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Gender Differences in Cardiovascular Risk in Adolescents with Type 1 Diabetes

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Abstract

Cardiovascular disease is seen at a younger age and at a higher prevalence in patients with type 1 diabetes (T1D) than in the general population. It is well described that women with T1D have a higher relative risk for cardiovascular disease than men with T1D, unlike that seen in the general population. The pathophysiology behind this is unknown. We did a cross-sectional study to examine gender differences in cardiovascular risk factors in adolescents with T1D between the ages of 13–20 years, compared to children of a similar age without T1D. All subjects underwent Dual Energy X-ray Absorptiometry (DXA scan) to measure body composition, and an HDI/Pulsewave CR-2000 test measure of arterial elasticity. Fasting serum lipids, apolipoprotein B and apolipoprotein C-III were measured in each subject. 29 children with T1D (10 F, 19 M) and 37 healthy children (18F, 19 M) participated. Although no gender differences for body mass index ($p = 0.91$) and A1C ($p = 0.69$) were seen, females with T1D had a significantly higher trunk % fat compared to males ($p = 0.004$). No gender differences were found ($p > 0.05$) for trunk % fat in adolescents without diabetes. There was no gender difference among any other cardiovascular risk factors in both children with and without diabetes. Thus we conclude that female adolescents with T1D have more centrally distributed fat which may contribute to their relatively higher cardiovascular risk. Attenuation of the central distribution of fat through exercise and dietary modifications may help ameliorate their subsequent cardiovascular disease burden.

Keywords

body composition; cardiovascular disease; type 1 diabetes

Introduction

Cardiovascular mortality is increased in patients with diabetes[1]. Patients with T1D have a cumulative mortality of 35% from coronary artery disease compared to 4% to 8% in the

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general population[2]. However, women with both type 1 and type 2 diabetes have a higher relative risk for cardiovascular disease (CVD) than men. In the UK diabetes cohort of almost 24,000 patients diagnosed with diabetes prior to 30 years of age, females had much higher standardized mortality ratios compared to males [3]. Data from Alleghany county in Pennsylvania show a similar trend with a standardized mortality ratio more than triple that seen in males [4]. The higher relative risk in women with type 1 diabetes (T1D) compared to men with T1D is seen across all ages, with the highest relative risk seen in the younger population [5]. In contrast, this gender difference is not evident in non-diabetic individuals. These data suggest that women with diabetes lose the gender protection enjoyed by premenopausal women without diabetes.

The reasons for increased susceptibility to coronary artery disease in women with diabetes are not clear. Different risk factors contribute to cardiovascular disease in men and women. In women with T1D, waist to hip ratio, physical activity and depressive symptomatology predicted coronary artery disease while renal disease was a better predictor in men[6, 7]. A study using electron beam computer tomography showed that women with diabetes have a significantly higher risk of coronary calcification compared with women without diabetes[8]. For men, however there was no significant difference in the coronary calcification score between individuals with and without diabetes. Also in men the strongest predictor of death due to cardiovascular disease (CVD) was a previous history of CVD irrespective of their diabetes status. Women with diabetes however, had a higher cardiovascular mortality even without a history of previous CVD compared to women without diabetes who have had a previous cardiovascular incident[9]. These and other data suggest that diabetes somehow increases the cardiovascular burden in a gender specific manner. The exact pathophysiology is yet to be elucidated.

The current study was done to examine the gender differences in cardiovascular risk factors (body composition, vascular elasticity, lipid profile, apolipoproteins B and C-III) in adolescents with T1D who are between the ages of 13 to 20 years. The control population consisted of subjects without diabetes across the same age range. We hypothesized that females with T1D will have more cardiovascular risk factors than males with T1D, whereas females without T1D will be relatively protected, as evident by fewer risk factors compared to their male counterparts.

Methods

Subjects

This was a cross-sectional study of adolescents with and without T1D between the ages of 13-20 years. Children across all weight ranges were recruited. Subjects with T1D were primarily recruited from diabetes clinics at the University of Oklahoma Health Sciences Center, and non-diabetic subjects were recruited through recruitment fliers and campus wide emails. A total of 77 children were enrolled, and 68 completed the study. Data from 2 participants were not included in the analysis: one subject was on oral contraceptive pills (inadvertently consented and screened, despite having an exclusion criterion per protocol), and the other because of markedly abnormal lipid data suggesting a possible genetic mutation in lipoprotein metabolism, also an exclusion criterion. The research protocol was approved by the Institutional Review Board at University of Oklahoma Health Sciences Center and all subjects signed an assent form prior to testing.

Inclusion criteria for children with T1D included a diagnosis of T1D for more than 3 years and an average HbA1c between 6.5 to 10.7% for the past 6 months. To control for potentially independent effects of extreme hyperglycemia, a cut-off HbA1c level of 10.7% was selected, which represents one standard deviation above the mean HbA1c achieved in

the adolescent population during the nationwide Diabetes Control and Complications Trial (DCCT). To control for the well-described changes in the rates of diabetic complications after puberty, children who were prepubertal or early pubertal (Tanner 1 and 2) were excluded. Children were excluded from the study if they had any other coexisting endocrine, genetic or metabolic disease, if they were on any medications, including those that could affect substrate metabolism (excluding insulin), psychotropic medications, weight loss medications, and oral contraceptives for female subjects. Inclusion criteria for Tanner stage and ages were identical for non-T1D children. Children were excluded from the study if they had impaired fasting glucose or had diabetes based on fasting glucose values. Additional exclusion criteria were otherwise identical to those listed above for the group with T1D. Two children with coexisting hypothyroidism and 1 child with Addison's disease, all well-controlled on physiological replacement hormonal therapy, were included in the study. Three diabetic participants treated for urinary microalbuminuria with angiotensin converting enzyme inhibitors (for an average of three years prior to entry into the study) were included.

Measurements

After obtaining appropriate consent and assent, each child underwent a history and physical exam by a board certified pediatrician. Height and weight were used to calculate BMI, waist and hip circumference were obtained on each subject, and the presence and degree of acanthosis nigricans were noted if present. Then they underwent testing for body composition, vascular elasticity indices and a blood draw for lipid profile and apolipoprotein values. All testing was done in the morning after an overnight fast and was done by an experienced nurse assigned to the study at the General Clinical Research Center at the University of Oklahoma Health Sciences Center.

Body composition was measured using dual energy x-ray absorptiometry scan (DXA; Hologic QDR 4500, Waltham, MA). Pulse wave analysis determination was made by HDI/Pulsewave CR-2000, Hypertension Diagnostics, Eagan, MN[10]. This technique uses a modified Windkessel model to derive information on proximal and distal arteries by analyzing the diastolic part of the arterial wave form [11]. Testing was done in fasting subjects when rested in the supine position. An average of three readings was calculated to derive the mean small artery elasticity index and large artery elasticity index. The test-retest intraclass reliability coefficient is $R=0.87$ for large artery elasticity index and $R=0.83$ for small artery elasticity index. [12]

Blood was analyzed for fasting lipids, apolipoprotein B, and apolipoprotein C-III levels. Total cholesterol, triglycerides and HDL-cholesterol were measured by standardized enzymatic procedure. VLDL-cholesterol and LDL-cholesterol were estimated by Freidewald formula and non HDL-cholesterol was calculated as total cholesterol-HDL-cholesterol. Blood was frozen for later analysis of apolipoprotein levels. Apolipoprotein levels were measured by immunoturbidimetry as described previously[13]. Since heparin-manganese has a high affinity for apoB, apoC-III in heparin manganese precipitate (HP) is apoC-III bound to apoB-containing lipoproteins. The apoC-III in heparin-manganese supernate (HS) is apoC-III bound to apoA-containing lipoproteins. ApoC-III is measured in the total plasma sample and in the precipitate following reconstitution to the original volume to obtain apoC-III HP. The value for apoC-III HS was derived by subtracting apoC-III HP from total plasma apoC-III.

Statistical Methods

Descriptive statistics were calculated for all demographic and clinical variables. Means and standard deviations, or medians and ranges, as appropriate, were computed and compared across genders for both diabetic and non-diabetic subjects. All continuous variables were

checked for normality and comparisons were made using a t test for means or a Wilcoxon-Mann-Whitney test for medians as appropriate. Controlling for other covariates, such as BMI, in a general linear model was not performed because several of the clinical variables were not normally distributed. All analyses were performed using the SAS 9.2 (Cary, NC) statistical package. Statistical significance was set at $p < 0.05$.

Results

29 adolescents and young adults with T1D and 37 children without T1D were recruited. The demographic information is given in Table 1. There was no significant difference ($p > 0.05$) in age, HbA1C, weight or body mass index (BMI) between males and females with T1D. Similarly there was no gender difference in these demographic variables in subjects without T1D.

There was no significant difference in total lean and fat mass between males and females with and without T1D (Table 2), but females with T1D had higher trunk% fat compared to their male counter parts ($p < 0.01$). This was not true of subjects without T1D ($p = 0.22$). There was a non-significant trend ($p = 0.09$) for trunk fat mass to be higher in female adolescents with T1D than males, but this trend was not evident in children without T1D ($p = 0.96$).

There were no significant differences ($p > 0.05$) in systolic or diastolic blood pressure between female and male adolescents with and without T1D (Table 3). Similarly there were no gender difference ($p > 0.05$) in vascular elasticity indices, both small and large, in children with and without T1D. Lipid profile and apolipoprotein variables did not differ significantly between the two genders in both subjects with and without T1D (Table 2). Similarly C-reactive protein did not differ significantly between males and females with or without T1D.

Discussion

Our study demonstrates a higher trunk fat % in girls with T1D compared to boys, but this gender difference is not present in subjects without T1D. A study done by Inberg et al using DXA measurements, showed higher BMI and fat mass in girls with T1D compared to controls which tracked over a period of 6 years[14]. However this group did not find any difference in regional adiposity between girls with and without T1D. This study did not include males, and unlike our study, girls with T1D in this study had a higher BMI compared to control subjects. Interestingly, a study done by the same group again using DXA measurements, showed no gender difference in body composition in adults with long standing T1D. In that study, the skin fold measurements revealed a higher abdominal fat in females with T1D compared to healthy controls but, this difference was not substantiated by DXA measurements[15]. They attributed it to stiffer and less elastic subcutaneous tissue. Abdominal distribution of fat is well known to be associated with an adverse cardiovascular risk in the general population [16]. In the Pittsburgh Epidemiology of Diabetes Complications cohort, subjects with T1D and coronary heart disease had higher trunk fat % mass [17]. Higher trunk % fat mass was associated with a higher prevalence of coronary heart disease only in females, which points to the importance of addressing this risk factor at a young age.

Our study did not show a significant gender difference in other traditional cardiovascular risk factors, including total cholesterol and triglyceride levels. Novel risk factors like apo-B or apo C-III levels also did not differ between the two genders among subjects either with or without T1D. Women with T1D have been described to have higher total cholesterol levels accounting for their disproportionately high cardiovascular mortality, though we could not

document this among our subjects[18]. This could be because our subjects were younger and their diabetes was relatively well controlled. Various apolipoprotein abnormalities have also been described in subjects with T1D including elevated apolipoprotein C and lipoprotein a levels[19, 20]. A 15-year follow-up study from Switzerland showed apolipoprotein B levels to be a strong predictor of cardiovascular mortality in T1D subjects[21]. However, all these studies have been done in adults with T1D. We could not demonstrate any gender difference in apolipoprotein profiles in our subjects with T1D and this could be because of either the smaller sample size or the relatively shorter duration of diabetes in our subjects.

In our study, there was no significant gender difference in vascular elasticity among both subjects with and without T1D. The lack of any gender difference in vascular elasticity in our subjects could be because we included only subjects with relatively well controlled T1D and the duration of diabetes was not long enough for them to manifest these subtle changes. A study by Ahlgren et al revealed higher stiffness of the central elastic arteries like aorta and common carotid artery compared to muscular arteries like the femoral artery in women with T1D compared to men with T1D. This gender difference was not observed in their control population without T1D [22]. Though these patients also had good control of their diabetes, they were older (between 21-61 years of age with an average duration of diabetes for almost 19 years. Endothelial dysfunction is closely associated with development of diabetic complications including diabetic nephropathy and retinopathy[23]. Though diabetes status has been shown to abolish the vascular protective effect of estrogen in female rats [24], this effect has not yet been documented in humans. Additional research needs to be done to study the gender differences in vascular properties in humans.

Limitations

We included only T1D subjects who had relatively good control of their diabetes and thus these data can't be generalized to those with poor control. However, the impact of poor control of diabetes on microvascular and macrovascular complications is already well-known and has been studied in large multi-centric trials. The small sample size is another limitation. However, the fact that the gender difference in body composition was seen only in the population with diabetes and not in the control population is interesting, and needs to be corroborated further in larger studies. Additionally, the menstrual phase of the female adolescents is not known in relation to their participation in the study. The effect of sex steroids on plasma lipids is questionable based on recent review of data[25]. However, all participants were Tanner stage 3 or above when they were enrolled in the study.

Conclusion and Clinical Significance

Female adolescents and young adults with T1D have more centrally distributed fat than their male counterparts, which may contribute to their relatively higher cardiovascular risk. Attenuation of the central distribution of fat through exercise and dietary modifications may help ameliorate their subsequent cardiovascular disease burden.

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Table 1

Demographic Data

Variable	T1D Females n=10	T1D Males n=19	p value	Healthy Females n=18	Healthy males n=19	p value
Age	17.3 (13.2-20.3)	15 (13.1-19.3)	0.09	15.1 (13.1-20.5)	15.4 (13.3-20.3)	0.89
BMI	24.7 ± 3.62 (18.0-28.8)	24.69 ± 5.68 (17.0-37.3)	0.91	27.53 (18.5-44.3)	30.51 (18.5-44.35)	0.39
HbA1C	8.59 ± 1.24	8.49 ± 1.18	0.69	5.24 ± 0.25	5.34 ± 0.29	0.28

Normally distributed parameters are presented as mean ± S D while parameters not having a normal distribution are presented as median with ranges in parenthesis; HDL: High Density Cholesterol; LDL: Low density cholesterol

Table 2

Body Composition and Lipid Parameters

Variable	T1D Females n=10	T1D Males n=19	p value	Healthy Females n=18	Healthy males n=19	p value
Trunk Fat (kg)	9.3 (4.2-16.1)	5.2 (1.6 - 19.2)	0.09	11.4 (2.6 - 23.8)	10.9 (1.9 - 31.1)	0.96
Trunk %Fat	30 (18.2-41.5)	15.6 (9.7 - 39.2)	<0.01	36.2 (10.2 - 48.3)	27.5 (8.9 - 52.5)	0.22
Triglyceride (mg/dl)	82.5 (36-141)	46.0 (32-173)	0.25	70.0(31-189)	92.5(50-191) *	0.23
HDL (mg/dl)	49.0(43-58)	38 (29-64)	0.28	40.0 (19.0-60.0)	35.5 (21-55) *	0.08
Total cholesterol (mg/dl)	170.3 ± 22.6	151.6 ± 32.5	0.12	159.6 ± 43.9	139 ± 25.5 *	0.1
LDL cholesterol (mg/dl)	97.0 (80-133)	91 (63-176)	0.18	90 (69-225)	82 (49-112) *	0.35

* n=18; Normally distributed parameters are presented as mean ± S.D while parameters not having a normal distribution are presented as median with ranges in parenthesis; HDL: High Density Cholesterol; LDL: Low density cholesterol

Table 3

Vascular Measures

Variable	T1D Females n=10	T1D Males n=19	p value	Healthy Females n=18	Healthy Males n=19	p value
DBP (mm/Hg)	63 ± 6	62 ± 8	0.65	61 ± 6	59 ± 8	0.53
SBP (mm/Hg)	110 (105-124)	117 (108-138)	0.18	114 (94-134)	116 (101-137)	0.34
LAEI	16 ± 4.6	17.4 ± 4.6	0.44	16.2 ± 3.5	18.3 ± 7.2	0.29
SAEI	7.1 (5.4-28.2)	9.9 (3.1-14.5)	0.12	8.8 (4.2-12.7)	11.0(5.4-14.4)	0.07

LAEI: Large Artery Elasticity Index; SAEI: Small Artery Elasticity Index; Normally distributed parameters are presented as mean ± S.D while parameters not having a normal distribution are presented as median with ranges in parenthesis; n=18