Effect of a Peroxisome Proliferator–Activated Receptor-γ Agonist on Myocardial Blood Flow in Type 2 Diabetes

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OBJECTIVE — The relationship between coronary endothelial function and insulin resistance remains speculative. We sought to determine whether pioglitazone, an insulin-sensitizing peroxisome proliferator–activated receptor (PPAR)-γ agonist, improves cardiac endothelial function in individuals with type 2 diabetes.

RESEARCH DESIGN AND METHODS — Sixteen subjects with insulin-treated type 2 diabetes and without overt cardiovascular disease were randomly assigned to receive either 45 mg of pioglitazone or matching placebo for 3 months. Rest and adenosine-stimulated myocardial blood flow (MBF) were quantified with [^{13}N]ammonia and positron emission tomography at baseline and study conclusion.

RESULTS — After 3 months, HbA_{1c} levels dropped by 0.68% in the pioglitazone group and increased by 0.17% in the placebo group ($P = 0.009$ for difference between groups). Triglyceride (−93 vs. −39 mg/dl, $P = 0.026$) and HDL concentrations (+4.8 vs. −6.0 mg/dl, $P = 0.014$) improved significantly in the pioglitazone group compared with placebo. Despite these favorable changes, there was no demonstrable change in baseline MBF (-0.05 ± 0.24 vs. -0.09 ± 0.24 ml · min^{-1} · g^{-1}, $P = 0.45$), adenosine-stimulated MBF (0.10 ± 0.75 vs. 0.14 ± 0.31 ml · min^{-1} · g^{-1}, $P = 0.25$), or coronary flow reserve (0.45 ± 1.22 vs. 0.35 ± 0.72 ml · min^{-1} · g^{-1}, $P = 0.64$) after 12 weeks of exposure to pioglitazone or placebo, respectively. Regression analysis revealed that lower glucose concentration at the time of the study was associated with higher coronary flow reserve ($P = 0.012$).

CONCLUSIONS — Pioglitazone treatment for 12 weeks in subjects with insulin-requiring type 2 diabetes had no demonstrable effect on coronary flow reserve despite metabolic improvements. Higher ambient glucose levels contribute to impaired vascular reactivity in individuals with diabetes.

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Abbreviations: MBF, myocardial blood flow; PET, positron emission tomography; PPAR, peroxisome proliferator–activated receptor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Atherosclerotic vascular disease and its sequelae remain the primary causes of mortality in patients with diabetes, accounting for 65–75% of deaths (1). Recent attention has focused on functional abnormalities of the endothelium as an early step in the pathogenesis of diabetic heart disease (2,3). Notably, studies on individuals with diabetes have demonstrated a consistently higher myocardial blood flow (MBF) at rest and impaired myocardial vasodilation capacity (4).

Endothelial dysfunction occurs when endogenous vasoconstrictors such as endothelin and angiotensin II dominate the action of naturally occurring vasodilators such as nitric oxide, an imbalance linked to known cardiovascular risk factors (5–7). Importantly, measures of endothelial dysfunction predict subsequent cardiovascular events (8). Insulin resistance has emerged as a putative common link between diabetes and endothelial dysfunction. Those with insulin resistance demonstrate impaired endothelial function (7,9) even before diabetes is diagnosed, and insulin induces vasodilation via endothelial nitric oxide release (10,11).

Thiazolidinediones activate the nuclear receptor peroxisome proliferator–activated receptor (PPAR)-γ to improve postreceptor insulin signaling and insulin sensitivity. Thiazolidinediones may also limit inflammation and atherosclerosis (12,13). We compared the effect of pioglitazone versus placebo on coronary blood flow, vascular resistance, and flow reserve using positron emission tomography (PET) at rest and after induced hyperemia. Concurrently, we characterized the effects of thiazolidinedione on serum markers of vascular relevance and predictors of coronary flow in 16 individuals with insulin-requiring type 2 diabetes.

RESEARCH DESIGN AND METHODS

— Twenty individuals with insulin-requiring type 2 diabetes

were recruited who met the following criteria: no clinical evidence of heart disease (i.e., angina or heart failure symptoms), no evidence of obstructive coronary artery disease on rest-stress myocardial perfusion PET imaging, no ischemic changes or left ventricular hypertrophy on resting electrocardiogram, no overt clinical evidence of cerebrovascular or peripheral vascular disease, no history of more than mild hypertension (blood pressure <160/95 mmHg), no overt nephropathy (serum creatinine <1.4 mg/dl), glycohemoglobin level of >7%, and no history of cardiomyopathy, valvular heart disease, or liver dysfunction. The Human Investigation Committee of Wayne State University approved the study protocol, and all participants gave written informed consent.

Eligible patients were randomly assigned to receive either pioglitazone or placebo for 12 weeks in a double-blinded fashion. Pioglitazone (Actos; Takeda Pharmaceuticals, Lincolnshire, IL) was initiated at 30 mg and titrated to 45 mg after 4 weeks; identical placebo pills were similarly titrated. Subjects continued their initial insulin regimen and other medications. Each subject was evaluated monthly by a diabetologist, and each subject submitted glucose data sheets weekly; insulin dosing was adjusted accordingly. Compliance with treatment assignment was assessed by pill count.

PET imaging

Rest and adenosine-stimulated MBF were assessed by PET imaging (Siemens EXACT/HR whole body PET tomography) before randomization and after 12 weeks of treatment. Before PET imaging, subjects were asked to fast (4 h) and to avoid caffeine-containing beverages, cigarettes, calcium channel blockers, β-blockers, arterial vasodilators, and theophylline-containing compounds (24 h).

MBF was measured at rest and during an adenosine (0.14 mg · kg^{-1} · min^{-1}) intravenous infusion using [^{13}N]ammonia as a flow tracer. A 15-min transmission scan was acquired for correction of photon attenuation. Beginning with the intravenous bolus administration of [^{13}N]ammonia (0.286 mCi/kg), serial images were acquired for 20 min as described previously (6,18). After 30 min, intravenous adenosine was infused for 4 min. After 2 min of the adenosine infusion, a second dose of [^{13}N]ammonia was injected, and images were recorded. Heart rate, blood pressure, and 12-lead electrocardiogram were recorded at baseline and every minute throughout adenosine infusion.

MBF assessment

To quantify regional MBF, regions corresponding to the three main coronary territories were automatically assigned to each of four mid-ventricular short-axis slices of the [^{13}N]ammonia images as before (14). A small circular region of interest at the center of the left ventricular blood pool was used to obtain the arterial input function. The corresponding regions of interest were then copied to the entire image sequences and regional myocardial tissue and blood pool time-activity curves were obtained. Regional MBF was calculated by fitting the [^{13}N]ammonia time activity curves with a previously validated three-compartment tracer kinetic model (15). An index of coronary vascular resistance was calculated by dividing the mean aortic blood pressure by MBF. The coronary flow reserve was defined as the ratio between adenosine-stimulated and basal MBF. To account for differences in workload, rest MBF was divided by the rate-pressure product; this corrected MBF was used to calculate the corrected coronary flow reserve. Because myocardial perfusion was homogeneous in all participants, regional estimates of MBF, flow reserve, and vascular resistance were averaged in each subject.

Laboratory analysis

Venous plasma and serum samples were obtained after overnight fast. Measurements included plasma glucose by the glucose oxidase method, glycohemoglobin level by high-performance liquid chromatography, VonWillebrand factor by immunoelectrophoresis, total insulin by a chemiluminescent competitive assay, and free fatty acids by colorimetric assay. Serum cholesterol and triglyceride concentrations were measured using standard enzymatic methods via automated analyzer. HDL cholesterol was measured using Equal HDL direct method (Technicon DAX; Bayer, Cambridge, MA), and LDL levels were calculated using the Friedewald formula (16).

Statistical analysis

Data are presented as mean ± SD. Using a sample size of 10 evaluable patients per group and a two-tailed α of 0.05, power was predicted to be 0.85 for the analysis for an expected 20% improvement in coronary flow reserve. Baseline characteristics were compared using the χ^2 or independent groups' *t* test. To account for the small sample size, the conservative Wilcoxon's rank-sum test was used to determine the statistical significance of changes in flow and laboratory values over time. Independent predictors of change in coronary flow reserve were ascertained using backwards regression analysis of variables with borderline statistical significance in the univariate analysis and those of particular interest to this study, including serum glucose at the time of study, glycohemoglobin, and diabetes duration. All tests were two-tailed. Statistical significance was defined as $\alpha = 0.05$ throughout. Analysis was performed using Stata 8 (Statacorp LP, College Station, TX).

RESULTS

Baseline characteristics

Twenty subjects (10 male and 10 female) were recruited; two subjects in each group who did not complete the protocol were excluded from analysis. Age ranged from 45 to 65 years (mean 54 years); BMI averaged 33.7 kg/m^2 (range 26.45–42.1). Randomization was equal in both arms except for sex, with more females randomized to pioglitazone ($P = 0.025$) (Table 1). Coronary flow variables did not differ by sex. Five subjects (31%) continued to smoke cigarettes. The mean duration of diabetes was 15 years (range 7–32). Three patients in the pioglitazone group and one patient in the placebo group had a hypoglycemic event requiring assistance from another person ($P = 0.26$). One patient in the pioglitazone arm developed dependent edema but continued with treatment. Two subjects in the placebo group and one in the treatment arm withdrew for personal reasons unrelated to the study. One female patient in the pioglitazone arm was withdrawn from the study after developing new onset congestive heart failure requiring hospitalization.

Biochemical parameters

After 3 months, HbA_{1c} dropped by 0.68% in the pioglitazone group and increased by 0.17% in the placebo group ($P = 0.009$ for difference between groups).

Table 1—*Baseline characteristics of study subjects*

	Placebo group	Pioglitazone group
n	8	8
Female*	1	6
Age (mean years)	52.5	56.5
BMI (kg/m^2)	32.3 ± 4.1	35.1 ± 7.1
HbA_{1c} (mean %)	7.65	7.35
Diabetes duration (years)	14.0 (11–32)	15.5 (7–30)
Daily insulin dose (mean units)	70.9	58.9
Curent smoking	2 (25)	3 (37)
Hypertension	6 (75)	6 (75)
β-Blocker	1 (12)	2 (25)
Ca-channel blocker	2 (25)	2 (25)
ACE inhibitor/adrenergic receptor binder	5 (62)	6 (75)
Statin	4 (50)	1 (12)
Aspirin	4 (50)	5 (62)
Metformin	3 (37)	1 (12)
Sulfonylurea	3 (37)	0 (0)

Data are means ± SD, mean (range), and *n* (%). *$P = 0.025$.

Triglyceride (−93 vs. −39 mg/dl; $P = 0.026$) and HDL concentrations (4.8 vs. −6.0 mg/dl, $P = 0.014$) improved significantly in the pioglitazone group compared with placebo (Table 2). No significant changes in serum total or LDL cholesterol, free fatty acids, or VonWillebrand factor concentrations were found. Insulin levels, influenced by patients' exogenous insulin dosing, did not change significantly. None of these parameters changed within the placebo group during the study.

Hemodynamic responses to adenosine infusion

Heart rate and rate-pressure product consistently increased during adenosine infusion (Table 3). The increase in rate-pressure product was statistically similar at baseline (19 ± 17%) and after 3 months (28 ± 21%). No significant differences in heart rate or rate-pressure product between baseline and follow-up studies occurred. Blood pressure (systolic and diastolic) at rest or during adenosine infusion, measured at baseline and after 3 months, did not differ significantly in either group.

MBF parameters

MBF at rest was similar in the pioglitazone and placebo groups (0.95 ± 0.23 vs. 0.90 ± 0.30 ml · min^{-1} · g^{-1}, respectively; $P = 0.50$), and remained unchanged after 12 weeks of therapy (0.90 ± 0.26 vs. 0.81 ± 0.19 ml · min^{-1} · g^{-1}, respectively; $P = 0.45$). During adenosine stimulation, MBF increased significantly and similarly in the pioglitazone and placebo groups (2.16 ± 0.42 vs. 2.02 ± 0.60 ml · min^{-1} · g^{-1}, respectively; $P = 0.63$; Table 3). After 12 weeks, adenosine-stimulated MBF was similar in the pioglitazone and placebo groups (2.26 ± 0.48 vs. 1.98 ± 0.50 ml · min^{-1} · g^{-1}, respectively; $P = 0.25$). The change in MBF over time in each group was not different between groups, even after correction for the workload achieved.

Consequently, coronary flow reserve was similar in the subjects assigned to pioglitazone and placebo both at baseline (2.32 ± 0.44 vs. 2.17 ± 0.67 ml · min^{-1} · g^{-1}, respectively; $P = 0.52$) and after 12 weeks of treatment (2.77 ± 1.29 vs. 2.52 ± 0.76 ml · min^{-1} · g^{-1}, respectively; $P = 0.64$). Vascular resistance during peak adenosine remained at 47 ± 15% of the rest resistance level. A decline in minimal vascular resistance was seen in both groups during the 12-week study period but did not reach statistical significance.

Predictors of changes in coronary flow reserve

In univariate analysis, the predictors of coronary flow reserve were serum glucose ($r = 0.43$, $P = 0.009$), total cholesterol ($r = 0.36$, $P = 0.036$), free fatty acid concentration ($r = 0.35$, $P = 0.048$), and triglyceride concentration ($r = 0.39$, $P = 0.022$). By stepwise multiple regression, the significant predictors of the coronary flow reserve were ambient glucose ($P = 0.019$; Fig. 1), a history of hypertension ($P = 0.005$), and a history of smoking ($P = 0.028$).

CONCLUSIONS — The major finding of this pilot study was that subjects

Table 2—*Changes in lipid and glycemic parameters*

	Placebo group		Pioglitazone group	
	Baseline	12 weeks	Baseline	12 weeks
n	8	8	8	8
Total cholesterol (mg/dl)	199.2 ± 45.0	192.6 ± 42.0	194.9 ± 40.5	182.9 ± 36.9
LDL (mg/dl)	134.0 ± 46.1	105.5 ± 16.1	98.3 ± 33.7	102.4 ± 16.7
HDL (mg/dl)	47.5 ± 18.5	41.5 ± 7.2	63.6 ± 25.9	68.4 ± 21.3†
Triglycerides (mg/dl)	242.7 ± 309.9	204.0 ± 148.6	191.7 ± 146.9	98.8 ± 56.9†
Free fatty acids (mEq/l)	0.64 ± 0.49	0.50 ± 0.34	0.70 ± 0.45	0.519 ± 0.245
HbA_{1c} (%)	7.65 ± 0.64	7.82 ± 1.21	7.35 ± 0.64	6.67 ± 0.64*†
VonWillebrand factor (U/dl)	75.7 ± 28.5	137.1 ± 96.0*	94.4 ± 63.4	141.1 ± 137.2
Insulin (μU/ml)	15.9 ± 26.1	16.75 ± 18.8	27.5 ± 21.1	31.5 ± 57.2
Plasma glucose at PET (mg/dl)	168.1 ± 42.9	170.5 ± 38.3	161.4 ± 80.1	142.7 ± 77.6

Data are means ± SD. *$P < 0.10$ vs. baseline; †$P < 0.05$ vs. placebo.

Table 3—*Physiologic parameters at rest and during adenosine infusion*

	Placebo group		Pioglitazone group	
	Baseline	12 weeks	Baseline	12 weeks
n	8	8	8	8
Heart rate (beats/min)				
Rest	74.6 ± 10.8	71.9 ± 12.8	71.7 ± 13.1	66.4 ± 9.9
Peak hyperemia	86.4 ± 13.9*	87.6 ± 11.3†	92.7 ± 16.5†	89.4 ± 14.1†
Systolic blood pressure (mmHg)				
Rest	148.9 ± 27.7	156.0 ± 36.6	146.4 ± 22.1	137.3 ± 26.3
Peak hyperemia	147.0 ± 29.7	152.5 ± 23.1	130.3 ± 25.1	132.7 ± 18.2
Mean aortic blood pressure (mmHg)				
Rest	101.8 ± 18.6	109.2 ± 19.0	101.5 ± 15.5	93.2 ± 13.1
Peak hyperemia	98.3 ± 18.9	102.2 ± 13.6	88.3 ± 13.1	86.7 ± 5.7
Rate pressure product (mmHg · $beat^{-1} \cdot min^{-1}$)				
Rest	11,302 ± 3427	11,424 ± 4,194	10,550 ± 2,538	9,020 ± 1,799
Peak hyperemia	12,936 ± 4289	13,515 ± 3,308	12,275 ± 4,019	11,746 ± 1,604†
Myocardial blood flow (ml · $min^{-1} \cdot g^{-1}$)				
Rest	0.901 ± 0.297	0.815 ± 0.192	0.953 ± 0.227	0.902 ± 0.259
Corrected rest	0.867 ± 0.454	0.763 ± 0.223	0.918 ± 0.158	0.980 ± 0.190
Peak hyperemia	1.836 ± 0.457†	1.975 ± 0.498†	2.159 ± 0.422†	2.264 ± 0.475†
Coronary vascular resistance (mmHg · $ml^{-1} \cdot min^{-1} \cdot g^{-1}$)				
Rest	119.74 ± 27.77	137.82 ± 27.10	110.59 ± 24.02	113.26 ± 13.36
Peak hyperemia	59.08 ± 30.14†	54.68 ± 15.29†	42.27 ± 9.40†	39.64 ± 10.50†
Coronary flow reserve (ratio)	2.174 ± 0.674	2.520 ± 0.76	2.321 ± 0.442	2.770 ± 1.29
Corrected coronary flow reserve (ratio)	2.333 ± 0.714	2.668 ± 0.645	2.428 ± 0.691	2.383 ± 0.735

Data are means ± SD. *$P < 0.05$ vs. rest; †$P < 0.01$ vs. rest.

with insulin-requiring type 2 diabetes showed no significant change in coronary flow reserve after pioglitazone treatment, despite significant improvements in known or postulated cardiovascular risk factors (specifically HDL, glucose, and triglycerides). After a 12-week treatment course, patients assigned to high-dose pioglitazone had similar changes in adenosine-stimulated coronary blood flow and vascular resistance compared with those in the placebo group. An association was noted between coronary flow reserve and ambient glucose levels.

Insulin resistance has a central role in type 2 diabetes, as well as its associated complications of obesity, hypertension, dyslipidemia and atherosclerotic heart disease. Thiazolidinediones have demonstrated significant benefits on both glycemic control and associated cardiovascular risk factors including hypertension (17) and biochemical markers of risk (18). Some of these vascular benefits may derive from changes in the endothelium where PPAR-γ is known to be expressed (19,20). Indeed, numerous in vitro studies reveal changes in PPAR-γ–regulated target genes (21) that would be expected to limit atherosclerosis and/or inflammation, including nitric oxide synthetase (22). Consistent with this, troglitazone inhibited the development of atherosclerosis in mice fed high-fat diets (23) and rosiglitazone decreased aortic atheroma formation in apolipoprotein E–deficient mice (24). Early human studies noted a reduction in carotid intimal media thickness after 12 weeks of treatment with troglitazone or pioglitazone (25,26). Although improvements in flow-mediated vasodilation were reported with troglitazone (12,27), a thiazolidinedione now withdrawn from the market, its structure contains the antioxidant α-tocopherol. Antioxidant benefits are implicated as one potential mechanism for troglitazone's effects (13). The effect of thiazolidinediones on human coronary circulation has not yet been defined and

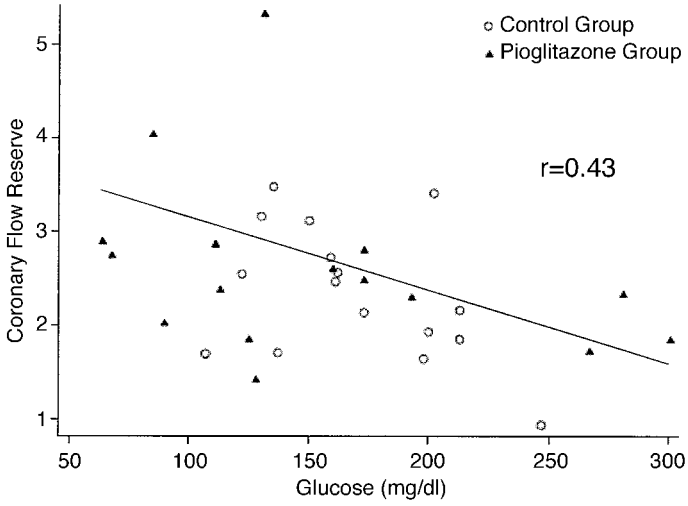

Figure 1—*Inverse relationship between coronary flow reserve and plasma glucose.*

the absence of a significant effect after pioglitazone treatment found here may suggest that thiazolidinediones (or other insulin sensitizers used under standard conditions) may not improve coronary microvascular function, within the limits of this study.

Although changes in arteriolar resistance in response to adenosine are largely mediated by direct vascular smooth muscle relaxation, reflecting primarily endothelium-independent vasodilation, there is evidence that 20–40% of the maximal vasodilator response caused by adenosine is related to nitric oxide release from the endothelium in response to shear stress (28,29). Thus, the vasodilator response to adenosine reflects the integrated effects of both vascular smooth muscle and endothelial cell function. Consequently, our findings suggest insulin resistance may not be related to either endothelium-dependent or independent coronary microvascular function.

Our results extend prior studies that examined the relationship between insulin resistance, diabetes, and endothelial function. Using high resolution brachial ultrasound, Tack et al. (30) demonstrated that troglitazone treatment for 8 weeks did not alter endothelium-dependent or independent vascular responses as measured by nitroglycerin-mediated brachial artery dilation in nondiabetic insulin-resistant subjects. Caballero et al. (27) also found no improvements in forearm blood flow using either flow or nitroglycerin-mediated dilation after 12 weeks of troglitazone in patients with established type 2 diabetes; flow-mediated dilation in the forearm did improve in a subgroup of individuals with recently diagnosed type 2 diabetes. After 16 weeks of rosiglitazone, one study noted a significant improvement in skin nitric oxide production without a significant change in skin perfusion (31). Our findings extend these observations by examining the coronary circulation in individuals who have progressed from insulin resistance to diabetes, demonstrating minimal difference between placebo- and pioglitazone-treated subjects in adenosine-stimulated coronary flow reserve.

Several factors should be considered in interpreting the results of this initial pilot study. As is often encountered in such studies, the total patient number is limited (to 16 patients in this case). This cohort may not be fully representative of the spectrum of individuals with insulin-requiring diabetes. Indeed, despite randomization, more females received pioglitazone (6 of 8) whereas more males received placebo (7 of 8). This sex difference could have resulted in confounding, although we note that within these small numbers, sex was not a predictor of MBF in the regression model. In addition, some of these patients were receiving insulin, which itself can contribute to variability in glucose concentrations, another potentially important variable in MBF that may have interfered with our ability to conclusively determine a relationship between glucose and MBF. Of note, although an inverse relationship was found between mean glucose and coronary blood flow, no relationship was apparent between HbA_{1c} and MBF.

Twelve weeks may be an insufficient exposure to the active drug to demonstrate changes in MBF, especially for one that acts by modulating gene transcription, as predicted for a PPAR-γ agonist. Nevertheless, prior studies have noted significant improvements in hepatic and peripheral insulin sensitivity to thiazolidinediones within similar time frames (32). Interestingly, lipid-lowering agents have been reported to rapidly alter MBF (33). The effect of pioglitazone on MBF may have been less than the predicted 20% change in myocardial perfusion used to power the study. PET-estimated coronary flow reserve is thought to be sufficiently precise to argue against test variability as accounting for the lack of change in MBF seen here. It is also worthwhile noting that the trend for improved blood flow did not reach statistical significance; perhaps a larger study may have demonstrated a thiazolidinedione benefit.

Consistent evidence exists (and is growing) for hyperglycemia as a key modulator of coronary vasodilator function in diabetes. An infusion of 50% dextrose in the brachial artery significantly attenuated the forearm blood flow response to methacholine in healthy humans without diabetes independent of their systemic insulin concentration (34). Kawano et al. (35) showed impaired flow-mediated brachial artery dilation after an oral glucose loading in subjects with normal and impaired glucose tolerance.

Several large-scale clinical trials are underway to test the effect of thiazolidinediones on clinical cardiovascular events. Several lines of evidence suggest that PPAR-γ agonists may reduce atherosclerosis and cardiovascular morbidity. Within the limits of this study, the data presented here suggest that reductions in biochemical risk factors and blood pressure may be the dominant contributors to such vascular benefits rather than changes in insulin resistance. Data suggest that pioglitazone and other thiazolidinediones decrease C-reactive protein levels (18), microalbuminuria (36), arterial pulse-wave velocity (18), systolic and diastolic blood pressure (17), and increase adiponectin levels (18), each of which is associated with cardiovascular risk reduction. These data provide rationale for ongoing research to determine the nature of the effect of this class of agents on the cardiovascular system.

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