Am J Physiol Heart Circ Physiol 305: H1584–H1591, 2013.
First published September 16, 2013; doi:10.1152/ajpheart.00420.2013.

CALL FOR PAPERS | *Sex and Gender Differences in Cardiovascular Physiology-Back to the Basics*

Impact of sex on the heart's metabolic and functional responses to diabetic therapies

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Submitted 20 May 2013; accepted in final form 13 September 2013

Lyons MR, Peterson LR, McGill JB, Herrero P, Coggan AR, Saeed IM, Recklein C, Schechtman KB, Gropler RJ. Impact of sex on the heart's metabolic and functional responses to diabetic therapies. *Am J Physiol Heart Circ Physiol* 305: H1584–H1591, 2013. First published September 16, 2013; doi:10.1152/ajpheart.00420.2013.—Increased myocardial lipid delivery is a determinant of myocardial substrate metabolism and function in animal models of type 2 diabetes (T2DM). Sex also has major effects on myocardial metabolism in the human heart. Our aims were to determine whether *1*) sex affects the myocardial metabolic response to lipid lowering in T2DM, *2*) altering lipid [fatty acid (FA) or triglyceride] delivery to the heart would lower the elevated myocardial lipid metabolism associated with T2DM, and *3*) decreasing lipid delivery improves diastolic dysfunction in T2DM. To this end, we studied 78 T2DM patients (43 women) with positron emission tomography, echocardiography, and whole body tracer studies before and 3 mo after randomization to metformin (MET), metformin + rosiglitazone (ROSI), or metformin + Lovaza (LOV). No treatment main effects were found for myocardial substrate metabolism, partly because men and women often had different responses to a given treatment. In men, MET decreased FA clearance, which was linked to increased plasma FA levels, myocardial FA utilization and oxidation, and lower myocardial glucose utilization. In women, ROSI increased FA clearance, thereby decreasing plasma FA levels and myocardial FA utilization. Although LOV did not change triglyceride levels, it improved diastolic function, particularly in men. Group and sex also interacted in determining myocardial glucose uptake. Thus, in T2DM, different therapeutic regimens impact myocardial metabolism and diastolic function in a sex-specific manner. This suggests that sex should be taken into account when designing a patient's diabetes treatment.

sex; type 2 diabetes; myocardial metabolism; metformin; rosiglitazone; Lovaza

TYPE 2 DIABETES (T2DM) IS a risk factor for heart failure even in the absence of coronary disease. Sex plays an important role in the interaction between T2DM and heart failure. Diabetic women have a higher heart failure risk than diabetic men, and the women's risk of death from heart failure is greater than that of the men (3, 9).

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To understand why sex may impact heart failure in diabetes, it is important to understand how abnormalities in myocardial substrate metabolism contribute to the diabetic cardiomyopathic process. In animal models of T2DM, excessive fatty acid (FA) delivery to the myocardium is an important causative event in the development of cardiomyopathy (25). Conversely, lowering plasma FA levels in animals with T2DM prevents heart lipid accumulation and cardiac dysfunction (25). Animals with T2DM treated with omega-3E fatty acids, which typically lower plasma triglyceride levels, also improved cardiac dysfunction, but only in the males (22). A study in T2DM men showed that lowering plasma FA levels (via weight loss) was associated with lower myocardial fat accumulation and improved diastolic function (23).

Sex has a major effect on fat turnover rates and plasma lipid delivery to the body. Women typically have much more body fat, higher whole body fat turnover rates, and higher plasma triglycerides than men (16). Moreover, sex plays a significant role in the myocardial metabolic response to a condition linked with diabetes: obesity (19). There are also sex-related differences in response to other medications, e.g., heart failure therapies (14). However, no studies have evaluated the effect of sex on the human heart's response to diabetic treatments that are designed to lower plasma fats.

The purpose of our study was to compare the sex-specific cardiac effects of adding therapies that primarily lower either plasma free FA levels (rosiglitazone) or triglyceride levels (Lovaza), respectively, to metformin (little impact on FA or triglyceride) in patients with T2DM. To this end, we performed a randomized, double-blind trial. Our primary endpoint was myocardial FA utilization change. We hypothesized that treatments that lower plasma free FA or triglyceride levels would lower myocardial FA utilization (and raise glucose metabolism) and improve diastolic function. We further hypothesized that sex would have a major impact on the myocardial response to diabetes treatment. This is the first study, to our knowledge, to demonstrate the effects of combination therapy on myocardial metabolism and diastolic function in patients with T2DM. It is the first to evaluate the sex-related differences in the metabolic response of the heart to diabetes treatment. And it is the first to put myocardial metabolism of the diabetic heart in

context with simultaneously measured whole body substrate turnover rates.

MATERIALS AND METHODS

Study design and population. Figure 1 shows the trial design. We studied 78 T2DM subjects, aged >18 <65 years. Thirty-six of the subjects were men and 42 were women, with all but 10 of the latter being postmenopausal (or surgically sterilized). All subjects signed informed consent before study participation, and the Washington University Human Research Protection Office approved the protocol. Entry criteria were a body mass index ≤40 kg/m^2 and normal stress echocardiograms. Exclusion criteria were thiazolidinedione treatment within 6 mo, insulin therapy >2 wk within 1 year, current smoking, corticosteroid use, hormone replacement medication, niacin or fibrate treatment within 6 mo, serum triglycerides >400 mg/dL, liver disease, creatinine >1.5 mg/dL for women or 1.6 mg/dl for men, or ≥2+proteinuria, lack of birth control for premenopausal women. There was a run-in period of 1 to 6 mo during which subjects were treated to a Hb_{A1c} ≤7% on metformin or metformin plus a sulfonylurea, glitinide, or α-glucosidase inhibitor, a blood pressure of ≤140/90 mm/Hg, and an LDL <130 mg/dl on a stable lipid lowering regimen. Subjects underwent imaging studies and then were randomized to maximal tolerated dose of metformin alone (MET), metformin plus rosiglitazone (ROSI), or metformin plus Lovaza (LOV). The investigators and patients were blinded to treatment assignment. Groups were matched for age, T2DM duration, Hb_{A1c}, body mass index, and menopausal status. After 4 mo of treatment, the imaging studies were repeated. All subjects provided written informed consent, approved by the Institutional Review Board at the Washington University School of Medicine. After the FDA black box warning regarding the increased cardiovascular risk associated with rosiglitazone was issued, the ROSI arm was halted. Thus the ROSI subgroup of men was necessarily smaller than the other groups.

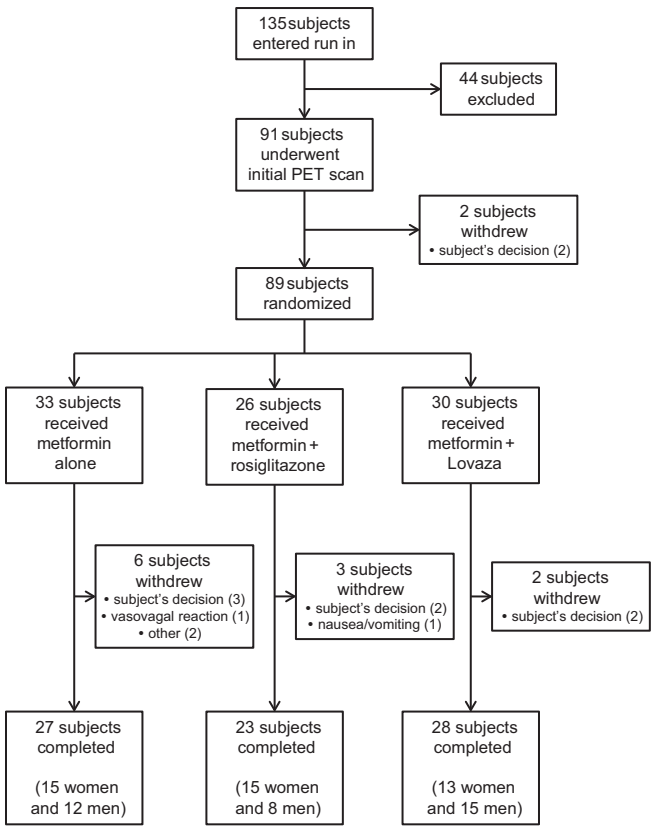

Fig. 1. Trial profile.

PET imaging studies and analysis. PET studies were performed in the resting state after a 12-h fast on a commercially available tomograph (Siemens ECAT 961 HR and ECAT 962 HR+; Siemens Medical Systems, Knoxville, Tennessee). Myocardial blood flow (MBF), myocardial oxygen consumption (MV_{O_2}), myocardial FA, and glucose metabolism were measured after ^{15}O-water, 1-^{11}C-acetate, 1-^{11}C-palmitate, and 1-^{11}C-glucose injections, respectively. [We used 1-^{11}C-glucose instead of ^{18}F-fluorodeoxyglucose because the former provides a more accurate assessment of myocardial glucose utilization (8).] In conjunction with well-validated compartmental models, MBF (in ml·g^{-1}·min^{-1}), $M\dot{V}_{O_2}$ (μmol·g^{-1}·min^{-1}), glucose uptake (in ml·g^{-1}·min^{-1}) and utilization (in nmol·g^{-1}·min^{-1}), and FA uptake (ml·g^{-1}·min^{-1}), utilization (in nmol·g^{-1}·min^{-1}) and oxidation (in nmol·g^{-1}·min^{-1}) measurements were performed as previously reported (19). In brief, myocardial FA or glucose uptake represents the fraction of substrate taken up by the heart corrected for MBF, whereas myocardial FA/glucose utilization multiplies substrate uptake by plasma substrate concentration.

Plasma measurements and body composition. Venous blood samples were obtained at predetermined intervals during the study to measure plasma substrates (glucose, free FAs, and lactate) and insulin levels. Plasma glucose concentration was measured by using the Cobas Mira analyzer (Roche Diagnostics, Indianapolis, IN). Insulin was measured by radioimmunoassay (Linco Research, St. Charles, MO), free FAs by an enzymatic colorimetric method (NEFA C kit; WAKO Chemicals, Richmond, VA), and lactate by using a photospectrometry kit (Sigma Chemicals, St. Louis, MO). Total body fat and fat-free mass (FFM) were determined by dual-energy X-ray absorptiometry (DEXA; Hologic QDR 2000/w; Waltham, MA).

Stable isotope studies. Concurrent with PET imaging, whole body metabolism measurements were performed with stable isotopes. Palmitate rates of appearance (R_a), disappearance (R_d), and clearance (i.e., R_d/concentration) were measured using 1-^{13}C-palmitate. Sixty minutes before the administration of 1-^{11}C-palmitate, a constant infusion (0.035 μmol·kg^{-1}·min^{-1}) of 1-^{13}C-palmitate (Cambridge Isotopes) bound to human albumin (Centeon, Kankakee, IL) was started and continued for 90 min. Blood samples were obtained before and during the isotope infusion. To assess whole body glucose kinetics, 3.5 h before the administration of 1-^{11}C-glucose a constant infusion of 6,6-^{2}H-glucose (priming dose: 18 μmol/kg; infusion rate: 0.22 μmol·kg^{-1}·min^{-1}; Cambridge Isotope Laboratories, Andover, MA) was started and maintained until the end of the data collection. Blood samples were obtained before and during the isotope infusions for glucose R_a, R_d, and clearance calculations.

Echocardiography. During the PET studies, subjects had a complete two-dimensional and Doppler echocardiographic examination using a Sequoia-C256 (Acuson-Siemens, Mountain View, CA). Ejection fraction was calculated using the modified Simpson's method. Left ventricular mass, ejection fraction, spectral Doppler (for E/A determination), and tissue Doppler-derived Em and Am were obtained and measured as previously described (12, 18). Em/Am, a validated marker of early diabetic cardiomyopathy, was calculated (2).

Statistical analysis. All analyses were performed using SAS, version 9. Data are shown as means ± standard error. Categorical variables among the groups were compared using χ^2 tests. Baseline continuous variables among the treatment groups were compared using one-way ANOVA. Differences at baseline between men and women were assessed using unpaired *t*-tests. The treatment group effect on the response to therapy was assessed using ANOVA. If a treatment effect was found among the three groups, paired comparisons were used to evaluate the differences between any two groups' responses to therapy. Because of known sex effects on myocardial metabolism, we performed subgroup analyses of the treatment effects within each sex using paired *t*-tests (19, 20). We also tested for

Table 1. *Baseline characteristics and whole body kinetics*

	Met		Rosi		Lov		*P* Value	
	Women	Men	Women	Men	Women	Men	Treatment Group, ANOVA	Sex, *t*-test
N	15	12	15	8	13	15		
Age, years	57.2 ± 2.3	56.5 ± 2.5	54.4 ± 2.5	55.7 ± 3.4	54.6 ± 2.5	57.6 ± 2.0	NS	NS
Diabetes mellitus duration, years	5.9 ± 1.0	5.5 ± 1.2	6.9 ± 1.2	4.6 ± 1.7	5.6 ± 1.1	6.7 ± 0.9	NS	NS
Hb_{A1c}, %	6.5 ± 0.2	6.3 ± 0.3	6.6 ± 0.2	6.8 ± 0.3	6.5 ± 0.2	6.7 ± 0.2	NS	NS
BMI, kg/m^2	32.0 ± 1.7	33.3 ± 0.9	35.9 ± 1.9	35.4 ± 2.2	34.7 ± 1.9	31.5 ± 1.6	NS	NS
FFM, kg	47.1 ± 2.1	70.3 ± 2.5	52.6 ± 2.3	70.9 ± 4.1	52.7 ± 3.1	67.1 ± 2.5	NS	<0.0001
Fat mass, kg	34.1 ± 2.9	30.9 ± 2.6	41.9 ± 3.6	35.9 ± 3.8	38.2 ± 3.2	27.4 ± 2.5	NS	<0.0001
Plasma, μmol/ml								
Glucose	6.0 ± 0.3	6.6 ± 0.5	7.0 ± 0.4	6.1 ± 0.3	5.9 ± 0.3	6.6 ± 0.4	NS	NS
Free FA	837 ± 87	554 ± 51	724 ± 47	565 ± 84	811 ± 57	606 ± 36	NS	<0.0001
Insulin	10.9 ± 1.7	16.9 ± 3.2	15.7 ± 3.3	20.3 ± 2.9	12.2 ± 2.3	13.4 ± 3.2	NS	NS
Total cholesterol, mg/dL	147 ± 7	143 ± 7	172 ± 8	141 ± 9	147 ± 5	129 ± 11	NS	0.006
Lipoprotein, mg/dL								
High density	47 ± 3	40 ± 3	49 ± 2	39 ± 2	52 ± 3	38 ± 3	NS	<0.0001
Low density	73 ± 5	74 ± 6	90 ± 6	67 ± 7	74 ± 4	64 ± 9	NS	0.04
Plasma triglycerides, mg/dL	135 ± 13	149 ± 18	164 ± 15	178 ± 28	109 ± 16	130 ± 18	NS	NS
Whole Body Substrate Kinetics								
Glucose R_a/FFM, μmol·min^{-1}·kg^{-1}	15.6 ± 0.8	13.4 ± 0.7	16.2 ± 0.9	13.1 ± 1.1	16.6 ± 0.9	14.5 ± 0.6	NS	0.001
Glucose clearance/FFM, ml·min^{-1}·kg^{-1}	2.7 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.3 ± 0.4	2.9 ± 0.1	2.3 ± 0.1	NS	<0.01
Palmitate R_a/FFM, μmol·min^{-1}·kg^{-1}	2.6 ± 0.3	1.7 ± 0.2	2.2 ± 0.1	1.6 ± 0.2	3.1 ± 0.4	1.9 ± 0.1	NS	<0.0001
Palmitate clearance/FFM, ml·min^{-1}·kg^{-1}	14.0 ± 1.4	12.9 ± 1.9	12.0 ± 1.0	10.7 ± 1.8	15.1 ± 1.4	11.6 ± 0.8	NS	NS

Values are means ± SD. Met, metformin; Rosi, rosiglitazone + metformin; Lov, lovaza + metformin; BMI, body mass index; FA, fatty acids; R_a, rate of appearance; FFM, fat-free mass; NS, not significant.

group × sex interactions in predicting study endpoints. $P < 0.05$ was considered significant.

RESULTS

Baseline measures. The randomized patients in the three treatment groups were very similar. Importantly, they did not differ in any baseline metabolic, hemodynamic, or cardiac measure except MBF, which was higher in the MET group compared with the LOV group (Tables 1 and 2). There was also no difference in the groups' racial or sex composition, or metformin dosage taken.

However, there were differences between men and women: most significantly, whole body glucose R_a, glucose clearance, and FA R_a were higher in women than in men (Table 1). MBF, $M\dot{V}O_2$, and myocardial FA uptake and metabolism were also higher in the women, whereas myocardial glucose uptake and metabolism were lower in the women than in the men (Table 2).

The MET group. The only changes from pre- to post-treatment as a group were a decrease in weight (pre, 94.2 ± 4.1; post, 93.2 ± 4.1 kg; $P = 0.02$) and a trend toward a decrease in systolic blood pressure (pre, 134 ± 3; post, 130 ± 2 mmHg; $P = 0.07$). There were no within-group changes in myocardial metabolism from pre- to post-treatment. Part of the reason for this is that the two sexes did not respond in the same way to the metformin treatment.

Neither whole body or myocardial FA metabolism changed after treatment in the MET women. However, in MET men whole body FA clearance (estimated by palmitate clearance) decreased (Table 4). Correspondingly, myocardial FA utilization (Fig. 2) and oxidation (pre, 98 ± 11; post, 115 ± 12 nmol/g/min; $P = 0.02$) increased. This higher myocardial FA utilization was associated with lower myocardial glucose uptake and utilization in the men (Figs. 3 and 4).

Myocardial glucose metabolism was unchanged in women (Figs. 3 and 4), likely in part because both whole body glucose

Table 2. *Myocardial blood flow and metabolism*

	Met		Rosi		Lov		*P* Value	
Baseline	Women	Men	Women	Men	Women	Men	Treatment Group	Sex
MBF, ml·g^{-1}·min^{-1}	1.26 ± 0.07	0.96 ± 0.06	1.10 ± 0.06	0.94 ± 0.05	1.07 ± 0.05	0.87 ± 0.03	0.02	<0.0001
$M\dot{V}O_2$, μmol·g^{-1}·min^{-1}	6.16 ± 0.41	4.60 ± 0.33	5.99 ± 0.25	5.01 ± 0.63	7.34 ± 0.52	4.86 ± 0.46	NS	<0.0001
Glucose uptake, ml·g^{-1}·min^{-1}	0.022 ± 0.004	0.039 ± 0.007	0.023 ± 0.003	0.030 ± 0.007	0.019 ± 0.004	0.021 ± 0.004	NS	<0.05
Glucose utilization, nmol·g^{-1}·min^{-1}	130 ± 24	278 ± 59	152 ± 19	184 ± 44	110 ± 24	147 ± 30	NS	0.02
FA uptake, ml·g^{-1}·min^{-1}	0.222 ± 0.009	0.201 ± 0.009	0.235 ± 0.009	0.211 ± 0.004	0.225 ± 0.011	0.209 ± 0.005	NS	0.004
FA utilization, nmol·g^{-1}·min^{-1}	187 ± 19	111 ± 11	167 ± 9	114 ± 17	182 ± 14	128 ± 9	NS	<0.0001
FA oxidation, nmol·g^{-1}·min^{-1}	159 ± 19	98 ± 11	141 ± 13	104 ± 14	170 ± 15	107 ± 11	NS	<0.0001
Percent change								
MBF	−12.1 ± 4.8*	5.6 ± 7.1	12.1 ± 5.2	7.3 ± 8.2	5.0 ± 6.5	9.7 ± 0.61	0.02**	NS***
$M\dot{V}O_2$	6.3 ± 7.8	1.4 ± 5.3	10.3 ± 9.3	−2.5 ± 7.3	−2.8 ± 7.3	10.2 ± 8.5	NS**	NS***

Values are means ± SD. MBF, myocardial blood flow; $M\dot{V}O_2$, myocardial oxygen consumption. *Intra-subgroup response (i.e., the change in the Met women from before to after treatment): $P < 0.05$; **treatment effect across 3 groups; ***treatment effects in a single sex.

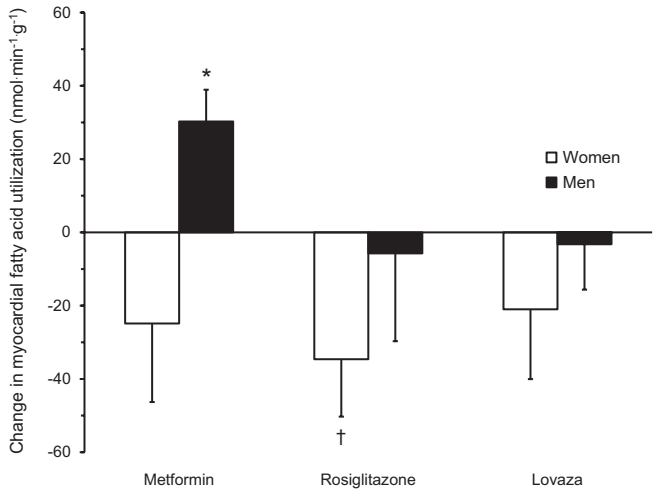

Fig. 2. Change in myocardial fatty acid (FA) utilization after treatment. There were significant intra-subgroup responses to therapy in the metformin-treated men (*$P = 0.006$) and in the rosiglitazone-treated women (†$P = 0.04$).

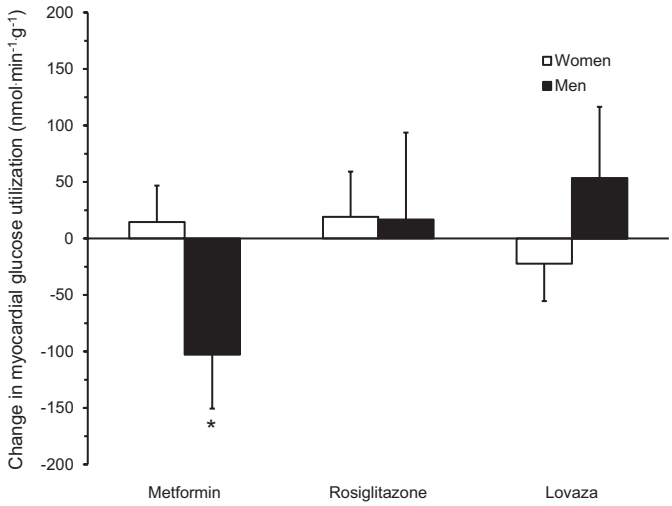

Fig. 4. Changes in myocardial glucose utilization after treatment. Metformin treatment significant reduced myocardial glucose utilization in men (*$P = 0.05$). There was also a trend ($P = 0.09$) toward a significant group*sex interaction.

R_a ($P < 0.05$) and glucose clearance trended higher ($P < 0.06$) after treatment (Table 4). Thus there was no change in plasma glucose level (Table 4) to drive a change in myocardial metabolism, despite MBF and hence glucose delivery decreasing (Table 2). The MET men, in contrast, trended toward having a lower myocardial glucose uptake and utilization after treatment (Figs. 3 and 4).

The ROSI group. In the ROSI group as a whole, there were several changes after treatment. As hypothesized, palmitate clearance/FFM increased, leading to decreased ($P = 0.02$) plasma FA levels (Table 4) and a trend ($P = 0.07$) toward decreased FA utilization (Fig. 2). In addition, FFM, LV mass, and high-density lipoprotein levels increased while plasma glucose, insulin, Hb_{A1c}, and diastolic blood pressure decreased (data by group—not broken down by sex—not shown).

Most of the change in the ROSI group as a whole was because the ROSI women experienced a significant increase in FA clearance, and consequent decreases in plasma FA levels and myocardial FA utilization (Table 4 and Fig. 2). Interestingly, the ROSI group men did not realize a change in whole body FA turnover, FA levels, or myocardial FA utilization (Table 4 and Fig. 2).

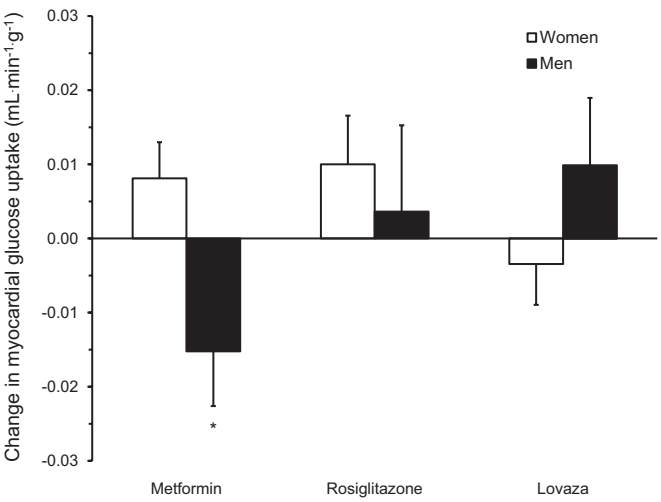

Fig. 3. Changes in myocardial glucose uptake after treatment. Metformin tended to reduce myocardial glucose uptake in men (*$P < 0.07$). There was also a significant group by sex interaction ($P = 0.04$) that was driven by differences between men and women in the response to therapy with metformin vs. Lovaza ($P = 0.01$).

Most of the decrease in plasma glucose levels in the ROSI group was in the women. This is likely because the women's whole body glucose clearance increased (Table 4). However, a trend toward an increase in the women's MBF ($P = 0.07$; Table 2) appears to have offset the decrease in plasma glucose, resulting in no change in myocardial glucose uptake or metabolism (Figs. 3 and 4). Again, the men did not respond in the same way. The ROSI men had no change in whole body glucose turnover and hence no change in plasma glucose (or insulin) levels (Table 4). Their MBF was similarly unchanged (Table 2). Thus their myocardial glucose metabolism was not altered (Figs. 3 and 4).

The LOV group. There were no changes in any metabolic measurement in the LOV group as a whole after treatment. Contrary with our original hypothesis, plasma triglyceride levels and consequently myocardial FA metabolism did not change. However, there was a beneficial increase in LV diastolic function (measured by Em/Am) in all LOV patients ($P = 0.02$; Table 3).

There were no whole body or myocardial metabolic changes in either the men or the women in the LOV group when the sexes were analyzed separately. However, the increase in the diastolic function of the LOV group appears to be the result of an improvement in the men ($P = 0.02$), because the women showed no improvement.

Treatment effect differences across the three groups. There were few treatment effects that were different across the three groups. Body mass index, fat free mass, and LV mass both increased in the ROSI group as compared with the other two groups (Tables 3 and 4). There were no differences among the groups' myocardial FA or glucose metabolism responses to treatment.

This overall lack of differences was likely due to the significant effects of sex on the response to treatment. To reinforce

Table 3. *Hemodynamics, cardiac structure, and function*

	Met		Rosi		Lov		*P* Value	
Baseline	Women	Men	Women	Men	Women	Men	Treatment Group	Sex
Rate-pressure product, mmHg*beats/min	8,793 ± 527	8,577 ± 639	9,099 ± 350	9,178 ± 559	7,856 ± 313	8,704 ± 421	NS	NS
Ejection fraction, %	61 ± 1	68 ± 1	61 ± 2	64 ± 2	65 ± 2	67 ± 1	NS	0.007
LV mass, g	208 ± 10	144 ± 7	201 ± 20	165 ± 11	190 ± 14	138 ± 7	NS	<0.0001
E/A	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	NS	NS
Em/Am	0.95 ± 0.04	0.87 ± 0.05	0.84 ± 0.06	0.90 ± 0.05	0.97 ± 0.04	0.92 ± 0.04	NS	NS
Percent change								
Rate-pressure product	−6 ± 2*	−1 ± 6	−9 ± 5	−3 ± 3	+1 ± 4	+1 ± 4	NS**	NS***
Ejection fraction	+1 ± 3	−2 ± 4	−2 ± 3	−2 ± 2	0 ± 3	0 ± 3	NS**	NS***
LV mass	−3 ± 3	−1 ± 4	+7 ± 8	+10 ± 5	−3 ± 5	−5 ± 5	0.02**	<0.06 (women)***
E/A	−4 ± 5	−3 ± 5	−9 ± 8	−2 ± 7	−2 ± 4	−4 ± 5	NS**	NS***
Em/Am	−3 ± 3	+3 ± 4	−7 ± 7	+4 ± 6	+7 ± 5	+7 ± 3*	NS**	0.02 (men)***

Values are means ± SD. LV, left ventricular. *Intra-subgroup response to therapy: $P < 0.05$ (i.e., the rate-pressure product change in the Met women and the E/A change in the Lov men from pre- to post-therapy were significant); **treatment effect across 3 groups; ***treatment effects in a single sex.

the sexes' within-group differences in response to each treatment (described above), there were several differences in treatment responses across the groups, if the sexes were evaluated separately (Tables 2–4, last column). Moreover, group and sex interacted in determining myocardial glucose uptake (Fig. 3).

DISCUSSION

In this groundbreaking study we demonstrated that in relatively healthy humans with T2DM, well-controlled with metformin, it is possible to further modify myocardial substrate metabolism by adding therapies designed to lower plasma FA levels (i.e., rosiglitazone) but not triglyceride levels (i.e., Lovaza). Moreover, we observed that the myocardial metabolic response was driven by the effects of these various therapies on whole-body substrate metabolism, which in turn altered substrate delivery to the heart (Fig. 5). Most importantly, we observed significant sex-related differences in the whole-body and myocardial metabolic response to these various therapies. These differences were frequently divergent and obscured the metabolic responses to the different therapies when the treatment effects were not evaluated by sex. Finally, there were differential treatment effects seen on LV structure and diastolic function that were different between men and women but were not correlated with changes in myocardial metabolism.

Divergent responses to metformin in myocardial FA metabolism between women and men appear to explain the lack of a treatment effect when the treatment groups were analyzed as a whole. We saw an increase in FA utilization and oxidation in men but not women. This finding in men makes sense given that their palmitate clearance decreased, and they had slightly higher plasma FA levels. These myocardial FA findings are in contrast with another study, which demonstrated a decline in FA oxidation with metformin (23). The reasons for this dis-

Table 4. *Postintervention percent change in characteristics and whole body metabolic values*

	Met		Rosi		Lov		*P* Value	
	Women	Men	Women	Men	Women	Men	Treatment Effect Across 3 Groups	Treatment Effects in a Single Sex
Hb_{A1c}	−1 ± 2	+2 ± 1	−4 ± 2*	−7 ± 4*	−3 ± 3	+1 ± 2	NS	NS
BMI	−1 ± 1	−1 ± 1	+1 ± 1	+4 ± 1*	−1 ± 1	0 ± 0	0.01	0.0007 (men)
FFM	−0.5 ± 1.0	0.8 ± 0.9	1.5 ± 0.8	2.1 ± 0.6*	−0.4 ± 1.0	−0.4 ± 0.7	0.06	NS
Fat mass	0.2 ± 1.4	0.5 ± 2.3	2.0 ± 1.9	4.1 ± 2.3	−2.0 ± 2.3	1.6 ± 1.0	NS	NS
Plasma glucose	−5 ± 5	−2 ± 9	−24 ± 6†	−1 ± 10	+3 ± 5	−4 ± 6	NS	0.01 (women)
Plasma free FA	−12 ± 9	+25 ± 12	−18 ± 8*	−20 ± 16	−9 ± 9	−6 ± 8	NS	0.04 (men)
Plasma insulin	−4 ± 12	−2 ± 8	−27 ± 8‡	−8 ± 15	0 ± 12	−4 ± 9	NS	<0.05 (women)
Total cholesterol	+5 ± 8	−9 ± 5*	+3 ± 8	+12 ± 5*	+2 ± 21	+2 ± 7	NS	0.05 (men)
High-density lipoprotein	+1 ± 13	−5 ± 4	+9 ± 5	+10 ± 4*	+4 ± 4	0 ± 6	NS	NS
Low-density lipoprotein	+8 ± 8	−11 ± 7	+3 ± 10	+19 ± 9	+2 ± 9	+7 ± 12	NS	NS
Plasma triglycerides	+16 ± 30	−10 ± 5	−5 ± 15	+1 ± 12	−2 ± 12	−5 ± 12	NS	NS
Whole Body Substrate Kinetics								
Glucose R_a/FFM, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	+14 ± 7*	−1 ± 5	−1 ± 7	−11 ± 7	−2 ± 5	−6 ± 6	0.08	NS
Glucose clearance/FFM, $ml \cdot min^{-1} \cdot kg^{-1}$	+17 ± 8	−6 ± 9	+27 ± 10*	−13 ± 21	−6 ± 6	+3 ± 11	0.05	0.02 (women)
Palmitate R_a/FFM, $\mu mol \cdot min^{-1} \cdot kg^{-1}$	+5 ± 24	+6 ± 15	+15 ± 10	−4 ± 16	−9 ± 21	0 ± 10	NS	NS
Palmitate clearance/FFM, $ml \cdot min^{-1} \cdot kg^{-1}$	+4 ± 15	−31 ± 13*	+35 ± 12§	+8 ± 37	−10 ± 19	+6 ± 14	0.07	NS

Values are means ± SD. Intra-subgroup response (e.g., Met women's glucose R_a/FFM pretreatment vs. posttreatment): *$P < 0.05$, †$P = 0.001$, ‡$P = 0.005$, §$P = 0.01$.

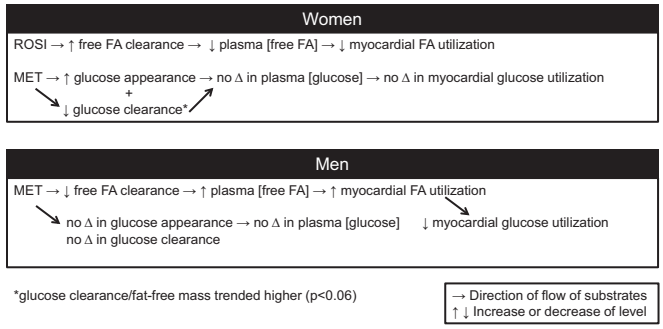

Fig. 5. A proposed scheme integrating the findings of this study on sex-specific treatment-induced changes in whole body substrate turnover leading to changes in plasma substrate concentration, thereby changing myocardial substrate metabolism rates. Likely inverse interaction between myocardial FA and glucose metabolism due to the Randle cycle in the men is also depicted. ROSI, rosiglitazone + metformin (MET).

parity are not clear. However, in our study subjects treated with metformin did not have a change in rate-pressure product, but in the other study cardiac work decreased. Thus their patients' requirements for myocardial metabolism may have decreased. Also, our subjects were taking metformin at baseline; their subjects were not. Differences in patient populations, metformin dose, and/or the mathematical techniques used to measure myocardial FA metabolism may also have played a role in the divergent results. The increased myocardial FA metabolism of the men in our MET group should contribute to reduced glucose uptake and utilization according to Randle cycle principles, just as we observed (21). This decline in myocardial glucose uptake and utilization with metformin in men is consistent with a prior report in men with T2DM (23).

Our divergent myocardial glucose metabolism response in the MET men and women is an entirely novel finding. Altered MBF or plasma glucose levels cannot explain it. The women's better-preserved myocardial glucose metabolism findings are concordant, though, with their increased whole-body glucose R_a. Our findings in the MET group of continued whole-body and myocardial metabolic changes suggest these effects continue long after therapy initiation. The continued metabolic response was not associated with a further improvement in glycemic control. How long before a new equilibrium is reached in whole-body and myocardial metabolism is unknown and requires longer-term studies.

The addition of rosiglitazone to metformin treatment did not result in an overall decrease in myocardial FA metabolism as we originally hypothesized. Again, this was in part due to men and women not responding in the same way to rosiglitazone. In diabetic women, rosiglitazone resulted in a significant decrease in myocardial FA utilization. This appears to be due to the FA level decrease. This decrease likely reflects the insulin-sensitizing effects of the drug on the women's adipose tissue as evidenced by their increased clearance of ^{13}C-palmitate. In men rosiglitazone did not alter whole-body or myocardial FA metabolism. This lack of an effect in men may relate to concomitant MET therapy decreasing their whole-body ^{13}C-palmitate clearance and increasing myocardial FA utilization. The relatively tight diabetes control at baseline may also have blunted the change in plasma FA levels from rosiglitazone in the group as a whole.

Rosiglitazone in combination with metformin also did not increase myocardial glucose uptake and utilization in either men or women. This is in contrast with previous studies, which showed insulin-stimulated myocardial glucose uptake increased with thiozoladinedione therapy (5, 13). This disparity likely reflects the difference between obtaining measurements under fasting conditions (current study) versus in response to insulin stimulation (prior studies) (5).

This study is the first to our knowledge to evaluate the effects of Lovaza on myocardial metabolism in humans with T2DM. Our study demonstrated that Lovaza had no significant effect on myocardial metabolism. This is likely due to the fact that we saw no change in plasma triglyceride or FA levels after treatment, because many subjects were treated for hyperlipidemia and thus had low plasma triglyceride levels pre-intervention. Moreover, we did not directly trace or measure triglyceride uptake by the myocardium since there is no currently available radiolabeled triglyceride tracer. Thus, although Lovaza had little effect on myocardial FA metabolism, it cannot be concluded that triglyceride is not an important contributor to myocardial lipid metabolism.

In the current study, different LV structural changes occurred with the therapies. LV mass increased with rosiglitazone in both men and women. This increase may be related to the volume retention effects of the thiozoladinedione agents (17). Although a consistent increase in LV mass is not observed in all studies of thiozoladinedione treatment (23), there are reports of increases in LV mass in animal studies (17). Alternatively, there may be a potential synergistic effect on LV mass when metformin and rosiglitazone are given concomitantly.

An improvement in LV diastolic function was observed in men after Lovaza treatment. This was not mediated by a hemodynamic change. This result agrees with findings in a type 1 diabetes rat model in which males exhibited greater diastolic function improvement than females (22). The mechanism of this sexually dimorphic response is not clear. At least theoretically, it could be due to differential activation of PPARs and/or inhibition of NF-κB by omega-3E fatty acids, since sex-related differences in the regulation of these pathways have been observed under other conditions (4, 24). Alternatively and/or in addition, some studies suggest sex-related differences in the generation of oxidative stress and the response to oxidative stress (6, 11), and oxidative stress is linked to cardiac dysfunction (1). The lack of correlation between functional and metabolic changes is at odds with studies in experimental models of T2DM (25) but consistent with reports in T2DM patients (23). The lack of correlation may reflect the fact that our patients were relatively healthy, well-controlled, and only exhibited mild diastolic dysfunction. The lack of correlation between the metabolic and structural/functional changes may also be due to the relatively short duration of the intervention.

Our finding of profound baseline sex-related differences in T2DM patients' MBF and myocardial substrate metabolism is concordant with previous findings in nondiabetic subjects (19, 20). As noted above, we also found sex-related differences in myocardial metabolism and diastolic function with specific T2DM therapies. The mechanisms responsible for these sex-related differences are not completely clear. However, it is known that estrogen modulates whole-body and specific organ

substrate metabolism. In liver and skeletal muscle, estrogen increases FA oxidation and decreases glucose oxidation, gluconeogenesis, and glycogenolysis (9, 15). In the heart, postmenopausal estrogen replacement therapy increases myocardial FA utilization and oxidation (7). Thus, it is possible that, for example, the failure of myocardial FA utilization to increase in response to metformin in the women may have been because all but two of the subjects in this group were postmenopausal. However, further study is needed to confirm the role of estrogen and other mechanisms by which sex affects the metabolic response to different T2DM therapies.

Limitations. The ROSI group had fewer subjects compared with the other two groups. As mentioned, this disparity was the result of stopping the ROSI arm earlier than the other two arms due to concerns raised about the cardiovascular safety of the drug. The extent to which this minimized the differences between therapies in the ROSI group is unclear. We studied only subjects in the resting state under fasting conditions after 3 mo of treatment. Further study is needed to determine the impact of these therapies on the myocardial metabolic and functional to respond to various stimuli such as alterations in the substrate environment, neurohumoral milieu, and cardiac work.

Conclusions. The findings of our study point to the need to assess not only overall outcomes but sex-specific outcomes as well. Clinical medicine is moving toward individualizing therapy, and this research provides strong evidence that sex effects should be considered as an important factor in determining the optimal therapy for patients with T2DM. Moreover, the presence of marked sex differences in the pattern of myocardial substrate use in diabetic patients under baseline conditions could affect the ability of the heart to respond to superimposed conditions that require particular substrate use (e.g., ischemia requires glucose). As a consequence, these sex-specific substrate preferences of the diabetic heart may contribute to the known sex-related cardiovascular outcomes differences in diabetes mellitus.

ACKNOWLEDGMENTS

We thank Kristin O'Callaghan for editorial assistance and Ava Ysaguirre for the preparation and submission of this article.

GRANTS

This work was supported by National Institutes for Health (Bethesda, Maryland) Grants PO1HL013851-43, MO1-RR000036-461599, P30-DK056341-08, and P60-DK020579-30.

DISCLOSURES

After finishing this study, Dr. Gropler received a grant from GlaxoSmithKline to perform a different study. All other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

Author contributions: M.R.L., J.B.M., A.R.C., I.M.S., and C.R. performed experiments; M.R.L., L.R.P., P.H., A.R.C., I.M.S., K.B.S., and R.J.G. analyzed data; M.R.L., L.R.P., J.B.M., P.H., A.R.C., I.M.S., C.R., K.B.S., and R.J.G. interpreted results of experiments; M.R.L., L.R.P., A.R.C., I.M.S., and R.J.G. prepared figures; M.R.L., L.R.P., and I.M.S. drafted manuscript; M.R.L., L.R.P., J.B.M., P.H., A.R.C., I.M.S., C.R., K.B.S., and R.J.G. approved final version of manuscript; L.R.P., J.B.M., P.H., A.R.C., and R.J.G. edited and revised manuscript; J.B.M., P.H., A.R.C., K.B.S., and R.J.G. conception and design of research.

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