Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 1215–1221
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Free fatty acids are associated with pulse pressure in women, but not men, with type 1 diabetes mellitus

Baqiyyah Conway[a], Rhobert W. Evans[a], Linda Fried[b], Sheryl Kelsey[a], Daniel Edmundowicz[c], Trevor J. Orchard[a,*]

[a]*Department of Epidemiology, The University of Pittsburgh, Pittsburgh, PA 15213, USA*
[b]*VA Pittsburgh Healthcare System, University Drive Division, Pittsburgh, PA 15240, USA*
[c]*Cardiovascular Institute, University of Pittsburgh Medical Center, Pittsburg, PA 15213, USA*

Received 15 January 2009; accepted 27 March 2009

Abstract

Cardiovascular disease (CVD) is the leading cause of death in type 1 diabetes mellitus (T1D). Pulse pressure, a measure of arterial stiffness, is elevated in T1D and associated with CVD. Free fatty acids (FFAs), elevated in women and abdominal adiposity, are also elevated in T1D and CVD. We thus examined the association of fasting FFAs 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 sex. Mean age and diabetes duration were 42 and 33 years, respectively, when CAC, visceral abdominal adiposity (VAT), and subcutaneous abdominal adiposity (SAT) were determined by electron beam tomography. Free fatty acids were determined by in vitro colorimetry. Pulse pressure was calculated as systolic blood pressure minus diastolic blood pressure. Free fatty acids were log transformed before analyses, and all analyses were controlled for serum albumin. Free fatty acids were associated with pulse pressure in women ($r = 0.24$, $P = .04$), but not in men ($r = 0.07$, $P = .55$). An interaction for the prediction of pulse pressure was noted between FFAs and both VAT ($P = .03$) and SAT ($P = .008$) in women, but only a marginal interaction with SAT ($P = .09$) and no interaction for VAT ($P = .40$) with FFAs were observed in men. In multivariable linear regression analysis allowing for serum albumin, age, height, heart rate, albumin excretion rate, hemoglobin A_{1c}, high-density lipoprotein cholesterol, hypertension medication use, FFAs, SAT, and the interaction between FFAs and SAT, the interaction between FFAs and SAT remained associated with pulse pressure in women (FFAs, $P = .04$; interaction term, $P = .03$), but not men (FFAs, $P = .32$; interaction term, $P = .32$). FFAs showed no association with log-transformed CAC. Although FFAs were not associated with CAC in either sex, they were associated with pulse pressure in women and their effect appeared to vary by abdominal adiposity, particularly SAT. This finding might help explain the loss of the sex difference in CVD in T1D.

1. Introduction

Free fatty acids (FFAs) are known to be elevated in type 1 diabetes mellitus (T1D) and obesity, particularly abdominal obesity [1,2], and to be associated with insulin resistance [3]. Insulin resistance in type 2 diabetes mellitus 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 FFA 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 nonexistent relationships were observed between the severity of CAC and abdominal fat [6]. In T1D, 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

The authors have no conflict of interest to declare.

* Corresponding author. Tel.: +1 412 383 1032; fax: +1 412 383 1020.
E-mail address: orchardt@edc.pitt.edu (T.J. Orchard).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.03.010

stiffness. All these measures are likely strongly interrelated. Sutton-Tyrrell et al [7] observed a direct relationship between visceral abdominal fat with arterial stiffness in a nondiabetes population; however, little is known about this association in T1D. 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 FFAs observed in T1D may also help explain the greatly increased risk of coronary artery disease in this population.

The purpose of this study was to assess the association of FFAs with CAC and arterial stiffness (pulse pressure) in an adult population of childhood-onset T1D and to determine whether any such association appeared to be mediated by body fat. As abdominal body fat and FFAs are known to vary by sex, these analyses are investigated sex specifically. To our knowledge, this has not been investigated in T1D.

2. 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 FFA measurements and underwent electron beam tomography (EBT) scanning of visceral abdominal fat and subcutaneous abdominal fat as part of the Insulin Resistance Study, a substudy of the 16-year follow-up, 150 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 A_{1c} (HbA_{1c}). High-density lipoprotein cholesterol (HDLc) was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method [12]. Cholesterol was measured enzymatically [13]. Free fatty acids were measured using the colorimetric method (Wako Pure Chemical Industries, Osaka, Japan). Urinary albumin was determined immunonephelometrically [14].

Coronary artery calcification 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 electrocardiographic signals at 80% of the R-R interval. Coronary artery calcification 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 *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 CAC, and adiposity indices, height, heart rate, glycemia indices, and albumin excretion rate. Free fatty acids, visceral abdominal adiposity (VAT), subcutaneous abdominal adiposity (SAT), BMI, CAC, 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 CAC. All analyses with FFAs were adjusted for serum albumin, as FFAs travel in serum bound to albumin. Free fatty acids and serum albumin were forced into all multivariable models. Analyses were conducted using SAS (Cary, NC) version 9.1.3. All procedures were approved by the Institutional Review Board of the University of Pittsburgh, and all participants provided informed consent.

3. Results

Twenty-nine percent of the 16th-year follow-up examination study participants provided nonfasting 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, $P = .02$, 0.99 vs 0.84 mmol/L, $P = .04$ in fasters vs nonfasters, respectively; data not shown). However, upon sex-specific examination, this age difference was found to be only in men. Fasting men were approximately 5 years younger than nonfasters (41.0 vs 45.8, $P = .004$). By contrast, there was no difference in age in women able or willing to come in for the examination fasting (42.5 vs 43.5, $P = .56$); however, fasting women, but not men, had significantly higher FFA levels than nonfasters. 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 sex-specific Pearson correlations between FFAs, pulse pressure, and CAC, and adiposity and glycemia indices, height, heart rate, and albumin excretion rate. Free fatty acids were positively correlated with pulse pressure in women ($r = 0.24$, $P = .04$) but not men

Table 1
Characteristics of study participants by fasting status at 16th-year follow-up examination

	Men (n = 101)		Women (n = 109)	
	Fasting (n = 74)	Nonfasting (n = 27)	Fasting (n = 76)	Nonfasting (n = 33)
Age, y	41.0 (6.9)†	45.8 (7.5)	42.5 (8.0)	43.5 (8.5)
VAT[a], cm^2	113.8 (66.5)	138.2 (68.6)	75.2 (52.4)	75.2 (43.9)
SAT[a], cm^2	241.0 (387.5)	203.1 (76.2)	255.1 (138.6)	300.5 (434.9)
BMI[a], kg/m^2	26.9 (3.9)	27.0 (3.5)	26.2 (5.0)	25.6 (4.6)
FFA[a], mmol/L	0.95 (0.50)	0.86 (0.38)	1.02 (0.48)*	0.82 (0.48)
HbA_{1c}, %	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 d)	0.67 (0.21)	0.68 (0.23)	0.58 (0.21)	0.57 (0.20)
AER[a], μg/min	220.0 (698.1)	94.3 (182.4)	99.0 (397.5)	52.0 (170.8)
Height, m	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[a]	169.7 (358.2)	252.0 (512.4)	229.3 (529.5)	154.4 (259.7)
Pulse pressure, mm Hg	50.8 (14.8)	54.2 (15.0)	50.2 (14.2)	51.9 (13.5)

Mean (SD). AER indicates albumin excretion rate.
[a] Natural logarithmically transformed before analyses.
* $P < .05$.
† $P < .01$.

($r = 0.07$, $P = .55$), but showed no association with CAC in either sex. Free fatty acids were associated with fasting glucose in both men and women, but showed no association with HbA_{1c}, albumin excretion rate, heart rate, height, or any of the adiposity indices. Correlates of pulse pressure and CAC are also presented in Table 2.

Figs. 1 and 2 show the Pearson correlation of FFAs with pulse pressure by tertiles of sex-specific SAT and VAT, respectively. An interaction was observed between FFAs and subcutaneous adiposity for pulse pressure in both men ($P = .09$) and women ($P = .008$), although it was only marginal in men. An interaction was also seen between FFAs and VAT in women ($P = .03$), but not in men ($P = .40$). Interactions between FFAs and adiposity were not observed for CAC.

After multivariable linear regression analyses with backward selection, FFAs remained significantly associated with pulse pressure in women, but not in men. After adding the interaction term between FFAs and subcutaneous adiposity to the final model, the interaction term was significant in women ($P = .03$), but not in men ($P = .32$) (Table 3).

Table 4 shows the multivariable linear regression analyses, with backward selection, of FFAs with CAC. Free fatty acids were not associated with CAC in either sex.

4. Discussion

The major finding of this study was that FFAs are associated with pulse pressure in women, but not CAC in

Table 2
Association of FFAs with pulse pressure, CAC, adiposity indices, and glycemia

	Men (n = 74)			Women (n = 76)		
	FFA	PP	CAC	FFA	PP	CAC
PP, mm Hg	0.07 (.55)			0.24 (.04)		
CAC score[a]	0.14 (.25)	0.34 (.003)		0.04 (.74)	0.38 (.0006)	
VAT[a], cm^2	0.15 (.22)	0.40 (.0005)	0.40 (.005)	0.10 (.38)	0.20 (.09)	0.18 (.12)
SAT[a], cm^2	0.17 (.15)	0.26 (.03)	0.24 (.04)	0.06 (.63)	0.14 (.23)	0.03 (.80)
BMI[a], kg/m^2	0.19 (.10)	0.17 (.13)	0.22 (.06)	0.09 (.41)	0.17 (.13)	0.03 (.77)
HbA_{1c}, %	0.14 (.24)	−0.01 (.93)	−0.03 (.78)	0.02 (.89)	0.19 (.10)	0.001 (.99)
Insulin dose[b], U/kg/d	0.25 (.08)	0.25 (.07)	0.11 (.41)	−0.14 (.35)	−0.39 (.006)	−0.07 (.63)
Glucose, mg/dL	0.33 (.005)	0.05 (.66)	−0.10 (.41)	0.49 (<.0001)	0.23 (.05)	0.11 (.33)
Triglycerides[a], mg/dL	0.39 (.0007)	0.15 (.20)	0.30 (.01)	0.04 (.74)	0.13 (.26)	0.22 (.05)
HDLc, mg/dL	0.02 (.85)	−0.02 (.85)	−0.21 (.07)	0.09 (.44)	0.16 (.17)	−0.01 (.91)
eGDR, mg/kg/min	−0.17 (.18)	−0.37 (.002)	−0.17 (.16)	0.10 (.40)	−0.41 (.0004)	−0.18 (.13)
AER[a], μg/min	0.14 (.25)	0.29 (.01)	0.13 (.28)	−0.08 (.50)	0.35 (.002)	0.21 (.07)
Height, m	0.14 (.24)	0.11 (.35)	0.09 (.45)	0.01 (.93)	0.12 (.33)	−0.11 (.35)
Heart rate, beats/min	0.13 (.27)	0.04 (.71)	−0.13 (.27)	−0.02 (.83)	0.22 (.05)	0.01 (.90)

Pearson r (P value). Analyses with FFA are controlled for serum albumin. PP indicates pulse pressure; eGDR, estimated glucose disposal rate.
[a] Natural logarithmically transformed before analysis.
[b] n = 55 for men and 47 for women.

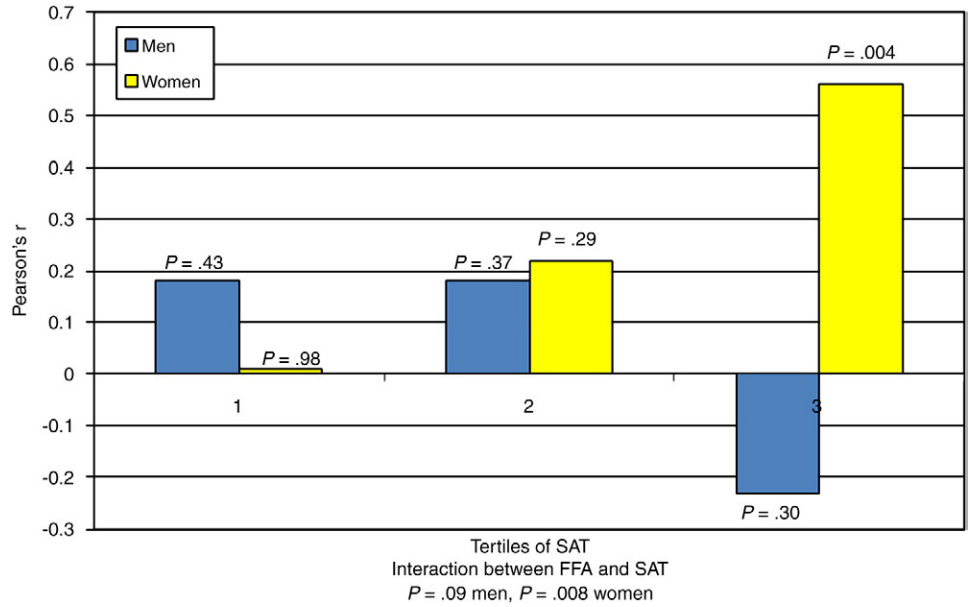

Fig. 1. Association of FFAs with pulse pressure by tertiles of SAT.

either sex, in T1D. We also observed that the relationship of FFAs with pulse pressure varied by level of SAT and VAT. Finally, we note that, although abdominal fat is not associated with FFAs in T1D, it does appear to modify the relationship between FFAs and arterial stiffness in women with T1D.

Pulse pressure, the difference between the systolic and diastolic blood pressure, is a measure of arterial distensibility, or stiffness. We found that FFAs were associated with an increase in brachial pulse pressure. Although little is known about the relationship of FFAs and arterial stiffness, Steinberg et al [17] found that FFAs caused endothelial dysfunction in healthy individuals. Nakayama et al [18] found that abnormal FFA 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

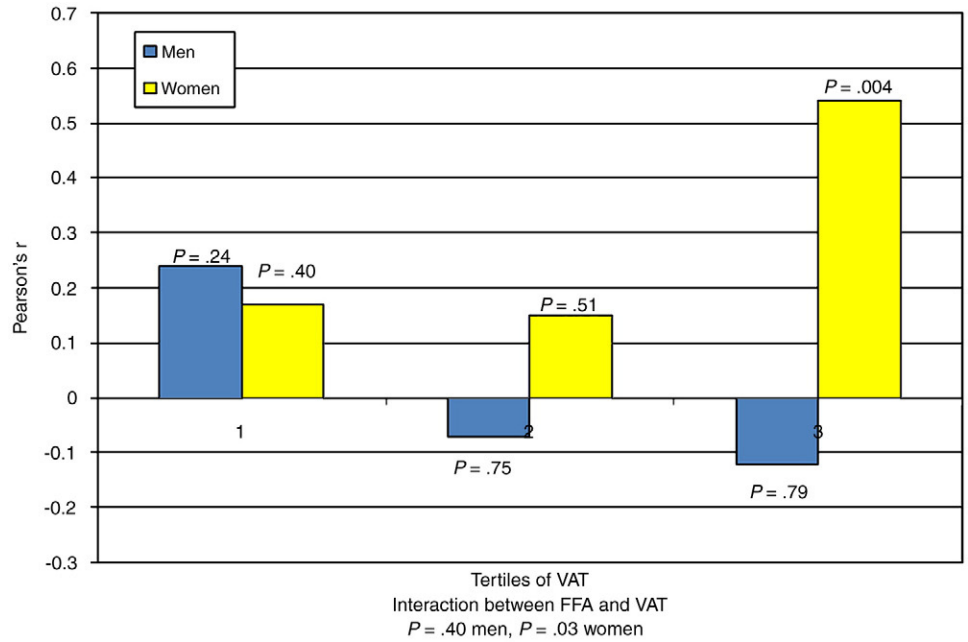

Fig. 2. . Association of FFAs with pulse pressure by tertiles of VAT.

Table 3
Multivariable-adjusted association of FFAs with pulse pressure in T1D: the EDC study

	Men (n = 70)	Women (n = 74)
	$B \pm$ SE (P value)	$B \pm$ SE (P value)
Interaction between FFA[a] and SAT[a]	−16.23 ± 39.4 (.68)	9.07 ± 4.14 (.03)
Serum albumin, g/dL	−3.44 ± 2.83 (.23)	−4.36 ± 2.32 (.06)
Age, y	0.95 ± 0.25 (.0003)	0.60 ± 0.17 (.0007)
Height, m	NS	0.40 ± 0.19 (.04)
Heart rate, beats/min	0.29 ± 0.14 (.04)	2.50 ± 0.72 (.0009)
Hypertension medication use	10.48 ± 3.74 (.007)	NS
FFA	94.5 ± 61.8 (.13)	−100.36 ± 48.9 (.04)
SAT	−8.36 ± 5.40 (.13)	4.26 ± 2.21 (.06)
Model R^2	0.41	0.45

Backward selection model controlled for FFAs and subcutaneous abdominal adiposity and also allowed for heart rate, HbA_{1c}, HDLc, and hypertension medication use. NS indicates not selected.

[a] Natural logarithmically transformed before analysis.

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 FFA-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 mellitus, Paolisso et al [9] observed ventricular premature complexes to increase with increasing FFA concentration and to decrease when FFAs were directly lowered. The increased FFAs accompanying hypoglycemia counterregulation or very low to absent insulin levels in T1D may also increase tissue ischemia. In nondiabetic men, elevated levels of FFAs were associated with ischemic heart disease [8]. Elevated levels of FFAs 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 Diabetes Mellitus Insulin-Glucose Infusion in Acute Myocardial Infarction (DIGAMI) study [24,25] randomized to insulin treatment may be due to insulin's suppression of FFA release into the circulation. In the EDC population, low daily insulin dose at baseline, but not HbA_{1c}, was independently predictive of the 18-year incidence of nonfatal coronary artery disease.

In our population, the adiposity relationship of pulse pressure with FFAs varied by sex. In the general population as well, a sex difference exists in adiposity, particularly visceral adiposity, and insulin resistance. Visceral adiposity and insulin resistance are both higher in men; nevertheless, FFAs tend to be slightly increased in women [26]. Sex differences among the general population are also observed in lipid metabolism [26]. Women, who store a greater proportion of FFAs in subcutaneous adipose tissue [27], particular upper body subcutaneous adipose tissue, compared with men, have a greater upper body FFA response to catecholamines [28] and an increased FFA response to fasting [29], whereas fasting glucose levels tend to be lower. Hojlund et al [30] observed during 72 hours of fasting that mean plasma FFAs were higher in women, whereas 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 (~63 mg/dL) in women, whereas they remained at approximately 4 mmol/L (~72 g/dL) or higher in men. Similar findings were noted by Soeters et al [31]. A sex difference in the counterregulatory response to hypoglycemia also exists both in the nondiabetic population [32-34] and in T1D [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 T1D. However, men experience a greater blunting of the autonomic nervous system counterregulatory responses to hypoglycemia after antecedent hypoglycemia [36]. Although the tightened glycemic control achieved in the intensive arm of the Diabetes Control and Complications Trial came at the expense of a 3-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 FFA 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 arterial stiffness observed in women with T1D [38,39] and may partially account for the loss of the sex difference in coronary artery disease in T1D.

Free fatty acids themselves, although related with pulse pressure, failed to show a relationship with CAC. We have previously suggested that the CAC in T1D might not simply reflect the obesity-/lipid-driven atherosclerosis, that is, the "traditional" product of atherosclerosis in response to

Table 4
Multivariable-adjusted association of FFAs with CAC in T1D: the EDC study

	Men (n = 70)	Women (n = 74)
	$B \pm$ SE (P value)	$B \pm$ SE (P value)
FFAs[a], mmol/L	−0.56 ± 0.48 (.25)	−0.60 ± 0.51 (.24)
Serum albumin, g/dL	−0.65 ± 0.48 (.18)	−0.18 ± 0.40 (.65)
Age, y	0.21 ± 0.04 (<.0001)	0.24 ± 0.03 (<.0001)
HDLc, mg/dL	−0.07 ± 0.02 (.002)	−0.03 ± 0.02 (.05)
Model R^2	0.36	0.48

Backward selection model also allowed for HbA_{1c}, log-transformed subcutaneous adiposity, and log-transformed albumin excretion rate. Results did not vary when log-transformed visceral adiposity was used in place of log-transformed subcutaneous adiposity.

[a] Natural logarithmically transformed before analysis.

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 2 distinct mechanisms of calcium deposition in the coronary arteries in T1D. 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 CAC in T1D [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, FFAs predict arterial stiffness in women with T1D, but do not predict CAC in either sex. As both low insulin dose and hypoglycemia increase the FFA flux in T1D, these findings may help explain the inconsistent, and generally null, findings of a relationship of HbA_{1c} and coronary artery disease in T1D, particularly in observational studies [42]. Given the recent failure of clinical trials to show a cardiovascular benefit, and in 1 study, an adverse association, of intensive glycemic control in diabetes [43-45], the results of our study may have important clinical implications. Both hypoglycemia and hyperglycemia need to be monitored, not just HbA_{1c}, to avoid elevated FFA flux to the myocardium and kidney and the subsequent myocardial damage.

Acknowledgment

This research was supported by National Institutes of Health grant DK34818. The authors would like to thank Beth Hauth for her help in assaying the FFAs. Finally, we would like to thank the EDC study participants for their dedicated participation in this research.

References

[1] Koutsari C, Jensen M. Free fatty acid metabolism and human obesity. J Lipid Res 2006;47:1643-50.
[2] Steinberg H, Gumbiner B. Pathophysiology of obesity and metabolic response to weight loss. In: Gumbiner B, editor. Obesity. Philadelphia: American College of Physicians; 2001. p. 50-66.
[3] Boden G. Gluconeogenesis and glycogenolysis in health and diabetes. J Investig Med 2004;52:375-8.
[4] Grundy S, Cleeman J, Daniels S, Donato K, Eckel R, Franklin B, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation 2005;13:322-7.
[5] Alberti K, Shaw P, the IDF Epidemiology Task Force Consensus Group. The metabolic syndrome—a new worldwide definition. The Lancet 2005;366:1059-62.
[6] Conway B, Miller R, Costacou T, Fried L, Kelsey S, Evans R, et al. Double-edged relationship between adiposity and coronary artery calcification in type 1 diabetes. Diabetes Vasc Dis Res 2007; 4:332-9.
[7] Sutton-Tyrrell K, Newman A, Simonsick EM, Havlik R, Pahor M, Lakatta E, et al. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension 2001;38:429-33.
[8] Pirro M, Mauriege P, Tchernof A, Cantin B, Dagenais G, Despres J, et al. Plasma free fatty acid levels and the risk of ischemic heart disease in men: prospective results from the Quebec Cardiovascular Study. Atherosclerosis 2002:377-84.
[9] Paolisso G, Gualdiero P, Manzella D, Rizzo M, Tagliamonte M, Gambardella A, et al. Association of fasting plasma free fatty acid concentration and frequency of ventricular premature complexes in nonischemic non–insulin dependent diabetic patients. Am J Cardiol 1997;80:932-7.
[10] Kurien V, Yates P, Oliver M. The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion. Eur J Clin Invest 1971;1:225-41.
[11] Pilz S, Scharnag H, Tiran B, Wellnits B, Seelhorst U, Boehm B, et al. Elevated plasma free fatty acids predict sudden cardiac death: a 6.85-year follow-up of 3315 patients after coronary angiography. Eur Heart J 2007;28:2763-9.
[12] National Institute of Health, Department of Health, Education and Welfare. Lipid Research Clinics Program. Washington, DC: US Govt. Printing Office; 1978. 1975 (NIH pub no. 75-628).
[13] Allain C, Poon L, Chan C, Richmond W, Fu P. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20(4):470-5.
[14] Ellis D, Buffone G. A new approach to the evaluation of proteinuric states. Clin Chem 1977;23:666-70.
[15] Callister T, Cooil B, Raya S, Lippolis N, Russo D, Raggi P. Coronary artery disease: improved reproducibility of calcium scoring with an electron-beam CT volumetric method. Radiology 1998;208: 807-14.
[16] Borhani N, Kass E, Langford H, Paynr G, Remington R, Stamler J. The hypertension detection and follow-up program. Prev Med 1976;5: 207-15.
[17] Steinberg H, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, et al. Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J Clin Invest 1998;100:1230-9.
[18] Nakayama H, Morozumi T, Nanto S, Shimonagata T, Ohara T, Takano Y, et al. Abnormal myocardial free fatty acid utilization deteriorates with morphological changes in the hypertensive heart. Jpn Circ J 2001;65:783-7.
[19] Fanelli C, Calderone S, Epifano L, De Vincenzo A, Modarelti F, Pampanelli S, et al. Demonstration of a critical role for free fatty acids in mediating counterregulatory stimulation of gluconeogenesis and suppression of glucose utilization in humans. J Clin Invest 1993;92: 1617-22.
[20] Sommerfield A, Wilkinson I, Webb D, Frier B. Vessel wall stiffness in type 1 diabetes and the central hemodynamic effects of acute hypoglycemia. Am J Physiol Endocrinol Metab 2007: E1274-9.
[21] Wright R, Firer B. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? Diabetes Metab Res Rev 2008;24:353-63.
[22] Mitchell G, Vasan R, Keyes M, Parise H, Wang T, Larson M, et al. Pulse pressure and risk of new-onset atrial fibrillation. JAMA 2007;297:709-15.
[23] Jouven X, Charles M, Desnos M, Ducimetiere P. Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population. Circulation 2001;104:756-61.
[24] Malmberg K, Rydén L, Efendic S, Herlitz J, Nicol P, Waldenström A, et al. Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. J Am Coll Cardiol 1995;26:57-65.
[25] Bhadriraju S, Ray KK, DeFranco AC, Barber K, Bhadriraju P, Murphy SA, et al. Association between blood glucose and long-term mortality in patients with acute coronary syndromes in the OPUS-TIMI 16 trial. Am J Cardiol 2006;97:1573-7.
[26] Mittendorfer B. Sexual dimorphism in human lipid metabolism. J Nutr 2005;135:681-6.

[27] Koutsari C, Snozek C, Jensen M. Plasma NEFA stage in adipose tissue in the postprandial state: sex-related and regional differences. Diabetologia 2008;51:2041-8.
[28] Jensen M, Cyre P, Johnson C, Murray M. Effects of epinephrine on regional free fatty acid and energy metabolism in men and women. Am J Physiol 1996;270:E259-64.
[29] Mittendorfer B, Horowitz JF, Klein S. Gender differences in lipid and glucose kinetics during short-term fasting. Am J Physiol Endocrinol Metab 2001;281:E1333-9.
[30] Hojlund K, Wildner-Christensen M, Eshoj O, Skjarbaek C, Holst J, Koldkjaer O, et al. Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. Am J Physiol Endocrinol Metab 2001;280:E50-8.
[31] Soeters M, Sauerwein H, Groener J, Aerts J, Ackermans M, Glatz J, Fliers E, Serlie M. Gender-related differences in the metabolic response to fasting. J Clin Endocrinol Metab 2007;92:3646-52.
[32] Amiel S, Maran A, Powne J, Umpleby A, MacDonald I. Gender differences in counterregulation to hypoglycemia. Diabetologia 1993;36:460-4.
[33] Davis S, Cherrington A, Goldstein R, Jacobs J, Price L. Effects of insulin on the counterregulatory response to equivalent hypoglycemia in normal females. Am J Physiol Endocrinol Metab 1993:E680-9.
[34] Diamond M, Jones T, Caprio S, Hallerman L, Meredith-Diamond M, Addabbo M, et al. Gender influences counterregulatory hormone response to hypoglycemia. Metabolism 1993;42:1568-72.
[35] Davis SN, Fowler S, Costa F. Hypoglycemic counterregulatory responses differ between men and women with type 1 diabetes. Diabetes 2000;49:65-72.
[36] Davis SN, Shavers C, Costa F. Gender-related differences in counter-regulatory responses to antecedent hypoglycemia in normal humans. J Clin Endocrinol Metab 2000;85:2148-57.
[37] Diabetes Control and Complications Trial Research Group. Epidemiology of severe hypoglycemia in the diabetes control and complications trial. Am J Med 1991;90:450-7.
[38] Ahlgren A, Astrand H, Sundkvist G, Lanne. Increased aortic stiffness is persistent in type 1 diabetic women: a follow-up study. Diabetologia 2005;48:780-3.
[39] Ahlgren A, Lanne T, Wollmer P, Sonesson B, Hansen F, Sundkvist G. Increased arterial stiffness in women, but not men, with IDDM. Diabetologia 1995;38:1082-9.
[40] Costacou T, Edmundowicz D, Prince C, Conway B, Orchard T. Progression of coronary artery calcium in type 1 diabetes. Am J Cardiol 2007;100:1543-7.
[41] Conway B, Wang J, Edigar M, Orchard T. Skin fluorescence and type 1 diabetes complications: a new marker of complication risk. Diabetes 2008:A287.
[42] Orchard T, Costacou T, Kretowski A, Nesto R. Type 1 diabetes and coronary artery disease. Diabetes Care 2006;29:2528-38.
[43] Taubes G. Diabetes. Paradoxical effects of tightly controlled blood sugar. Science 2008;322:365-7.
[44] The Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-59.
[45] The ADVANCE Collaborative Group. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-72.