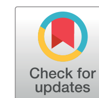

Skeletal Muscle Microvascular-Linked Improvements in Glycemic Control From Resistance Training in Individuals With Type 2 Diabetes

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OBJECTIVE

Insulin increases glucose disposal in part by enhancing microvascular blood flow (MBF) and substrate delivery to myocytes. Insulin's microvascular action is impaired with insulin resistance and type 2 diabetes. Resistance training (RT) improves glycemic control and insulin sensitivity, but whether this improvement is linked to augmented skeletal muscle microvascular responses in type 2 diabetes is unknown.

RESEARCH DESIGN AND METHODS

Seventeen (11 male and 6 female; 52 ± 2 years old) sedentary patients with type 2 diabetes underwent 6 weeks of whole-body RT. Before and after RT, participants who fasted overnight had clinical chemistries measured (lipids, glucose, HbA_{1c}, insulin, and advanced glycation end products) and underwent an oral glucose challenge (OGC) (50 g × 2 h). Forearm muscle MBF was assessed by contrast-enhanced ultrasound, skin MBF by laser Doppler flowmetry, and brachial artery flow by Doppler ultrasound at baseline and 60 min post-OGC. A whole-body DEXA scan before and after RT assessed body composition.

RESULTS

After RT, muscle MBF response to the OGC increased, while skin microvascular responses were unchanged. These microvascular adaptations were accompanied by improved glycemic control (fasting blood glucose, HbA_{1c}, and glucose area under the curve [AUC] during OGC) and increased lean body mass and reductions in fasting plasma triglyceride, total cholesterol, advanced glycation end products, and total body fat. Changes in muscle MBF response after RT significantly correlated with reductions in fasting blood glucose, HbA_{1c}, and OGC AUC with adjustment for age, sex, % body fat, and % lean mass.

CONCLUSIONS

RT improves OGC-stimulated muscle MBF and glycemic control concomitantly, suggesting that MBF plays a role in improved glycemic control from RT.

CARDIOVASCULAR AND METABOLIC RISK

Type 2 diabetes is linked to poor lifestyle (physical inactivity and diet) (1) and genetic (2) factors resulting in insulin resistance and hyperglycemia. Aerobic exercise training improves glucose tolerance (3), insulin sensitivity (4), and lipid profiles (5). In addition, resistance training (RT) also improves glycemic control (6,7), reduces abdominal

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adiposity (8), lowers central blood pressure (9), and improves insulin action in skeletal muscle, including GLUT-4 content, insulin signaling, and insulin-stimulated glucose clearance (10). However, mechanisms explaining improved metabolic parameters from RT are not fully understood (reviewed in [6]) but appear, at least in part, to be independent of those seen with aerobic training (6).

Insulin increases muscle microvascular blood flow (MBF), which facilitates muscle glucose uptake via improved delivery of insulin and glucose to myocytes. We have observed increased MBF in response to insulin using both the contrast-enhanced ultrasound (CEU) technique during microbubble infusion (11) and a biochemical marker of microvascular recruitment (1-methyl xanthine metabolism) (12). These increases in MBF have been attributed to increased circulating insulin, demonstrated via hyperinsulinemic-euglycemic clamp (11,13) or pancreatic secretion after a meal (14). However, these insulin-stimulated responses of MBF are impaired in insulin resistant rats (15) and obese humans (16).

We have shown that impaired insulin-mediated MBF is an early event in the development of insulin resistance and precedes myocyte insulin resistance (17). Activity-restricted nonhuman primates display blunted muscle MBF responses to intravenous glucose tolerance tests (18). This is complimented by our work showing that exercise training in rats augments microvascular insulin sensitivity independent of muscle capillary density changes (19). Whether muscle MBF can be improved by RT in humans has not yet been investigated. Furthermore, it is unknown whether improvements in glycemic control with RT are associated with improvements in muscle MBF. Therefore, we sought to investigate the effects of RT on indices of glycemic control and muscle microvascular responses in patients with type 2 diabetes. We hypothesized that the improvement in glycemia after short-term RT is related to improved muscle microvascular function rather than changes in overall body composition.

RESEARCH DESIGN AND METHODS

This pre-post design study was approved by the University of Tasmania Human Research Ethics Committee (no. H14086). All participants gave written informed consent. The study was carried out in accordance with the Declaration of Helsinki as revised in 2008. It was calculated that 16 people would be needed to detect a 30% increase in MBF in response to the oral glucose challenge (OGC) (power = 0.8, $\alpha = 0.05$) (16). We recruited 18 people to account for a 10% drop-out rate. Eighteen sedentary people (7 female and 11 male; self-reported <30 min of moderate exercise per week) with clinically diagnosed type 2 diabetes were recruited through community advertisement. Participants were included in the study if they were between 18 and 60 years of age, had a clinical diagnosis of type 2 diabetes, and were normal weight to overweight (BMI 19–35 kg/m^2). Participants were excluded from the study if they participated in any kind of resistance exercise or performed more than low-intensity walking or if they were current smokers. Additional exclusion criterion included having a BMI >35 kg/m^2 or a personal history of smoking, cardiovascular disease, stroke, myocardial infarction, uncontrolled hypertension (seated brachial blood pressure $>160/100$ mmHg), peripheral arterial disease, pulmonary disease, arthritis/muscular skeletal disease, malignancy within the past 5 years, or severe liver disease. One female participant dropped out of the study after the first day of RT owing to muscle soreness. Her data were excluded from the final analysis. All methods described were obtained prior to, and after, 6 weeks of RT.

Screening Visit

Participants underwent a comprehensive medical examination to confirm eligibility, which included a 12-lead electrocardiogram, completion of a medical questionnaire, and evaluation of fasting blood glucose, HbA_{1c}, and lipid levels. Participants' height and weight were recorded. The International Physical Activity Questionnaire was used to assess level of physical activity, and participants were instructed not to change medications or dose while participating in this study. Maximum handgrip strength of the dominant arm was measured in triplicate using a handheld dynamometer. Participants completed a 3-day food record for monitoring of diet in the week before the first day of testing at the Menzies Institute for Medical Research, Clinical Research Centre, University of Tasmania.

Clinic Visit

Participants fasted overnight and refrained from alcohol and exercise for 48 h prior to the clinic visit. Medications for type 2 diabetes were stopped for 48 h prior to testing. A catheter was placed in the antecubital vein of the nondominant arm. Subjects were studied in a temperature-controlled room laying in a semirecumbent position. Subjects remained in bed for 30 min prior to, and throughout, clinical testing and were instructed to refrain from using their arms so as to minimize effects of movement (or contraction) on hemodynamic measurements.

OGC

A 2-h OGC (50 g glucose) was performed to assess glucose tolerance as previously reported (20). A fasting blood sample was taken prior to ingestion of the glucose drink (time: 0 min). Blood samples were taken at 15, 30, 60, 90, and 120 min post–glucose ingestion, thereby enabling measurement of a 2-h area under the curve (AUC) for glucose and insulin, and insulin sensitivity indices. Insulin sensitivity, using fasting blood glucose and insulin levels, was calculated according to HOMA of insulin resistance (HOMA-IR) (21) and QUICKI (22) indices. Disposition (23) and hepatic insulin resistance (24) indices were assessed by analysis of glucose and insulin responses during the OGC.

Muscle MBF

Microvascular perfusion in skeletal muscle was assessed by CEU as previously reported (15,16,25). A linear array transducer (L9-3) interfaced with an ultrasound system (iU22; Philips Medical Systems, North Ryde, New South Wales, Australia), was positioned over the right deep flexor muscle of the forearm. Low mechanical index (0.10) real-time imaging was performed. Definity microbubbles (Lantheus Medical Imaging, Melbourne, Australia) were diluted (1.5 mL added to 30 mL saline) and continuously infused intravenously at 0.03 mL/min/kg body wt. Microbubble replenishment curves after a high-energy ultrasound pulse were collected. Muscle microvascular responses were measured at baseline and then repeated 1 h after the OGC. Immediately after the 1-h OGC CEU measurements, participants underwent voluntary forearm contraction at 50% maximum handgrip strength, and CEU imaging was performed again. A handgrip dynamometer

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was used, as it allows isometric contraction of the deep flexor muscle bed—the same muscle bed used for imaging microvascular responses to the OGC. Therefore, a direct comparison between OGC versus contraction-mediated microvascular responses can be measured in the same muscle bed to determine whether improvements in MBF in response to the OGC after RT were due to microvascular remodeling (i.e., capillary density) or augmentation of microvascular insulin sensitivity.

Digital images were analyzed off-line using Qlab (Philips Medical Systems). Images were background subtracted (0.5 s frames for post-OGC and 0.25 s for contraction) to eliminate signal from larger blood vessels and tissue per se and curve fitted as previously described (15). Skeletal muscle MBF was determined at baseline and 1 h post-OGC and during forearm contraction.

Skin and Subcutaneous MBF

Skin and subcutaneous MBF was assessed by high-power laser Doppler flowmetry (LDF) as previously described (26). Briefly, the LDF probe (CP1; Moor Instruments, Devon, U.K.) was placed ~10 cm distal of the antecubital fold on the lateral side of the right forearm. LDF flux was recorded before and during the first hour of the OGC with the Vascular Monitor System (Moor Instruments). Data (expressed as flux/mmHg) were averaged in 10-min blocks.

Brachial Artery Flow

Brachial artery measurements were made using a high-frequency L12-5 linear array transducer (iU22 Philips ultrasound). Brachial artery diameter was measured online in high-definition zoom in triplicate using two-dimensional imaging of the longitudinal artery (diameter assessed as the distance between each inside edge of the arterial intima). Brachial flow velocity was determined using pulse-wave Doppler quantified by automated tracing software online and averaged over 10–12 heartbeats. Brachial artery blood flow was calculated from the diameter and velocity measurements. Brachial artery responses were measured at baseline and 1 h after the OGC.

Resting Metabolic Rate

After the initial 30-min rest period, resting metabolic rate was measured and calculated as previously described (20) using a MasterScreen CPX metabolic cart (CareFusion, Hoechberg, Germany). Briefly, a rubber mask was placed over the nose and mouth, and breath gas measurements were analyzed using JLab software (version 5.3) after calibration for volume and gas composition.

Blood Analysis

HbA_{1c}, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured at a nationally accredited pathology laboratory (Royal Hobart Hospital). Blood glucose was measured using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin was measured using an ELISA (Mercodia, Uppsala, Sweden). Fasting plasma free fatty acid (FFA) levels were determined using an enzymatic assay kit (Wako Pure Chemical Industries, Osaka, Japan). Total levels of advanced glycation end product (AGEs) in plasma were assayed using the OxiSelect AGE Competitive ELISA kit (Cell Biolabs, Inc., San Diego, CA).

Body Composition

Participants underwent a whole-body scan by DEXA (Discovery W, Hologic, Bedford, MA). Total body fat, total body fat percentage, trunk fat, and lean mass were calculated using Hologic Apex System software, version 4.0.2.

Central Hemodynamics

Each participant was fitted with a Mobil-O-Graph monitor (I.E.M., Stolberg, Germany) validated to measure aortic stiffness and brachial and central blood pressure (27). Recordings were taken in triplicate at baseline and every 10 min during the first hour of the OGC.

Exercise Intervention

RT was based on our previous work showing that similar training for 6 weeks lowers fasting blood glucose in healthy participants without changes in body weight (7). RT was performed 3 days/week at a local fitness center in Hobart, Tasmania, Australia (All Aerobic Fitness). All exercises were fully supervised by a trained exercise physiologist and the weights adjusted for individual ability. The trainer also kept track of training participation, length, and progression. Training involved full-body RT on Monday and Friday, using a mixture of free weights and resistance machines. One set of each resistance exercise was performed as recommended by the American College of Sports Medicine for strength training in sedentary (novice) people, with each set of resistance exercise performed to complete muscle failure (6–15 repetitions), including leg press, lateral pull-down, chest press, weighted lunges, seated row, back fly, bicep curl, incline chest press, dumbbell shoulder press, leg extension, leg curl, dips, lateral shoulder raise, triceps extension, dumbbell deadlift, and push-ups, respectively. Time to complete each session was limited to 60 min. All resistance exercises were recorded and the load incrementally increased (maintained between 65 and 85% of calculated 1 repetition maximum) as strength increased to ensure progression. Core and stability exercises were performed on Wednesday of each week and continually modified to match increased strength and fitness. Wednesday workouts used resistance-focused techniques and included dumbbell sit-ups, medicine ball toss, leg lifts, plank positions, burpees, and weighted farmer's walk. Leg press, bench press, and deadlift were used to assess strength before and after RT. Strength (calculated 1 repetition maximum) before and after training was estimated via the Epley formula (28) as previously described (7). Compliance to RT was determined as performing no fewer than 15 of the 18 workouts, as well as not missing any workouts in the last 2 weeks of participation.

Statistical Analyses

All data are expressed as means ± SEM. Student paired *t* test was used to compare measurements pre- and post-RT. When data were not normally distributed, the signed rank test was performed. The training-related effects were assessed using a two-way repeated-measures ANOVA (interactions: time, 0- and 60-min OGC; group, pre-RT and post-RT) followed by a Student-Newman-Keuls post hoc test for all of the continuous variables (microvascular, macrovascular, glucose, insulin, and AGE responses to an OGC). Mixed linear regression analysis allowing for repeated measurements on individuals to evaluate fasting glucose, HbA_{1c}, and glucose tolerance (OGC AUC) outcomes with microvascular responses (to OGC) after RT adjusted for age, sex, brachial artery blood flow, mean arterial pressure, BMI, percent body fat, and percent lean mass (with the number of confounders varying from 3 to 4). For analysis of the change before and after in outcomes and for analysis of its relation to change in microvascular response, the latter was partitioned into two components (variables): the mean of

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the measurements for an individual participant and the deviation from that mean for each period (pre-RT/post-RT); coding in this way allows simultaneous estimation of change for microvascular response over period (deviation component), as well as an overall relationship between individuals (mean component) (29). Significance was set at $P < 0.05$. Tests were performed using the SigmaStat statistical program (Systat Software, San Jose, CA) and R 3.3.1.

RESULTS

Anthropometrics and Blood Analyses

Participant characteristics at the time of enrolment are reported in Table 1. Participants' age ranged from 27 to 60 years, with all but one participant over the age of 40 years. Additional information on medication use and other comorbidities is shown in Supplementary Table 1. After 6 weeks of RT, participants had a small but significant reduction in body fat, with a significant increase in body lean mass. Fasting glucose, HbA_{1c}, total cholesterol, triglycerides, and HOMA-IR were significantly lower after RT, whereas LDL, HDL, FFA, QUICKI, disposition index, hepatic insulin resistance index, resting metabolic rate, and fasting plasma insulin levels were unchanged. Relative strength (kg lifted/kg body wt) increased by ~44% with RT (Table 1). Resting central and peripheral hemodynamic responses remained unchanged after RT (Table 1).

Table 1—Characteristics of study participants before and after RT

	Pre-RT	Post-RT	*P*
Age (years)	52 ± 2		
Sex (male/female)	11/6		
Diabetes duration (years)	7 ± 1		
Height (cm)	172 ± 2		
Weight (kg)	92 ± 4	92 ± 4	0.622
BMI (kg/m^2)	31.2 ± 1.1	31.1 ± 1.1	0.733
Body composition			
Percent body fat	32.6 ± 1.7	31.7 ± 1.8	0.005
Percent trunk fat	35.1 ± 1.6	33.8 ± 1.7	0.007
Body fat (kg)	27.6 ± 2.0	27.1 ± 2.0	0.021
Percent body lean mass	64.9 ± 1.6	65.8 ± 1.7	0.005
Body lean mass (kg)	54.4 ± 2.7	55.7 ± 2.8	0.013
Fasting blood glucose (mmol/L)	10.0 ± 0.8	8.7 ± 0.6	0.005
HbA_{1c}			
%	7.7 ± 0.3	7.3 ± 0.3	0.003
mmol/mol	61 ± 3	56 ± 3	
Plasma insulin (pmol/L)	55.7 ± 7.9	54.5 ± 7.4	0.627
Insulin sensitivity indices			
HOMA-IR	3.7 ± 0.7	3.1 ± 0.5	0.040
QUICKI	0.33 ± 0.01	0.33 ± 0.01	0.207
Disposition index	1.2 ± 0.6	0.8 ± 0.2	0.306
Hepatic insulin resistance index	22.4 ± 5.7	20.1 ± 7.1	0.459
Plasma lipids			
Total cholesterol (mmol/L)	4.8 ± 0.3	4.4 ± 0.2	0.024
LDL (mmol/L)	2.9 ± 0.3	2.6 ± 0.2	0.274
HDL (mmol/L)	1.4 ± 0.2	1.2 ± 0.1	1.000
TG (mmol/L)	1.8 ± 0.3	1.4 ± 0.2	0.016
FFA (mmol/L)	0.55 ± 0.06	0.54 ± 0.07	0.829
Resting metabolic rate (kcal/day)	1,910 ± 101	1,884 ± 100	0.854
Relative strength (kg/kg body wt)	2.43 ± 0.20	3.49 ± 0.27	<0.001
Brachial blood pressure			
SBP (mmHg)	137 ± 3	134 ± 3	0.175
DBP (mmHg)	87 ± 2	85 ± 2	0.345
Pulse pressure (mmHg)	50 ± 2	47 ± 1	0.262
Central blood pressure			
SBP (mmHg)	129 ± 3	125 ± 3	0.192
DBP (mmHg)	88 ± 3	86 ± 2	0.279
Pulse pressure (mmHg)	41 ± 2	40 ± 2	0.354
Heart rate (bpm)	67 ± 2	65 ± 2	0.297
Augmentation index (%, adjusted for HR 75 bpm)	18.7 ± 2.8	17.8 ± 2.5	0.685
Pulse wave velocity (m/s)	7.8 ± 0.2	7.6 ± 0.2	0.071
Peripheral vascular resistance (mm Hg/mL × min)	1.3 ± 0.1	1.2 ± 0.1	0.600

Data are mean ± SEM (n = 17). Student paired t test or signed rank test if data not normally distributed. DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; TG, triglycerides.

Vascular Responses

Forearm muscle MBF did not change after the OGC before training; however, after RT, MBF was enhanced after the OGC (Fig. 1*A*). ΔMBF (OGC-basal) was significantly higher post-RT versus pre-RT (Fig. 1*B*).

As expected, MBF increased markedly in response to acute forearm contraction at 50% maximum handgrip strength (Fig. 1*C*), and this was unaffected by 6 weeks of RT. Similarly, ΔMBF in response to contraction was similar before and after RT (Fig. 1*D*).

Brachial artery blood flow was higher after the OGC prior to RT, and this was significantly blunted after RT (Fig. 1*E*). These effects occurred despite a similar increase in heart rate with the OGC pre- and posttraining (pre-RT 66 ± 2 to 70 ± 2 bpm, $P < 0.001$; post-RT: 64 ± 2 to 70 ± 2 bpm, $P < 0.001$). However, the change in brachial artery blood flow in response to the OGC was not significantly different after RT (Fig. 1*F*).

Skin microvascular responses to OGC were assessed by LDF. LDF conductance did not change in response to the OGC during the first 50 min but was significantly elevated by 60 min and was similar post-RT (Fig. 1*G*). Consequently, the LDF conductance AUC was not significantly different pre-RT versus post-RT (Fig. 1*H*).

Glucose, Insulin, and AGE Responses During the OGC

Blood glucose levels during the OGC were significantly lower at each time point post-RT (Fig. 2*A*). Consequently, the area under the glucose-time curve (Fig. 2*B*) was also lower post-RT ($P < 0.005$). Plasma insulin levels during the OGC were not different at any time point before or after RT (Fig. 2*C*), and area under the insulin-time curve did not change with RT either (Fig. 2*D*).

Total plasma AGE levels were markedly reduced after 6 weeks of RT, and as expected, these levels did not change during OGC (Fig. 2*E*). The AGE AUC during the 2-h OGC was significantly reduced post-RT (Fig. 2*F*).

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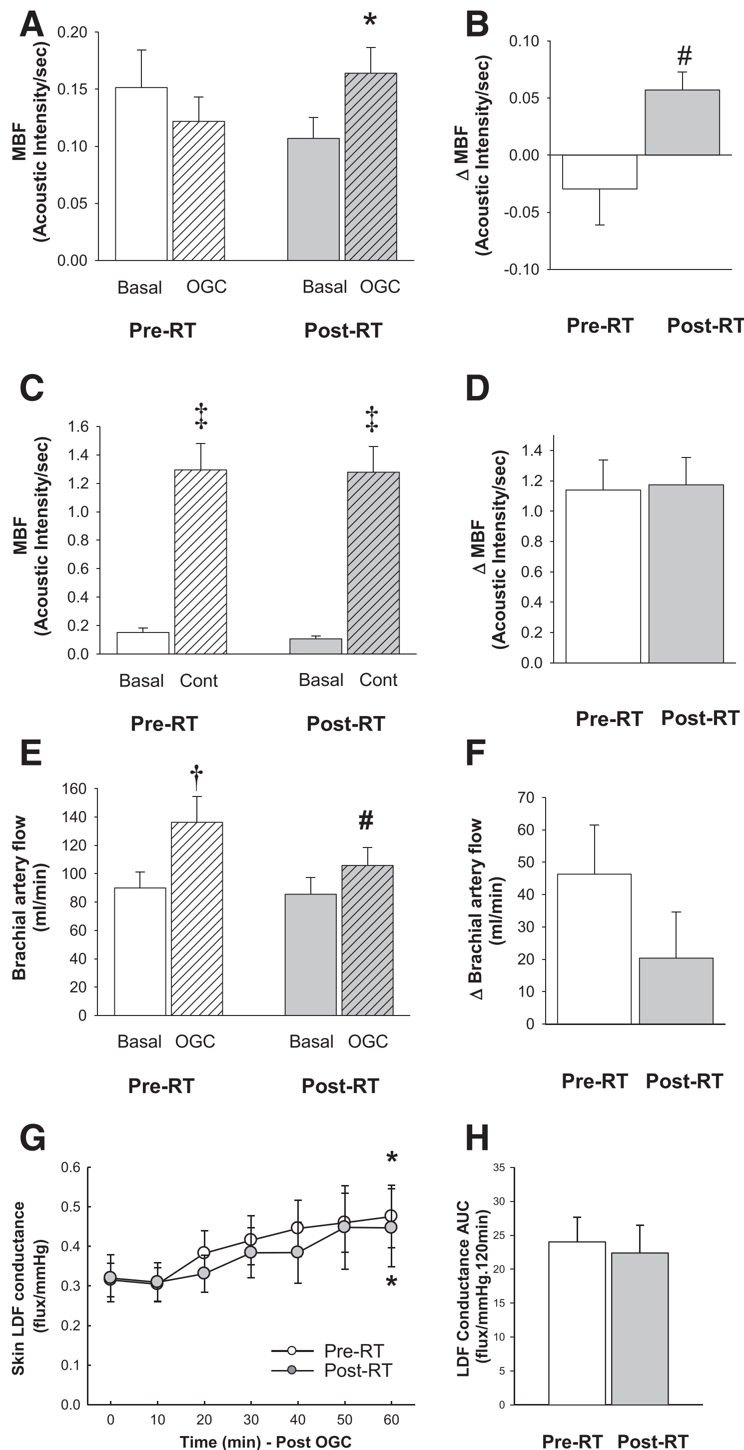

Figure 1—Vascular responses before and after RT. *A*: Skeletal muscle MBF at basal and 60 min post-OGC before (pre-RT) and after (post-RT) exercise training. *B*: Change in MBF from basal to 60 min post-OGC before and after exercise training. *C*: Skeletal muscle MBF at basal and during acute contraction (Cont) at 50% maximum handgrip strength before and after exercise training. *D*: Change in MBF from basal to contraction before and after exercise training. *E*: Brachial artery blood flow at basal and 60 min post-OGC before and after exercise training. *F*: Change in brachial artery blood flow from basal to 60 min post-OGC before and after exercise training. *G*: Microvascular skin LDF conductance timelines in response to an OGC. *H*: AUC for LDF conductance during the OGC. Data are means ± SEM for each group (n = 16). Repeated-measures two-way ANOVA was used to determine whether there were differences between treatment groups over the time course of the experiment or Student t test (or signed rank test if data not normally distributed) was used for single point measurements. When a significant difference was found, pairwise comparisons by the Student-Newman-Keuls test were used to determine treatment differences. *$P < 0.05$ vs. basal, ‡$P < 0.001$ vs. basal, †$P < 0.005$ vs. basal, #$P < 0.05$ vs. pre-RT. sec, seconds.

Diet and Medication

Dietary habits as assessed by 3-day food record were not different before or after RT (Supplementary Table 2). Participants' medication and dose did not change during participation (self-reported) in this study.

Glycemic Control

Improvements in fasting blood glucose levels, HbA_{1c} levels, and glucose tolerance (OGC AUC) were significantly influenced by a higher skeletal muscle MBF after RT (Table 2). Specifically, an individual improvement in MBF response by 0.1 AI/s with RT translates to fasting blood glucose decreasing by 0.95 mmol/L (P = 0.003) and HbA_{1c} by 0.16% (1.76 mmol/mol) (P = 0.025) and glucose tolerance improving by ~5% (AUC reduced by 77.1 mmol/L × 120 min, P = 0.021) over pre-RT levels when adjusted for age, sex, percent body fat, and percent lean mass. Improvements in fasting blood glucose levels, HbA_{1c} levels, and glucose tolerance remained significantly influenced by MBF after RT when adjusted for other covariates including BMI, brachial artery blood flow, and mean arterial pressure (Table 2).

The overall (between subjects) relationship between MBF and blood glucose or glucose tolerance AUC was also significant when adjusted for three to four covariates at a time (Table 2). However, the overall (between subjects) relationship between MBF and HbA_{1c} was not significantly associated despite significant changes pre- versus post-RT.

Univariate analysis showed that improvements in total AGE (P = 0.67) levels could not be explained by improvements in MBF in muscle. This was also confirmed when applied to the mixed linear regression analysis (data not shown).

CONCLUSIONS

This study provides the first evidence that exercise training (of any kind) improves muscle MBF in subjects with type 2 diabetes. Specifically, 6 weeks of RT caused an increased MBF in response to an OGC. This microvascular improvement with RT was accompanied by improvements in glycemic control. Thus, RT-enhanced microvascular responsiveness in muscle may contribute to the metabolic improvements observed with RT. As insulin levels in response to a glucose load did not change with RT, the improved MBF responsiveness to an OGC is thought to be related

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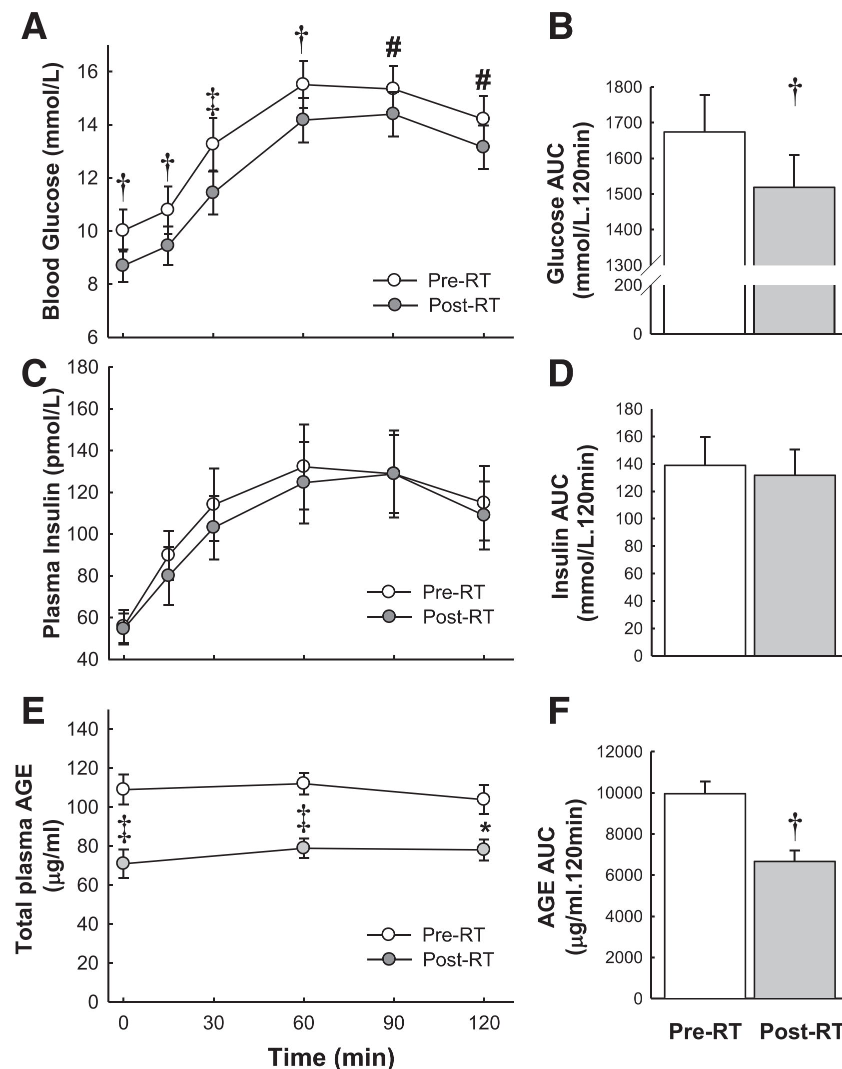

Figure 2—Glucose, insulin, and AGE levels during an OGC before and after RT. Blood glucose (*A*), plasma insulin (*C*), and total plasma AGE levels (*E*) during a 2-h OGC. AUC for each is represented in *B*, *D*, and *F*, respectively. Data are means ± SEM for each group (n = 17). Repeated-measures two-way ANOVA was used to determine whether there were differences between treatment groups over the time course of the experiment or Student t test (or signed rank test if data not normally distributed) was used for single point measurements. When a significant difference was found, pairwise comparisons by the Student-Newman-Keuls test were used to determine treatment differences. ‡$P < 0.001$, †$P < 0.005$, *$P < 0.01$, #$P < 0.05$ vs. pre-RT.

to improved microvascular insulin sensitivity. Mixed linear regression analysis of the improved skeletal muscle MBF response after RT was significantly associated with reductions in fasting blood glucose, HbA_{1c}, and glucose tolerance after adjustment for sex, age, BMI, percent body fat, percent lean mass, mean arterial pressure, and/or brachial artery blood flow.

The current findings on vascular and metabolic function occurred with an increase in relative strength and without changes in medications or dietary intake. The acute effect of diabetes medication (e.g., metformin versus sulfonylurea) is not expected to impact the clinical results reported here because all diabetes medication was withheld for 48 h prior to testing. Whether certain medications have any chronic actions that are still apparent even after the 48-h washout period is unknown. Thus, a limitation of the study is that not all participants were on the same diabetes medication. As 3-day food records did not indicate any dietary changes during the 6-week intervention, and physical activity (outside of participation in RT) did not change, the cardiometabolic improvements noted are attributed to the RT program. However, like other exercise interventions (30), a caveat to the study is that a nonexercising control group, or an attention control group, was not used. We have used mixed linear regression analysis to dissect effects post-RT (within subjects) as well as overall effects (between subjects). Whether changes in metabolic and vascular responses were solely due to the RT will need to be confirmed in a larger, controlled clinical trial.

Previous work has shown that insulin-mediated increases in skeletal muscle microvascular responses are blunted in insulin resistant rats (15) and humans (31), implicating reduced microvascular responses in whole-body insulin resistance. Prior to RT in the current study, we found no change in skeletal muscle MBF from baseline to 1 h after the OGC (Fig. 1*A*) despite a two- to threefold increase in plasma insulin concentration. This is not surprising, given that we previously found that a key feature of both insulin resistance and type 2 diabetes is an inability of insulin to increase MBF in skeletal muscle (31,32). Importantly, we found that RT was able to improve skeletal muscle MBF in response to a similar insulin concentration and that this was associated with improved glucose clearance from the blood. Thus, our data highlight that RT improves glycemic control in subjects with type 2 diabetes by improving insulin-stimulated MBF in muscle, thus enhancing glucose disposal.

MBF in response to OGC and RT differs between muscle (CEU) and skin (LDF). Skin MBF increased by 60 min during the OGC and was not altered after RT (Fig. 1*G* and *H*). Others have reported a similar stimulation in skin MBF during an oral glucose tolerance test (75 g glucose) (33) and the euglycemic-hyperinsulinemic clamp (skin MBF assessed by capillary videomicroscopy) (13). However, our study suggests that RT alters vascular insulin sensitivity favoring muscle MBF over skin in response to an OGC.

The connection between exercise and glucoregulatory control has been well-documented (34), although specific mechanisms are lacking (35). Our study demonstrates for the first time that improvements in MBF after RT can predict the improvements in glycemic control (fasting blood glucose, HbA_{1c}, and glucose tolerance) after RT in people with type 2 diabetes (Table 2). Although total AGE levels were significantly reduced after RT, the lack of association between MBF

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Table 2—Mixed linear regression analysis to evaluate impact of MBF on fasting blood glucose, HbA_{1c}, and glucose tolerance (OGC AUC)

Variable	β	95% CI	*P*
Fasting blood glucose, mmol/L			
MBF change with RT (0.1 AI/s)			
Model 1	−0.82	−1.43, −0.21	0.001
Model 2	−0.96	−1.67, −0.28	0.001
Model 3	−0.95	−1.63, −0.28	0.003
MBF overall (0.1 AI/s)			
Model 1	−2.76	−4.44, −1.09	0.008
Model 2	−2.72	−4.32, −1.12	0.006
Model 3	−2.52	−4.15, −0.88	0.006
Glucose tolerance (AUC), mmol/L × 120 min			
MBF change with RT (0.1 AI/s)			
Model 1	−85.8	−149.8, −21.9	0.009
Model 2	−90.7	−158.7, −22.7	0.031
Model 3	−77.1	−149.6, −4.71	0.021
MBF overall (0.1 AI/s)			
Model 1	−328.2	−574.9, −81.5	0.008
Model 2	−288.6	−550.8, −26.3	0.009
Model 3	−294.9	−546.2, −43.6	0.037
HbA_{1c}, % (mmol/mol)			
MBF change with RT (0.1 AI/s)			
Model 1	−0.11 (−1.24)	−0.30, 0.07 (−3.27, 0.79)	0.008
Model 2	−0.15 (−1.67)	−0.37, 0.07 (−4.09, 0.75)	0.019
Model 3	−0.16 (−1.76)	−0.39, 0.07 (−4.29, 0.78)	0.025
MBF overall (0.1 AI/s)			
Model 1	−1.10 (−12.0)	−1.91, −0.28 (−20.9, −3.1)	0.231
Model 2	−0.98 (−10.7)	−1.79, −0.16 (−19.6, −1.8)	0.177
Model 3	−0.93 (−10.1)	−1.74, −0.117 (−19.0, −1.3)	0.175

Model 1: analyses adjusted for sex, age, and BMI. Model 2: analyses adjusted for sex, age, brachial blood flow, and mean arterial pressure. Model 3: analyses adjusted for sex, age, percent body fat, and percent lean mass. AI/s, acoustic intensity per second.

and AGE suggests that other factors are involved in reducing AGE levels such as enzymatic modifications after RT (e.g., reducing oxidative stress) (36).

There were associations between MBF and HbA_{1c} levels in response to RT; however when assessed between subjects (overall effect), this association was lost. We believe this discrepancy is due to *1*) HbA_{1c} representing an average of the 3 previous months of blood glucose and *2*) all participants being on different combinations/doses of diabetes medications—therefore, HbA_{1c} levels reflect how well these medications control glucose over a 3-month period. Therefore it is not surprising that we saw significant associations between MBF and HbA_{1c} with 6 weeks of RT and no overall associations between participants. We anticipate that an overall association would have been evident if we trained people with type 2 diabetes who were drug naïve.

Before RT, participants had elevated brachial artery blood flow (i.e., total flow) in response to the OGC, which was reduced after RT (Fig. 1*E*). We have demonstrated that MBF in skeletal muscle increases independent of changes in total limb blood flow, e.g., during physiological doses of insulin (37) and low-intensity contraction (14,38). Conversely, total limb blood flow can also increase without changes in MBF (e.g., adrenaline) (39). Therefore, the skeletal muscle MBF increases in response to the OGC post-RT without a concomitant increase in total limb blood flow is not surprising. Therefore, we speculate that in individuals with type 2 diabetes, RT improves glucose tolerance by rerouting flow to the nutritive microvascular bed within skeletal muscle to augment substrate (e.g., glucose and insulin) and oxygen delivery to the myocytes.

Forearm contraction at 50% of maximal handgrip strength stimulates MBF in muscle (14), which was confirmed in the current study and did not change with RT (Fig. 1*C* and *D*). Although we did not perform histological analysis of capillary density, contraction-mediated microvascular recruitment serves as a surrogate measure of capillary density. As contraction-induced increases in microvascular responses were not different from pre- to post-RT, it is believed that 6 weeks of RT did not augment capillary density—unlike previous aerobic exercise findings (40). We propose that the augmentation in MBF in response to the OGC is due to enhanced recruitment of the microvasculature in response to insulin (rather than changes in capillary density per se) and therefore there is a greater surface area for glucose disposal in skeletal muscle. This is supported by recent work demonstrating that 4 h after an acute bout of exercise (single-legged exercise for 1 h) insulin-mediated microvascular recruitment and insulin-stimulated glucose disposal are augmented (25). The current study demonstrates a similar chronic effect of training on microvascular and metabolic response in type 2 diabetes 48 h after the last bout of exercise.

It has been shown that RT lowers central blood pressure in obese young men (9). However, the current study did not find any changes in central or peripheral hemodynamics (Table 1). It is important to note that participants on antihypertensive medications remained on these medications during all testing. It is possible that these drugs masked potential hemodynamic changes. Also of note is that increased MBF with training occurred independently of changes in either central or peripheral hemodynamics.

In summary, this study demonstrated that 6 weeks of RT enhanced muscle microvascular responses in subjects with type 2 diabetes, and this microvascular improvement is significantly linked to improved glycemic regulation. Furthermore, increased microvascular responsiveness to insulin is a likely mechanism contributing to the improved glycemic control noted in humans with type 2 diabetes after RT.

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Author Contributions. R.D.R., C.K.R., S.R., and M.A.K. were responsible for the conception and design of the research. R.D.R., D.H., T.G., S.J.B., R.M.D., J.E.S., K.A.S., A.A.B., M.B., D.P., S.H., and M.A.K. performed the experiments. P.O. conducted the linear regression analyses. All authors (including G.J., H.A.-A., and S.M.R.) analyzed and interpreted data. R.D.R. and M.A.K. drafted the manuscript. All authors revised the manuscript, with R.D.R. and M.A.K. having final approval. R.D.R. and M.A.K. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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