ± 0.8	11.4 ± 1.0	9.8 ± 0.4 Apo E 3/4 r = - 0.396; p = 0.129	11.4 ± 0.6 [a]
HDL_3-c (mg/dl)	30.0 ± 1.3	33.6 ± 1.9	35.7 ± 2.9	38.0 ± 2.9	31.7 ± 1.5	37.0 ± 2.7 [a]
LDL-c (mg/dl)	150.4 ± 14.7	148.1 ± 18.1	151.0 ± 12.7	153.0 ± 15.2	149.3 ± 11.5	152.0 ± 14.7
sd LDL-c (mg/dl)	39.2 ± 7.5	34.2 ± 5.9	58.1± 8.8	40.5 ± 10.4	36.8 ± 4.8	48.6 ± 7.0
HDL-c:TC ratio	0.16 ± 0.03	0.26 ± 0.05	0.21 ± 0.02	0.31 ± 0.05	0.21 ± 0.03 Apo E 3/4; r = - 0.445 p = 0.084	0.29 ± 0.05 Apo E 2/4, r = 0.551 p = 0.051
HDL-c:LDL-c ratio	0.29 ± 0.03	0.39 ± 0.03	0.31 ± 0.05	0.36 ± 0.03	0.34 ± 0.03 Apo E 4/4; r = - 0.625 p = 0.098	0.34 ± 0.03
Lp(a) (mg/dl)	9.5 ± 3.7	9.1 ± 3.9	13.1 ± 7.4	8.9 ± 8.6	9.3 ± 3.8	10.9 ± 8.2
FFA (μmol/l)	281.4 ± 35.9	296.4 ± 38.6	424.7 ± 35.5	419.6 ± 41.6	288.6 ± 37.8	422.8 ± 42.7 [a]
Leptin (ng/ml)	17.4 ± 3.3	15.9 ± 3.3	81.0 ± 20.3	84.4 ± 33.2	16.8 ± 3.0	82.7 ± 23.2 [a]
Adiponectin (μg/ml)	16.6 ± 2.1	17.6 ± 2.3	17.2 ± 1.9	17.3 ±1.6	17.0 ± 2.0	17.2 ± 1.7
C-reactive protein (mg/L)	5.9 ± 1.2	5.9 ± 1.0	7.9 ± 1.6	9.2 ± 2.1	5.9 ± 1.0	8.5 ± 1.7

[a] significantly different from males (mean of visits one and two). There were no significant differences for a given gender between visits one and two. Females had significantly different values for a given parameter at visit one and at visit two where a superscript is indicated in the last column.

higher in the females than males. The role of HDL_3-c in atheroma formation and hence atherosclerotic risk is not clear[3]. However, despite these differences it would appear that there is an absence of gender difference in the level of risk is manifested in the gender statistically identical and low levels of HDL-c: TC and HDL-c: LDL-c ratios between males and females coupled with statistically identical and elevated levels of the pro-atherogenic total cholesterol, triglycerides, LDL-c, sd LDL-c that were observed. These ratios are a measure of arterial wall cholesterol influx versus

efflux and it is apparent that these low ratios reflect the possibility of enhanced plaque formation derived from such greater influx. Elevated total cholesterol manifests in increased levels of LDL-c[30] while elevated triglycerides result in increased levels of the very highly pro-atherogenic sd LDL-c.[31] sd LDL-c represents a very high risk of atheroma formation via aggressive cholesterol influx into the arterial wall and hence atheroma formation.[3] Further females presented a statistically higher level of FFA which contributes to the pro-atherosclerotic impact of elevated blood serum glucose,[6,32] however this was not manifested in the extent and severity of atherosclerosis as measured by the marker CRP which was identically (statistically) elevated between males and females. Lp(a) mean levels are not in the pro-atherosclerotic range (above 20-30 mg %) [23] and thus are not in need of address. Elevated Lp(a) may result in increased arterial wall cholesterol influx and hence atheroma formation . Lp(a) has variously been reported to enhance thrombus and embolus residence time or to decrease platelet aggregration resulting in thrombus formation (for review see Barre 2007).[33]

Elevated leptin and decreased adiponectin are associated with higher triglycerides, cholesterol and LDL-c and lower levels of HDL-c. [8-19] Leptin is inversely correlated with HDL-c[11,17] but this is in dispute.[34-36] Regardless, the higher levels of HDL-c in females was also associated with a higher level of leptin. Buyukbese and associates [37] has noted a positive significant correlation between leptin and HDL-c in female type 2 diabetics. The absence of gender difference in adiponectin was apparently without gender impact on the various lipid levels.

The tendency toward higher triglycerides and lower HDL_2-c, HDL-c, the HDL:c:TC ratio in males carrying the apo E 3/4 genotype was observed. This is consistent with the observation that as triglycerides increase HDL-c decreases with, in the case of the present data, HDL_2-c apparently being more sensitive to the elevations of triglycerides, due in part, it may appear to the presence of the apo E 3/4 genotype. The apo E 4/4 genotype conferred a tendency toward lower HDLc:LDL-c ratio in males though it not clear why apo E 3/4 did confer a similar trend in the HDL-c:TC ratio. In females, apo E 4/4 gave significantly higher triglycerides while apo E2/4 generated a trend toward a higher HDLc: TC ratio. Thus it appears that in females the presence of apo E2 is necessary to counteract, via an elevated HDLc:TC ratio, the impact of apo E4 on the cardiovascular impact of elevated triglycerides. Indeed in both males and females departure from the apo E 4/4 genotype may confer a degree of cardiovascular protection. This is consistent with the work of Oh and Barrett-Connor[25]and Reznik and associates.[26]

The mean HDL-c and LDL-c levels and the ratios of HDL-c: TC and HDL-c : LDL-c do not meet the clinical guidelines set by the Canadian Diabetes Association[38] consistent with the work of Harris and associates[39] examining type 2 diabetes management in Canada. Thus much more aggressive intervention ranging from improved diet, aerobic exercise, reduced or preferably eliminated smoking and alcohol consumption are required. Should these approaches fail increased doses of anti-atherosclerotic drugs or drugs that have not yet been tried in a given patient will be necessary.

Such aggressive intervention is required to reduce or eliminate atheromatous plaque. When such plaque ruptures, collagen fibrils in the media of the artery are exposed to blood platelets. Collagen fibrils cause aggregation of these blood platelets resulting in thrombus and/or embolus formation which in turn may precipitate myocardial infarction. It has been previously observed that this population of type 2 diabetics has dramatically shortened bleeding times thus enhancing the risk of platelet aggregration and subsequent thrombus and/or embolus formation thus enhancing the opportunity for myocardial infarction.[40]

It is concluded that leptin was associated with higher HDL-c in females, adiponectin had no gender impact on lipid profile, departure from the apo E4/4 genotype confers a degree of improved lipid profile and hence reduced cardiovascular risk in females and perhaps in males, and that despite higher but still low levels of HDL-c and HDL_2-c in females and a lack of gender difference in the low HDL-c : TC and HDL-c : LDL-c ratios and the CRP levels, these type 2 diabetic patients (both males and females) are, as a total population, at equally significant risk of atherosclerosis and its sequela, myocardial infarction resulting from atheromatous occlusion and/or atheromatous plaque rupture leading to thrombus and/or embolus formation. Failure to address this risk equally aggressively in males and females will result in many more cases of unnecessary morbidity and mortality on Cape Breton thus presenting even greater challenges to already over-stretched healthcare budgets and attempts to improve the better but still struggling Cape Breton economic outlook. However, this was only a very small sample size and a larger study here on Cape Breton will more definitely address the hypothesis.

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