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Free Fatty Acids are Associated with Pulse Pressure in Women, but not Men, with Type 1 Diabetes

Baqiyah Conway, PhD,

3512 Fifth Ave, 2nd Fl, Pittsburgh, PA 15213, 412-383-1112, The University of Pittsburgh,
Department of Epidemiology

Rhobert W Evans, PhD,

502 Parran Hall, 130 DeSoto St, Pittsburgh, PA 15213, 412-624-2020

Linda Fried, MD MPH,

VA Pittsburgh Healthcare System, University Drive Division, Mailstop 111F-U, Pittsburgh, PA
15240, 412-360-6000

Sheryl Kelsey, PhD,

A525 Crabtree Hall, 130 DeSoto St, Pittsburgh, PA 15213, 412-624-5157

Daniel Edmundowicz, MS MD FACC, and

Cardiovascular Institute, University of Pittsburgh Medical Center, Pittsburgh, PA 15213,
412-802-3014

Trevor J Orchard, MD, M. Med. Sci

3512 Fifth Ave, 2nd Fl, Pittsburgh, PA 15217, OrchardT@edc.pitt.edu, Tel: 412-383-1032; Fax:
412-383-1020

Abstract

Background—Cardiovascular disease (CVD) is the leading cause of death in type 1 diabetes (T1D). Pulse pressure, a measure of arterial stiffness, is elevated in T1D and associated CVD. Free fatty acids (FFA), elevated in women and abdominal adiposity, are also elevated in T1D and CVD. We thus examined the association of fasting FFA with pulse pressure and coronary artery calcification (CAC—a marker of coronary atherosclerotic burden) in an adult population (n=150) of childhood onset T1D and whether any such associations varied by abdominal adiposity and gender.

Methods—Mean age and diabetes duration were 42 and 33 yrs, respectively when CAC, visceral adiposity (VAT), and subcutaneous abdominal adiposity (SAT) were determined by electron beam tomography. FFA were determined by in vitro colorimetry. Pulse pressure was calculated as SBP minus DBP. FFA were log-transformed before analyses and all analyses were controlled for serum albumin.

Results—FFA were associated with pulse pressure in women ($r=0.24$, $p=0.04$), but not in men ($r=0.07$, $p=0.55$). An interaction for the prediction of pulse pressure was noted between FFA and both VAT ($p=0.03$) and SAT ($p=0.008$) in women, but only a marginal interaction with SAT ($p=0.09$) and no interaction for VAT ($p=0.40$) with FFA observed in men (see Figure). In multivariable linear

Correspondence to: Trevor J Orchard.

The Internal Review Board of the University of Pittsburgh approved this research and all participants provided informed consent.

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regression analysis allowing for serum albumin, age, height, heart rate, albumin excretion rate, HbA1c, HDLc, and hypertension medication use, FFA, SAT, and the interaction between FFA and SAT, the interaction between FFA and SAT remained associated with pulse pressure in women (FFA $p=0.04$, interaction term $p=0.03$), but not men (FFA $p=0.32$, interaction term $p=0.32$). FFA showed no association with log-transformed CAC.

Conclusion—Though FFA were not associated with CAC in either gender, they were associated with pulse pressure in women and their effect appeared to vary by abdominal adiposity, particularly SAT. This finding might help to explain the loss of the gender difference in CVD in T1D.

Introduction

Free fatty acids (FFAs) are known to be elevated in type 1 diabetes and obesity, particularly abdominal obesity (1,2), and to be associated with insulin resistance (3). Insulin resistance in type 2 diabetes and in the general population is associated with a markedly increased risk of coronary artery disease (4,5). However, T1D is a disease characterized by an abnormality in fuel utilization. Both intermittent insulin deficiency/absence and excess are characteristic of this disease, and both contribute to excess free fatty acid production; therefore, the adiposity-FFA relationship observed in the general population may be very different in T1D.

Coronary artery calcification (CAC) is a subclinical marker of coronary artery disease. In the Epidemiology of Diabetes Complications (EDC) Study, inverse and non-existent relationships were observed between the severity of coronary artery calcification and abdominal fat (6). In type 1 diabetes it is uncertain to what extent calcification of the coronary arteries is due to atherosclerosis of the intima layer of the arterial wall (which may in part relate to obesity and other cardiovascular risk factors) or calcification of the medial layer, perhaps due to abnormal calcium metabolism. Another subclinical measure of cardiovascular disease is arterial stiffness. All these measures are likely strongly interrelated. Sutton-Tyrrell (7) observed a direct relationship between visceral abdominal fat with arterial stiffness in a non-diabetes population; however, little is known about this association in type 1 diabetes. As FFAs have been postulated to be a mediator of insulin resistance and are predictive of ischemic heart disease (8), cardiac arrhythmias (9,10) and sudden cardiac death (11) in the general population, the elevated levels of free fatty acids observed in type 1 diabetes may also help to explain the greatly increased risk of CAD in this population.

The purpose of this study was to assess the association of free fatty acids with coronary artery calcification and arterial stiffness (pulse pressure) in an adult population of childhood onset type 1 diabetes and to determine whether any such association appeared to be mediated by body fat. As abdominal body fat and free fatty acids are known to vary by gender, these analyses are investigated gender-specifically. To our knowledge, this has not been investigated in type 1 diabetes.

Methods

The EDC study is an ongoing study examining the long term complications of T1D in 658 individuals diagnosed before the age of 17 years with T1D at Children's Hospital of Pittsburgh between 1950 and 1980. This current report is based on a subset ($n=210$) of this population who had free fatty acid measurements and underwent electron beam tomography (EBT), VAT, subcutaneous abdominal fat (SAT) via EBT scanning as part of the Insulin Resistance Study, a substudy of the 16-year follow-up, one hundred fifty of whom were also fasting. These fasting participants form the basis of the main analyses.

Fasting blood samples were assayed for lipids, lipoproteins, and hemoglobin A1c. High-density lipoprotein (HDL) cholesterol was determined by a heparin and manganese procedure, a

modification of the Lipid Research Clinics method (12). Cholesterol was measured enzymatically (13). FFA were measured using the colorimetric method (Wako Pure Chemical Industries, Ltd). Urinary albumin was determined immuno-nephelometrically (14).

CAC was measured using EBT (GE-Imatron C-150, Imatron, South San Francisco, CA). Threshold calcium determination was set using a density of 130 Hounsfield units in a minimum of 2 contiguous sections of the heart. Scans were triggered by ECG signals at 80% of the R-R interval. CAC volume scores were calculated based on isotropic interpolation (15). Direct measurements of abdominal adiposity (visceral and subcutaneous abdominal adipose tissue surface area) were also taken by EBT scanning. Scans of abdominal adipose tissue were taken between the fourth and fifth lumbar regions, which were located by counting from the first vertebra below the ribs. Two 10 mm thick scans were taken during suspended respiration. The images were then analyzed using commercially available software for all pixels corresponding to fat density in Hounsfield units in the appropriate anatomical distribution (subcutaneous or visceral). Height was measured using a stadiometer.

Blood pressure was measured by a random-zero sphygmomanometer according to a standardized protocol (16) after a 5-minute rest period. Blood pressure levels were analyzed, using the mean of the second and third readings. Brachial pulse pressure was calculated (systolic blood pressure-diastolic blood pressure).

The student's *t* test was used to compare characteristics of study participants by fasting status. Further analyses were limited to participants providing fasting blood samples. Pearson correlations were used to assess the relationship between FFA, brachial pulse pressure, and coronary artery calcification, adiposity indices, height, heart rate, glycemia indices, and albumin excretion rate. FFA, VAT, SAT, BMI, coronary artery calcification, and albumin excretion rate were log-transformed before analyses. Multiple linear regression analyses with backward elimination was used to determine independent predictors of pulse pressure and coronary artery calcification. All analyses with FFA were adjusted for serum albumin as FFA travel in serum bound to albumin. FFA and serum albumin were forced into all multivariable models. Analyses were conducted using SAS version 9.1.3 (Cary, North Carolina). All procedures were approved by the Institutional Review Board of the University of Pittsburgh and informed and all participants provided informed consent.

Results

Twenty-nine percent of the sixteenth-year follow-up exam study participants provided non-fasting blood samples. Table 1 shows the characteristics of the study participants by fasting status. Overall, there were no differences by fasting status, with the exception of age and FFA levels (41.7 vs. 44.5 years, $p=0.02$, 0.99 vs. 0.84 mmol/l, $p=0.04$ in fasters vs. non-fasters, respectively, data not shown). However, upon gender-specific examination, this age difference was found to be only in men. Fasting men were approximately five years younger than non-fasters (41.0 vs. 45.8, $p=0.004$). By contrast, there was no difference in age in women able or willing to come in for the exam fasting (42.5 vs. 43.5, $p=0.56$); however, fasting women, but not men, had significantly higher free fatty acid levels than non-fasters. There were no other differences by fasting status in men and women. The remaining analyses are restricted to the 150 fasting participants.

Table 2 shows the gender-specific Pearson correlations between free fatty acids, pulse pressure, coronary artery calcification, adiposity and glycemia indices, height, heart rate, and albumin excretion rate. Free fatty acids were positively correlated with pulse pressure in women ($r=0.24$, $p=0.04$) but not men ($r=0.07$, $p=0.55$), but showed no association with coronary artery calcification in either gender. Free fatty acids were associated with fasting glucose in both men

and women, but showed no association with HbA1c, albumin excretion rate, heart rate, height, or any of the adiposity indices. Correlates of pulse pressure and coronary artery calcification are also presented in Table 2.

Figures 1 and 2 show the Pearson's correlation of free fatty acids with pulse pressure by tertiles of gender-specific subcutaneous and visceral abdominal adiposity, respectively. An interaction was observed between free fatty acids and subcutaneous adiposity for pulse pressure in both men ($p=0.09$) and women ($p=0.008$), although only marginal in men. An interaction was also seen between free fatty acids and visceral abdominal adiposity in women ($p=0.03$), but not in men ($p=0.40$). Interactions between free fatty acids and adiposity were not observed for coronary artery calcification.

After multivariable linear regression analyses with backward selection, free fatty acids remained significantly associated with pulse pressure in women, but not in men. After adding the interaction term between free fatty acids and subcutaneous adiposity to the final model, the interaction term was significant in women ($p=0.03$), but not in men ($p=0.32$) (Table 3).

Table 4 shows the multivariable linear regression analyses, with backward selection, of free fatty acids with coronary artery calcification. Free fatty acids were not associated with coronary artery calcification in either gender.

Discussion

The major finding of this study was that free fatty acids are associated with pulse pressure in women, but not coronary artery calcification in either gender in type 1 diabetes. We also observed that the relationship of free fatty acids with pulse pressure varied by level of subcutaneous and visceral abdominal adiposity. Finally we note that although abdominal fat is not associated with free fatty acids in type 1 diabetes it does appear to modify the relationship between free fatty acids and arterial stiffness in women with type 1 diabetes.

Pulse pressure, the difference between the systolic and diastolic blood pressure, is a measure of arterial distensibility, or stiffness. We found that free fatty acids were associated with an increase in brachial pulse pressure. Although little is known about the relationship of free fatty acids and arterial stiffness, Steinberg et al (17) found that free fatty acids caused endothelial dysfunction in healthy individuals. Nakayama et al (18) found that abnormal free fatty acid metabolism was associated with diastolic, but not systolic, dysfunction in individuals with essential hypertension. Free fatty acids account for a substantial proportion of the counterregulatory defense against hypoglycemia (19), a known player in endothelial dysfunction and cardiac ischemia. Acute hypoglycemia causes an increase in systolic blood pressure and a decrease in diastolic blood pressure, and therefore an increase in pulse pressure (20,21). Although mean fasting glucose levels in our study were well out of the hypoglycemia range, preclinic nocturnal hypoglycemia and subsequent counterregulation cannot be ruled out.

The free fatty acid associated increase in pulse pressure may lead to cardiac arrhythmias, ischemia, and sudden cardiac death. In the Framingham Heart Study, pulse pressure, but not mean arterial pressure, significantly predicted atrial fibrillation (22). In patients with type 2 diabetes, Paolisso et al (9) observed ventricular premature complexes to increase with increasing free fatty acid concentration and to decrease when free fatty acids were directly lowered. The increased free fatty acids accompanying hypoglycemia counterregulation or very low to absent insulin levels in type 1 diabetes may also increase tissue ischemia. In nondiabetic men, elevated levels of free fatty acids were associated with ischemic heart disease (8). Elevated levels of free fatty acids have also been associated with sudden cardiac death (11,23). Free fatty acid inundation of the myocardium is observed in Acute Coronary Syndromes and the better outcomes in patients in the first DIGAMI study (24,25) randomized to insulin treatment

may be due to insulin's suppression of free fatty acid release into the circulation. In the EDC population, low daily insulin dose at baseline, but not HbA1c, was independently predictive of the 18 year incidence of non-fatal coronary artery disease.

In our population, the adiposity relationship of pulse pressure with free fatty acids varied by gender. In the general population as well, a gender difference exists in adiposity, particularly visceral adiposity, and insulin resistance. Visceral adiposity and insulin resistance are both higher in men; nevertheless, free fatty acids tend to be slightly increased in women (26). Gender differences among the general population are also observed in lipid metabolism (26). Women, who store a greater proportion of FFA in subcutaneous adipose tissue (27), particular upper body subcutaneous adipose tissue, compared to men, have a greater upper body FFA response to catecholamines (28) and an increased free fatty acid response to fasting (29), while fasting glucose levels tend to be lower. Hojlund et al (30) observed during 72 hours of fasting mean plasma free fatty acids were higher in women while mean glucose levels were lower in women throughout the duration of the fast. After approximately 36 hours of fasting, mean glucose levels were approximately 3.5 mmol/l (~63mg/dl) in women, while they remained at approximately 4 mmol/l (~72mg/dl) or above in men. Similar findings were noted by Soeters et al (31). A gender difference in the counterregulatory response to hypoglycemia also exists both in the non-diabetic population (32,33,34) and in type 1 diabetes (35). Women have a reduced sympathetic nervous system response to hypoglycemia (36). This decreased epinephrine, norepinephrine, growth hormone, and subsequent endogenous glucose production response to declining glucose levels would be expected to produce a greater frequency of hypoglycemia in women with type 1 diabetes. However, men experience a greater blunting of the autonomic nervous system counterregulatory responses to hypoglycemia following antecedent hypoglycemia (36). Although the tightened glycemic control achieved in the intensive arm of the Diabetes Control and Complications Trial (DCCT) came at the expense of a three-fold increase in severe hypoglycemic events, there was no difference in the prevalence of hypoglycemia between men and women (37). Although women have decreased catecholamine response to hypoglycemia, they have an increased FFA response to catecholamines and the enhanced free fatty acid response to hypoglycemia in women (29) may account for the resistance women exhibit to the blunting effects of antecedent hypoglycemia. Nevertheless, this may come at the expense of the increased stiffness observed in women with type 1 diabetes (38,39) and may partially account for the loss of the gender difference in coronary artery disease in type 1 diabetes.

Free fatty acids themselves, although related with pulse pressure, failed to show a relationship with coronary artery calcification. We have previously suggested that the coronary artery calcification in type 1 diabetes might not simply reflect the obesity/lipid driven atherosclerosis, i.e. the "traditional" product of atherosclerosis in response to abnormal lipoprotein levels, but may also reflect advanced glycation end products (AGEs) in the subendothelial matrix or the vascular medial layer (6). These findings suggest two distinct mechanisms of calcium deposition in the coronary arteries in type 1 diabetes. One may be related to the progressive lipoprotein associated plaque accumulation and inflammatory response as seen in obesity driven insulin resistance. We have previously shown body mass index to predict progression of coronary artery calcification in type 1 diabetes (40). The second may represent AGEs associated with poor glycemic control and potentially associated with clinical cardiovascular events. As we have also recently observed a strong relationship between skin fluorescence (a marker of AGEs) and CAC (41), both processes are likely to play a role.

In conclusion, free fatty acids predict arterial stiffness in women with type 1 diabetes, but do not predict CAC in either gender. As both low insulin dose and hypoglycemia increase the free fatty acid flux in type 1 diabetes, these findings may help to explain the inconsistent, and generally null, findings of a relationship of HbA1c and coronary artery disease in type 1

diabetes, particularly in observational studies (42). Given the recent failure of clinical trials to show a cardiovascular benefit, and in one study, an adverse association, of intensive glycemic control in diabetes (43,44), the results of our study may have important clinical implications. Both hypoglycemia and hyperglycemia need to be monitored, not just HbA1c, in order to avoid elevated free fatty acid flux to the myocardium and kidney and the subsequent myocardial damage.

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Figure 1.
Association of Free Fatty Acids (FFA) with Pulse Pressure by Tertiles of Subcutaneous Abdominal Adiposity (SAT).

Figure 2.
Association of Free Fatty Acids (FFA) with Pulse Pressure by Tertiles of Visceral Abdominal Adiposity (VAT).

Table 1Characteristics of Study Participants by Fasting Status at 16th Year Follow-up Exam, mean (SD)

	Men (n=101)		Women (n=109)	
	Fasting (n=74)	Non-fasting (n=27)	Fasting (n=76)	Non-fasting (n=33)
Age, years	41.0 (6.9) ‡	45.8 (7.5)	42.5 (8.0)	43.5 (8.5)
VAT [*] , cm ²	113.8 (66.5)	138.2 (68.6)	75.2 (52.4)	75.2 (43.9)
SAT [*] , cm ²	241.0 (387.5)	203.1 (76.2)	255.1 (138.6)	300.5 (434.9)
BMI [*] , kg/m ²	26.9 (3.9)	27.0 (3.5)	26.2 (5.0)	25.6 (4.6)
FFA [*] , mmol/l	0.95 (0.50)	0.86 (0.38)	1.02 (0.48) †	0.82 (0.48)
HbA1c, %	8.0 (1.4)	8.2 (1.6)	7.9 (1.3)	7.3 (1.2)
Glucose, mg/dl	159.7 (89.1)	188.9 (98.5)	158.1 (83.4)	164.5 (83.9)
Dose, U/kg/dy	0.67 (0.21)	0.68 (0.23)	0.58 (0.21)	0.57 (0.20)
AER [*] , µg/min	220.0 (698.1)	94.3 (182.4)	99.0 (397.5)	52.0 (170.8)
Height, meters	174.5 (6.1)	174.7 (7.5)	162.4 (6.7)	161.2 (8.4)
Heart rate, beats/min	73.6 (11.5)	75.4 (9.6)	76.6 (11.6)	77.0 (13.2)
CAC score [*]	169.7 (358.2)	252.0 (512.4)	229.3 (529.5)	154.4 (259.7)
Pulse Pressure, mmHg	50.8 (14.8)	54.2 (15.0)	50.2 (14.2)	51.9 (13.5)

* Natural logarithmically transformed before analyses

†
p<0.05‡
p<0.01

VAT=visceral abdominal adiposity, SAT=subcutaneous abdominal adiposity, BMI=body mass index, FFA=free fatty acids, AER=albumin excretion rate, CAC=coronary artery calcification

Table 2

Association of Free Fatty Acids (FFA) with Pulse Pressure (PP), Coronary Artery Calcification (CAC), Adiposity indices, and Glycemia.
Pearson's r (p-value)

	Men (n=74)			Women (n=76)		
	FFA	PP	CAC	FFA	PP	CAC
Pulse Pressure, mmHg	0.07 (0.55)			0.24 (0.04)		
CAC score *	0.14 (0.25)	0.34 (0.003)		0.04 (0.74)	0.38 (0.0006)	
VAT *, cm ²	0.15 (0.22)	0.40 (0.0005)	0.40 (0.005)	0.10 (0.38)	0.20 (0.09)	0.18 (0.12)
SAT *, cm ²	0.17 (0.15)	0.26 (0.03)	0.24 (0.04)	0.06 (0.63)	0.14 (0.23)	0.03 (0.80)
BMI *, kg/m ²	0.19 (0.10)	0.17 (0.13)	0.22 (0.06)	0.09 (0.41)	0.17 (0.13)	0.03 (0.77)
HbA1c, %	0.14 (0.24)	-0.01 (0.93)	-0.03 (0.78)	0.02 (0.89)	0.19 (0.10)	0.001 (0.99)
Insulin dose, U/kg/dy **	0.25 (0.08)	0.25 (0.07)	0.11 (0.41)	-0.14 (0.35)	-0.39 (0.006)	-0.07 (0.63)
Glucose, mg/dl	0.33 (0.005)	0.05 (0.66)	-0.10 (0.41)	0.49 (<0.0001)	0.23 (0.05)	0.11 (0.33)
Triglycerides, mg/dl *	0.39 (0.0007)	0.15 (0.20)	0.30 (0.01)	0.04 (0.74)	0.13 (0.26)	0.22 (0.05)
HDLc, mg/dl	0.02 (0.85)	-0.02 (0.85)	-0.21 (0.07)	0.09 (0.44)	0.16 (0.17)	-0.01 (0.91)
eGDR, mg/kg/min	-0.17 (0.18)	-0.37 (0.002)	-0.17 (0.16)	0.10 (0.40)	-0.41 (0.0004)	-0.18 (0.13)
AER, µg/min *	0.14 (0.25)	0.29 (0.01)	0.13 (0.28)	-0.08 (0.50)	0.35 (0.002)	0.21 (0.07)
Height, meters	0.14 (0.24)	0.11 (0.35)	0.09 (0.45)	0.01 (0.93)	0.12 (0.33)	-0.11 (0.35)
Heart rate, beats/min	0.13 (0.27)	0.04 (0.71)	-0.13 (0.27)	-0.02 (0.83)	0.22 (0.05)	0.01 (0.90)

Analyses with FFA are controlled for serum albumin HDLc=high density lipoprotein cholesterol AER=albumin excretion rate

* Natural logarithmically transformed before analysis

** n=55 for men and 47 for women

Table 3

Multivariable Adjusted Association of Free Fatty Acids with Pulse Pressure in Type 1 Diabetes: The Epidemiology of Diabetes Complications Study

	Males (n=70)	Females (n=74)
	B ± SE (p-value)	B ± SE (p-value)
Interaction between FFA* and SAT*	-16.23 ± 39.4 (0.68)	9.07 ± 4.14 (0.03)
Serum albumin, g/dl	-3.44 ± 2.83 (0.23)	-4.36 ± 2.32 (0.06)
Age, years	0.95 ± 0.25 (0.0003)	0.60 ± 0.17 (0.0007)
Height, meters	NS	0.40 ± 0.19 (0.04)
Heart rate, beats/min	0.29 ± 0.14 (0.04)	2.50 ± 0.72 (0.0009)
Hypertension medication use	10.48 ± 3.74 (0.007)	NS
FFA	94.5 ± 61.8 (0.13)	-100.36 ± 48.9 (0.04)
SAT	-8.36 ± 5.40 (0.13)	4.26 ± 2.21 (0.06)
<i>Model R-square</i>	0.41	0.45

* Natural logarithmically transformed before analysis NS=not selected FFA=free fatty acids SAT=subcutaneous abdominal adiposity Backward selection model controlled for free fatty acids and subcutaneous abdominal adiposity and also allowed for heart rate, HbA1c, high density lipoprotein cholesterol and hypertension medication use.

Table 4

Multivariable Adjusted Association of Free Fatty Acids with Coronary Artery Calcification in Type 1 Diabetes: The Epidemiology of Diabetes Complications Study

	Males (n=70)	Females (n=74)
	B ± SE (p-value)	B ± SE (p-value)
Free fatty Acids* (mmol/l)	-0.56 ± 0.48 (0.25)	-0.60 ± 0.51 (0.24)
Serum albumin, g/dl	-0.65 ± 0.48 (0.18)	-0.18 ± 0.40 (0.65)
Age, years	0.21 ± 0.04 (<0.0001)	0.24 ± 0.03 (<0.0001)
High density lipoprotein cholesterol, mg/dl	-0.07 (0.002) ± 0.02	-0.03 ± 0.02 (0.05)
<i>Model R-square</i>	0.36	0.48

* Natural logarithmically transformed before analysis NS=not selected Backward selection model also allowed for HbA1c, log-transformed subcutaneous adiposity, and log-transformed albumin excretion rate. Results did not vary in log-transformed visceral adiposity was used in place of log-transformed subcutaneous adiposity.