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Are the metabolic benefits of resistance training in type 2 diabetes linked to improvements in adipose tissue microvascular blood flow?

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the manuscript. All authors (including C.K.R. and G.J) contributed to writing the manuscript. D.H. and M.A.K. are the guarantors of this work and, as such, have full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Running head: Resistance training and adipose tissue blood flow

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ABSTRACT

The microcirculation in adipose tissue is markedly impaired in type 2 diabetes (T2D). Resistance training (RT) often increases muscle mass and promotes a favourable metabolic profile in people with T2D, even in the absence of fat loss. Whether the metabolic benefits of RT in T2D are linked to improvements in adipose tissue microvascular blood flow is unknown. Eighteen sedentary people with T2D (7F/11M, 52±7 years) completed six weeks of RT. Before and after RT, overnight-fasted participants had blood sampled for clinical chemistries (glucose, insulin, lipids, HbA1c and pro-inflammatory markers), underwent an oral glucose challenge (OGC, 50g glucose x 2hr) and a DEXA scan to assess body composition. Adipose tissue microvascular blood volume and flow were assessed at rest and 1hr post-OGC using contrast-enhanced ultrasound. RT significantly reduced fasting blood glucose ($p=0.006$), HbA1c ($p=0.007$), 2-hr glucose area under the time curve post-OGC ($p=0.014$) and HOMA-IR ($p=0.005$). This was accompanied by a small reduction in total body fat ($p=0.002$), trunk fat ($p=0.023$) and fasting triglyceride levels ($p=0.029$). Lean mass ($p=0.003$), circulating TNFα ($p=0.006$) and soluble VCAM-1 ($p<0.001$) increased post-RT. There were no significant changes in adipose tissue microvascular blood volume of flow following RT, however those who did have a higher baseline MBF post-RT also had lower fasting triglyceride levels ($r=-0.476$, $p=0.045$). The anthropometric, glycemic and insulin sensitizing benefits of six weeks of RT in people with T2D are not associated with an improvement in adipose tissue microvascular responses, however there may be an adipose tissue microvascular-linked benefit to fasting triglyceride levels.

INTROUDCTION

Resistance training (RT) is recommended for people with type 2 diabetes (T2D) to improve overall cardiometabolic health (1, 16). Specifically, RT improves insulin sensitivity, glycemic control, circulating lipids, body composition (i.e. increases muscle mass and reduces body fat) and is protective against cardiovascular disease through a variety of potential mechanisms (e.g. lowers blood pressure, aortic stiffness, and improves endothelial function) (26).

Skeletal muscle is an important site for glucose disposal in response to insulin (32), and an increased microvascular blood flow (MBF) improves delivery of glucose and hormones (such as insulin) to the myocyte to improve glucose disposal (19). We have recently demonstrated that six weeks of RT markedly enhances skeletal muscle MBF in T2D subjects in responses to an oral glucose challenge (OGC) (27). Importantly, this enhanced skeletal muscle microvascular response was tightly linked to improvements in overall glycemic control including reductions in fasting blood glucose and HbA1c levels, and improvements in glucose tolerance following an OGC. Although body composition was also affected by RT, vascular and metabolic changes were not related with changes in body composition (27). This novel finding positions the microvasculature in skeletal as an important regulator of overall glucose homeostasis.

Albeit less than skeletal muscle, adipose tissue is also a site for glucose disposal following a meal (17). Perhaps more importantly, adipose tissue is a key site for the release of non-esterified fatty acids (NEFAs) and a storage site for triglycerides (13). Similar to skeletal muscle, adipose tissue has a dynamic microvascular blood supply to help promote the delivery and release of nutrients such as oxygen, glucose and

lipids (7). We (15) and others (33), have recently reported impairments in MBF and the recruitment of capillaries (microvascular blood volume, MBV) in response to an OGC in central subcutaneous adipose tissue of people with T2D. These microvascular impairments in adipose tissue, in particular MBF, were associated with a greater degree of obesity, insulin resistance, hypertriglyceridemia, elevated NEFA levels, hyperglycemia and glucose intolerance (15). Therefore, improving microvascular function in adipose tissue may be a novel approach to prevent pathogenesis of obesity related complications such as insulin resistance, dyslipidemia and glucotoxicity.

While previous studies have demonstrated that exercise training improves microvascular flow (and consequently metabolic function) in skeletal muscle, there have been no studies assessing the impact of exercise training on adipose tissue microvascular responses in people with T2D. In the present study, we sought to determine if six weeks of RT reverses impaired adipose tissue microvascular responses noted in sedentary people with T2D (15) and whether this is paralleled by improvements in insulin resistance, hyperglycemia and dyslipidemia.

METHODS

The study was carried out in accordance with the Declaration of Helsinki as revised in 2008. The study protocol was approved by the Tasmania Health & Medical Human Research Ethics Committee. Participants from this study were recruited as part of a previously-published exercise study (27). However, two participants from the previous study (27) were removed due to poor adipose tissue ultrasound image quality, and three additional participants were recruited and underwent the 6 weeks of RT for the current adipose tissue study.

Screening Visit

Sedentary (self-reported <30 min of moderate exercise per week) people with T2D were recruited through community advertisement. On screening, participants were invited to the Menzies Institute for Medical Research Clinical Centre to establish eligibility by using a medical questionnaire. Participants were included in the study if they were between 18 and 60 years of age, had a clinical diagnosis of T2D, and had a BMI of 18 - 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. 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 five years, or severe liver disease.

A prior power calculation determined that 16 people would be needed to detect a 30% improvement in MBF in response to RT (power = 0.8, $\alpha = 0.05$). This estimate was based on our previous work (15) where healthy people increase adipose tissue MBV

and MBF by ~30% in response to an OGC, whereas T2D did not increase MBV or MBF at all. We anticipated that 6 weeks of training would correct this microvascular dysfunction in adipose tissue and would stimulate MBV and MBF by a similar 30%. Therefore, twenty people with T2D completed the RT program. Data from two participants were excluded due to low quality ultrasound images (insufficient microbubble signal to accurately quantify MBF). Data from eighteen people (52 ± 7 years, 7F/11M) were used for the final analysis.

Clinic Visit

After the screening visit, participants were invited back after an overnight fast for testing. Participants refrained from exercise and alcohol 48 hr prior to testing and caffeine on the morning of the study. Diabetes medications were stopped for 48 hr prior to testing. Participants were asked to complete a physical activity questionnaire (IPAQ) to confirm eligibility that they were sedentary. All participants underwent a variety of testing procedures as described below.

Body composition

Subjects underwent a whole body scan by dual-energy X-ray absorptiometry (Discovery W, Hologic, Bedford MA, USA) to assess body composition before and after RT. Total body fat, total body fat percentage, trunk fat and lean mass were calculated using Hologic Apex System Software version 4.0.2 as previously reported (31). Height and weight were also measured.

Clinical Chemistries and Oral Glucose Challenge (OGC)

After a 12h overnight fast, subjects were placed in a semi-recumbent position in an adjustable bed. A small polyethylene catheter was placed into an antecubital vein of one arm for blood sampling between 8 a.m. and 10 a.m. Before the oral glucose

challenge (OGC, 50 g glucose), plasma and serum samples were collected and sent to Royal Hobart Hospital Pathology for the measurement of lipids and HbA1c. After the ingestion of 50 g of glucose (GLUCO SCAN), blood samples were collected at 15, 30, 60, 90, and 120 min for the measurement of glucose, NEFA and insulin concentrations. The blood collection tubes were immediately immersed in ice and centrifuged at 2400 *g* for 10 min. All plasma and serum samples were frozen and stored at -80°C until analysis. Plasma glucose was measured by using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was assayed by using ELISA (Mercodia, Sweden). Plasma NEFA levels were determined by using an enzymatic assay kit (Wako Pure Chemical Industries, Osaka, Japan).

Assessment of adipose tissue microvascular blood flow

Central (truncal) subcutaneous adipose tissue microvascular blood flow was assessed by real-time contrast enhanced ultrasound (CEU). A linear array transducer (L9-3) interfaced with an ultrasound system (iU22; Philips Medical Systems, Australia) was placed horizontally over the abdomen (immediately right of the umbilicus) and the beam focused on the subcutaneous adipose tissue depot. Microbubbles (Lantheus Medical Imaging, Melbourne, Australia) were diluted (1.5ml added to 30ml saline) and continuously infused intravenously at 2.0-2.6 ml/min (equating to 0.03 ml/min/kg body weight) for adipose tissue imaging. Once the systemic microbubble concentration reached steady-state (5 min), a high energy destructive pulse of ultrasound was transmitted to instantaneously destroy microbubbles within the volume of adipose tissue being imaged. The reflow dynamics of microbubbles into adipose tissue microvasculature was assessed in real-time at baseline and then repeated 1hr post-OGC. We have chosen the 1hr post-OGC time point because we have previously demonstrated that differences in the microvascular actions of the

OGC can be detected at this time point and are correlated to changes in metabolism and anthropometric measures (15).

Gain settings (90%), mechanical index (0.11 for continuous and 1.30 for flash), compression (C=30), depth and focus were identical in pre-RT *versus* post-RT. Adipose tissue within the abdomen was imaged and the region of interest drawn within the adipose tissue bed that was visible as per our previous publication (15). Digital image analysis was performed off-line using Qlab (Philips Medical Systems, Australia). Images were background subtracted (using the 0.5 sec frame) as published previously to eliminate signal from larger blood vessels and tissue *per se* (15). Analysis of the data was performed identically for baseline and 1 hr after OGC. Background-subtracted acoustic-intensity *versus* time was fitted to the function: $y = A(1-e^{-\beta(t-tb)})$ where: y is acoustic-intensity at time t, tb the background time, A is plateau acoustic intensity (microvascular blood volume, MBV), and β is the rate constant (a measure of microvascular re-filling rate). Microvascular blood flow (MBF) was determined by $A \times \beta$. While the investigators performing the analysis of MBF data by CEU were not blinded to the group allocation, the analysis was performed independently by two investigators (D.H and D.R). Both sets of analysis produced the same finding, that 6 weeks of RT did not alter microvascular response in adipose tissue. Only the analysis from D.H was used for the publication.

Inflammatory cytokines/markers

Plasma concentrations of tumour necrosis factor alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin 6 (IL-6), C-reactive protein (CRP), monocyte chemoattractant protein-1 (MCP-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were

determined using commercially available ELISA (ELISAKIT, Australia). All measurements were conducted as per manufacturer's instructions.

Resistance Training (RT) Intervention

The RT programme used in this study was in accordance with recommendations from the American College of Sports Medicine (ACSM) and based on previous RT studies (27, 28). Exercise training was performed three days per week at the same time at a local fitness centre in Hobart, Tasmania, Australia (All Aerobic Fitness). The training regime was divided into a full body RT workout on Monday and Friday, with core, alternative strength and stability exercises on Wednesday. The full body workout used a mixture of free-weights and resistance machines. One set of each resistance exercise was performed to complete muscle failure (6-15 reps) and included: 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. Core, alternative strength, and stability exercise workouts used a range of resistance-focused techniques including, but not limited to, dumbbell sit-ups, dynamic medicine ball movements, weighted farmer’s walk, and a series of floor exercises, including leg-lifts, 3-way plank position, burpees, and exercise ball movements. Workouts were continually monitored and modified to match increased strength and fitness with load progression.

Each session was limited to one hour. All resistance exercises were recorded with the load incrementally increased [to achieve muscle failure between 5 and 12 repetitions, indicative of maintaining workout loads of between 65%-85% of calculated 1 repetition maximum (1RM)] as strength was increased to ensure training progression.

Statistics

Data are presented as the means ± standard deviation. Student's paired t-test was used to compare end point measurements between Pre-RT and Post-RT. When data were not normally distributed, a Wilcoxon Signed Rank Test was performed. For all continuous variables, a two-way repeated measures ANOVA (interactions: time: 0 and 60 min group: pre-RT and post-RT) followed by a Student–Newman–Keuls post-hoc was performed. Pearson bivariate correlations were used to evaluate associations. Spearman correlations were used to evaluate associations when data were not normally distributed. Significance was set at $p<0.05$. Tests were performed using SigmaStat™ statistical program (Systat Software, San Jose, CA, USA).

RESULTS

Characteristics of subjects before and after RT

The characteristics of participants before and after RT are presented in Table 1. Six weeks of RT resulted in significant reductions in total body fat (p = 0.002) and trunk fat (p = 0.023), and an increase in lean mass (p=0.003). These changes in body composition occurred without changes in overall body weight or BMI. Fasting blood glucose (p = 0.006), HbA1c (p = 0.007), HOMA-IR (p = 0.005) and fasting triglyceride levels (p = 0.029) were significantly lower following RT, whereas fasting plasma insulin, QUICKI, blood pressure, total cholesterol, HDL, LDL and NEFA were unaffected.

Glucose, insulin and NEFA responses to the OGC before and after RT

Figure 1 shows the time course of blood glucose, plasma insulin and plasma NEFA levels before and after a 50 g OGC. Following RT, plasma glucose levels were significantly lower during the OGC except at 90 min (Figure 1A) and the area under the glucose time curve (Figure 1B) was significantly lower (p = 0.014). Plasma insulin levels during the OGC at 15, 30 and 60 min post-OGC, and area under the insulin time curve (p = 0.036) were significantly lower after RT (Figure 1C/D). Plasma NEFA levels during the OGC were significantly lower from 30 min post-RT. The area under the curve for plasma NEFA was significantly lower after RT (Figure 1 E/F). However, the incremental AUC for glucose, insulin and NEFA in response to the OGC were not significantly lower following RT (data not shown).

Adipose tissue MBF responses to OGC before and after RT

Adipose tissue microvascular responses to the OGC before and after RT are shown in Figure 2. Baseline MBV (p = 0.102), β (p = 0.885), and MBF (p = 0.225) did not

change following RT. Similarly, there were no significant changes in MBV, β or MBF responses to the OGC after six weeks of RT (Figure 2).

Effect of RT on circulating pro-inflammatory markers

Pro-inflammatory cytokines measured by ELISA before and after RT are shown in Figure 3. There was a significant increase in TNF-α and sVCAM-1 following six weeks of RT. However, there were no statistically significant differences observed in IL-6, CRP, MCP-1, or IL-1β after RT.

Correlates of Adipose Tissue MBV and MBF

Adipose MBV and MBF were correlated with measures that were significantly improved following RT. Changes in fasting blood glucose, glucose AUC during the OGC, HbA1c, insulin AUC during the OGC, NEFA AUC during the OGC, TNFα, sVCVAM-1 levels, HOMA IR and truncal adiposity did not correlate with changes in the microcirculation (MBV and MBF) in adipose tissue following RT (Table 2). However, there was a negative correlation ($r=-0.476$, $p=0.045$) between changes in baseline adipose MBF and changes in fasting triglyceride levels following RT (Table 2 and Figure 4). However, this relationship is influenced by one individual with unusually elevated triglyceride level and lower baseline MBF following RT. The correlation is no longer significant ($r= -0.377$, $p=0.131$) if this data point is removed from the analysis.

DISCUSSION

The current study demonstrates that six weeks of RT in people with T2D produced favorable effects of glycemic regulation, insulin sensitivity and body composition, however these effects occurred without a concomitant increase in adipose tissue MBV or MBF at rest or during an OGC. However those who did respond with a higher baseline MBF also had lower fasting triglyceride levels (r=-0.476, p=0.045). The anthropometric, glycemic and insulin sensitizing benefits of six weeks of RT in people with T2D are not associated with an improvement in adipose tissue microvascular responses, however there may be an adipose tissue microvascular-linked benefit to fasting triglyceride levels.

There are very few studies that have investigated the effects of chronic exercise interventions on human adipose tissue blood flow. To date, most studies on human adipose tissue blood flow, such as those of Frayn and colleagues (10-12), have used ^{133}Xenon washout which measures the disappearance of the isotope injected into adipose tissue, where faster disappearance reflects higher blood flow in adipose tissue. Using this technique it has been reported that adipose tissue blood flow is higher in trained *versus* sedentary healthy individuals (29, 30). Adipose tissue blood flow (using microspheres) has also been reported to be markedly higher in subcutaneous, mesenteric, parametrial and retroperitoneal fat depots of trained *versus* untrained rats (8). Given this finding, it would be reasonable to assume that exercise training interventions would likewise increase adipose tissue blood flow, however the evidence so far is not clear. Sixteen weeks of endurance exercise training in young healthy lean men improves aerobic capacity by ~25%, but does not improve body composition (fat mass or lean muscle mass) or resting or epinephrine stimulated adipose tissue blood flow (14). Similarly, 12 weeks of aerobic exercise training in

306 healthy older women produced a significant increase in exercise capacity, but again,
307 this improvement was not associated with changes in body composition or resting
308 adipose tissue blood flow (21). There have also been mixed findings regarding the
309 impact of chronic exercise training (12-16 weeks) on adipose tissue blood flow in
310 overweight/obese individuals when assessed indirectly using microdialysis (6, 24).
311 We reason that this lack of association between chronic exercise training and adipose
312 tissue blood flow may be due to indirect blood flow measurements which do not
313 assess flow at the microvascular level (which is the critical site for nutrient exchange).
314 In addition, previous studies have been conducted in healthy subjects where the
315 microcirculation is already functioning normally. In contrast, people with T2D have
316 impaired microvascular function, which in skeletal muscle, has been shown to
317 improve with exercise training. Given this, we hypothesised that microcirculation in
318 adipose tissue may respond in a similar way and that RT may help to restore this
319 impaired vascular function.

320 Over the past 15 years we have demonstrated the importance of microvascular blood
321 flow in determining insulin's metabolic effects in skeletal muscle, independent of
322 changes in total limb blood flow (3, 5, 18-20, 27, 34, 35). This was made possible in
323 part with the adaptation of the contrast enhanced ultrasound (CEU) technique for
324 skeletal muscle. In the present study we have used novel real-time CEU imaging to
325 assess microvascular blood flow responses in adipose tissue. This is an important
326 distinction from other techniques because nutrient exchange occurs at the
327 microvascular level. The CEU technique has the capacity to isolate the measurement
328 to the microcirculation and dissect different perfusion components – in particular,
329 microvascular blood volume (MBV – the number of capillaries being perfused),
330 microvascular flow velocity (β – the filling rate of the capillaries being perfused) and

331 microvascular blood flow (MBF – which is the product of MBV and β) (19, 36). Thus
332 using this technique we have been able to dissect different adipose tissue
333 microvascular responses in people with T2D and which components are altered
334 following six weeks of RT. We were surprised to find that adipose tissue
335 microvascular responses (MBV, β and MBF) were not altered following RT.

336 We have previously shown that chronic exercise training of rats improves skeletal
337 muscle microvascular responses to insulin in the absence of changes in muscle
338 capillary density (25). Others have reported that activity restricted non-human
339 primates have a lower skeletal muscle microvascular response to an intravenous
340 glucose challenge when compared to the normal activity group (4). We have
341 previously shown that adipose tissue MBF differs between healthy subjects and those
342 with T2D (15), and that 6 weeks of RT improves skeletal muscle MBF in those with
343 T2D, likely through improved insulin sensitivity (27). Taken together, it is reasonable
344 to believe that the same 6-week RT program might also improve MBF in adipose
345 tissue via the same insulin-related response seen in skeletal muscle (27). Therefore,
346 changes in MBF of subcutaneous adipose tissue was assessed fasting and post-OGC.
347 However, we observed that of the eighteen people tested, there was a range in their
348 adipose tissue MBF responses following RT – with an overall effect as being
349 negligible. Our study focused on central subcutaneous adipose tissue. Whether the
350 microvasculature in other fat depots (e.g. visceral) - which have different metabolic
351 demands - respond to 6 weeks of RT is not known and should be followed-up.
352 Nevertheless, our previous work demonstrates clear differences in MBV and MBF in
353 central subcutaneous adipose tissue between healthy and T2D subjects.

354 There are several possibilities as to why we did not see any significant improvements
355 in adipose tissue microvascular responses in adipose tissue in the majority of people

with T2D following RT. First, the length of training may not have been long enough to cause sufficient fat loss to see improvements in adipose tissue MBV or MBF at rest or during the OGC. This is particularly important given that the degree of obesity is negatively associated with adipose MBV and MBF (2, 15). Second, this type of exercise training (RT rather than aerobic exercise) may not be sufficient to sensitize the microcirculation to respond to the OGC. Although we have demonstrated marked improvements in skeletal muscle MBF following six weeks of RT in people with T2D (27), the regulation of the microcirculation between skeletal muscle and adipose tissue are clearly different. These tissue specific differences could also be due to both skeletal muscle cells and its vasculature being physically trained during RT, whereas adipose tissue is "passively trained". Third, the fat loss (albeit small) may not have caused concomitant microvascular remodelling. It is well known that during adipose tissue expansion (hypertrophy) capillary density declines (9) and therefore reducing adipocyte size may not necessarily alter capillary density. However, given that we did not do adipose tissue histology to determine adipocyte size or capillary density, we can only speculate at this stage. Fourth, we observed that following six weeks of RT, circulating TNFα and sVCAM-1 levels were significantly elevated (Figure 3). The effect of RT on systemic inflammation in people with T2D is divergent and appears to be dependent on the length of time training with some studies showing reductions after 9 months (23) whereas other showing increases after 3 weeks (22). Our cohort of T2D participants presented with a more inflammatory state after 6 weeks despite avoiding exercise for 48 hrs after the last bout of training before returning to the clinic for cardiometabolic testing. We have previously demonstrated that the pro-inflammatory cytokine TNFα can cause skeletal muscle microvascular insulin resistance in healthy rats (37). Whether the elevated TNFα and sVCAM-1 levels

observed post-RT caused microvascular insulin resistance in adipose tissue is not known and warrants further investigation. However, as TNFα plays a primary role in tissue repair, and the type and intensity of this RT program promotes acute muscle damage, it is possible that the elevated levels of TNFα are more indicative of muscular tissue repair rather than a pathogenic state. The improved skeletal muscle MBF noted in our previous study, and the lack of declining MBF in adipose in these findings support this notion. Additional studies could be performed varying the length of time between the last RT bout and clinical testing. Lastly, this study did not utilize a non-exercising control group. As such, it is not possible to determine if MBF in adipose would have declined in this time frame without RT. Therefore, results should be reproduced in a larger, controlled clinical trial and additional follow-up studies should be performed to determine the role of acute exercise or other pharmacological stimuli on adipose tissue MBF responses at various stages of the T2D continuum.

In summary, our findings demonstrate that the anthropometric, glycemic and insulin sensitizing benefits of six weeks of RT in people with T2D are not associated with an improvement in adipose tissue microvascular responses, however there may be an adipose tissue microvascular-linked benefit to fasting triglyceride levels. In addition, a pro-inflammatory phenotype after exercise training did not prevent the metabolic benefits of RT. Consequently, we can conclude that while targeting microvascular function in skeletal muscle may be a novel approach to preventing the pathogenesis of obesity, the role of microvascular function in adipose tissue is still uncertain.

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DISCLOSURE

No potential conflicts of interest relevant this article are declared by the authors.

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531 **FIGURE CAPTIONS**

532 **Figure 1: Blood glucose and insulin levels in response to a 50g OGC before and**
533 **after RT in people with T2D**. Blood glucose (A) plasma insulin (C) and plasma
534 NEFA (E) timelines in response to an OGC, and 2-hr glucose (B) insulin (D) and
535 NEFA (F) area under the time curve. Data are means ± SD for each group (n=18).
536 Repeated-measures two-way ANOVA was used to determine if there were differences
537 between treatment groups over the time course of the experiment, or Student's paired
538 t-test (or Signed Rank Test if data not normally distributed) was used for single point
539 measurements. When a significant difference was found, pairwise comparisons by the
540 Student–Newman–Keuls test was used to determine treatment differences. **P* < 0.05
541 vs. control; †*P* < 0.01 vs. Pre-RT, ‡P < 0.001.

542 **Figure 2: Adipose tissue microvascular blood volume (MBV), microvascular**
543 **filling rate (β) and microvascular blood flow (MBF) responses to an OGC before**
544 **and after RT in people with T2D.** MBV (A), β (B) and MBF (C) at baseline (time 0-
545 min) and after OGC (time 60-min). Data are presented as individual data points for
546 each participant and also expressed as means ± SD for each group (n=18). Repeated-
547 measures two-way ANOVA was used to determine if there were differences between
548 treatment groups over the time course of the experiment. When a significant
549 difference was found, pairwise comparisons by the Student–Newman–Keuls test was
550 used to determine treatment differences. Dotted (baseline) and solid lines (post OGC)
551 represent baseline and post OGC responses respectively in healthy people from a
552 previous published study by the authors (15).

553 **Figure 3: Fasting plasma pro-inflammatory cytokines before and after RT in**
554 **people with T2D.** TNF-α (A), IL-1β (B), IL-6 (C), CRP (D), MCP-1 (E) and
555 sVCAM-1 (F) concentrations before and after RT in people with T2D. Data are means

± SD (n=18). Student's paired t-test (or Signed Rank Test if data not normally distributed) was used for single point measurements.

Figure 4. Relationship between changes in baseline adipose tissue MBF and triglyceride levels following 6 weeks of RT. Spearman correlation was used to assess relationship between variables (n=18).

Characteristic	Pre-RT	Post-RT	P value
Age (years)	52 ± 7	-	-
Sex	7F/11M	-	-
Diabetes Duration (years)	9 ± 5	-	-
Diabetes Medication			
Lifestyle only (%)	1 (6)	-	-
Metformin (%)	17 (94)	-	-
Sulphonylurea (%)	2 (11)	-	-
Insulin (%)	2 (11)	-	-
GLP-1 RA (%)	2 (11)	-	-
DPP4 inhibitor (%)	1 (6)	-	-
SGLT2 inhibitor (%)	1 (6)	-	-
Height (cm)	170.9 ± 8.0	-	-
Weight (kg)	94.7 ± 26.0	90.5 ± 16.4	0.421
BMI (kg/m^2)	31.9 ± 7.4	30.8 ± 4.3	0.596
Body Fat			
Total fat (%)	32.1 ± 6.6	31.1 ± 16.8	**0.002**
Trunk fat (%)	34.1 ± 6.2	33.1 ± 6.2	**0.023**
Lean mass (%)	65.6 ± 6.7	66.5 ± 7.0	**0.003**
Fasting blood glucose (mmol/L)	10.2 ± 3.3	9.0 ± 3.0	**0.006**
Fasting plasma insulin (pmol/L)	111.9 ± 67.3	98.4 ± 55.6	0.108
HbA1c			
%	7.78 ± 1.58	7.44 ± 1.45	**0.007**
Insulin Sensitivity Indices			
HOMA-IR	7.76 ± 5.24	5.72 ± 4.08	**0.005**
QUICKI	0.30 ± 0.03	0.31 ± 0.02	0.078
Blood Pressure			
SBP (mmHg)	133 ± 15	130 ± 11	0.388
DBP (mmHg)	84 ± 11	83 ± 9	0.602
Lipids			
Total cholesterol (mmol/L)	4.69 ± 1.03	4.50 ± 0.99	0.260
Triglyceride (mmol/L)	1.82 ± 0.98	1.47 ± 0.66	**0.029**
HDL (mmol/L)	1.27 ± 0.46	1.28 ± 0.43	0.808
LDL (mmol/L)	2.60 ± 0.80	2.55 ± 0.86	0.734
NEFA (mmol/L)	0.59 ± 0.20	0.56 ± 0.27	0.485

Table 1: Characteristics of study participants before and after RT. Data expressed as Mean ± SD (n=18). Student's t-test (or Signed Rank Test if data not normally distributed) was used to determine differences. ACEi (angiotensin converting enzyme inhibitor), ARB (angiotensin receptor blocker), DPP4 (dipeptidyl peptidase 4), GLP-1 RA (glucagon-like peptide-1 receptor agonist), HDL (high

density lipoprotein), LDL (low density lipoprotein), NEFA (non-esterified fatty acid), SGLT2 (sodium-glucose cotransporter 2).

Variable	Δ Baseline MBV		Δ Baseline MBF		Δ OGC MBV		Δ OGC MBF	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**
Δ Fasting glucose (mM)	0.041	0.872	0.077	0.754	0.208	0.407	-0.022	0.931
Δ Glucose AUC (mM.2hr)	-0.047	0.852	-0.154	0.535	0.347	0.158	0.207	0.409
Δ HbA1c (%)	-0.257	0.320	-0.156	0.540	0.384	0.128	0.125	0.633
Δ Insulin AUC (pM.2hr)	-0.012	0.962	0.426	0.076	-0.256	0.305	0.270	0.278
Δ HOMA IR	0.061	0.805	0.028	0.908	-0.385	0.112	-0.271	0.270
Δ Fasting Triglyceride (mM)	0.176	0.484	**-0.476**	**0.045***	0.246	0.326	-0.109	0.667
Δ NEFA AUC (mM.2hr)	-0.436	0.071	-0.032	0.895	0.220	0.380	-0.036	0.887
Δ Trunk fat (%)	-0.022	0.930	0.053	0.831	-0.063	0.803	-0.022	0.932
Δ TNFα (pg/ml)	0.237	0.343	-0.224	0.365	-0.191	0.448	-0.025	0.921
Δ sVCAM-1 (ng/ml)	-0.255	0.301	0.077	0.754	0.106	0.668	0.086	0.729

572

573 **Table 2:** Correlates of adipose tissue MBV and MBF and variables altered by 6 weeks of RT. Pearson correlation was used between normally
574 distributed variables. Spearman correlation was used if any of the variables were not normally distributed. Δ represents post-RT minus pre-RT.
575 AUC indicates area under the curve; HOMA IR, homeostatic model assessment of insulin resistance; MBF, microvascular blood flow; MBV,
576 microvascular blood volume; NEFA, non-esterified fatty acids; OGC, oral glucose challenge; sVCAM, soluble vascular cell adhesion molecule;
577 and TNF, tumor necrosis factor. * bold indicate a significant correlation.

578

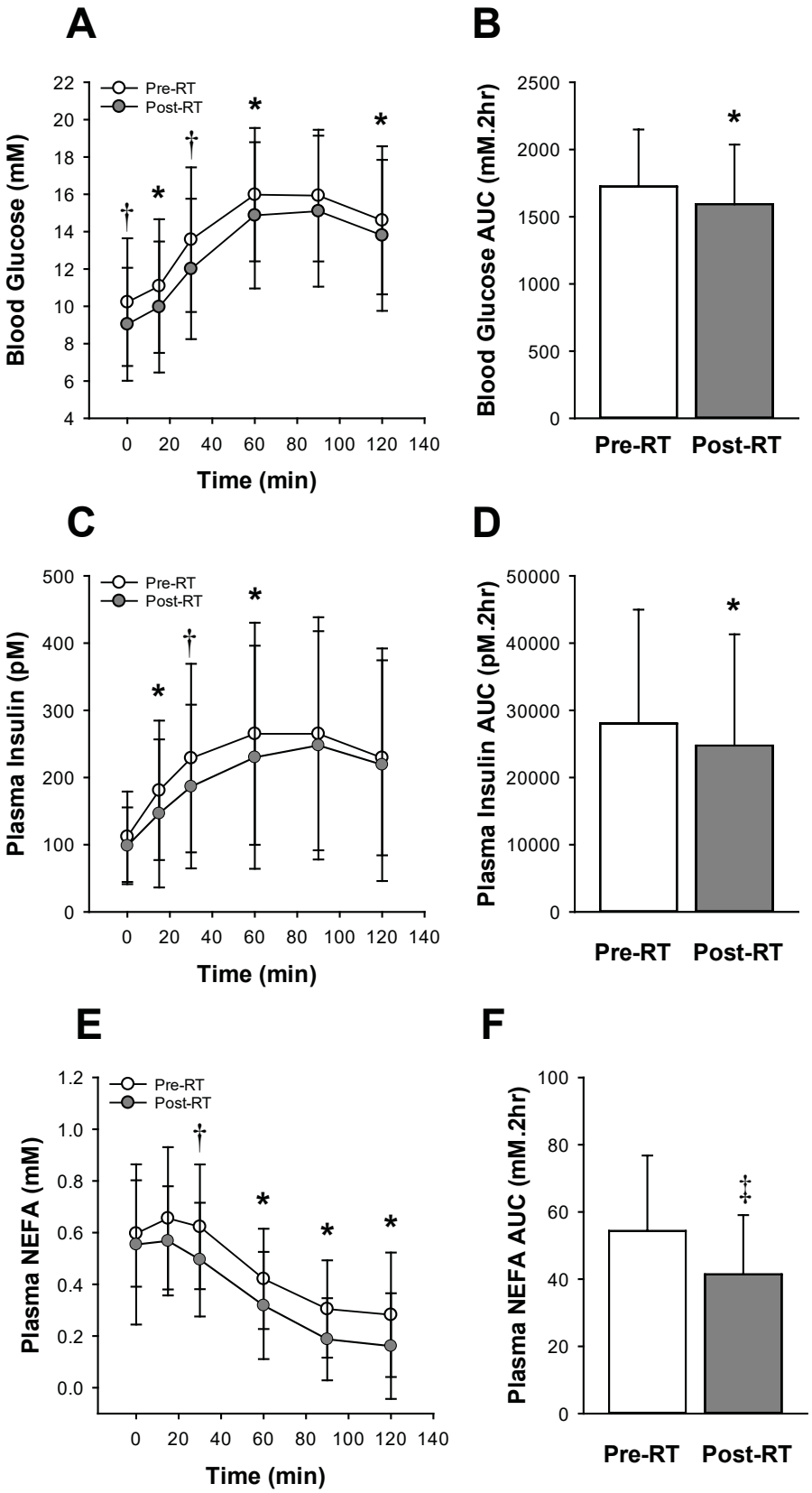

A
Pre-RT
Post-RT
Blood Glucose (mM)
Time (min)
B
Blood Glucose AUC (mM.2hr)
Pre-RT
Post-RT
C
Plasma Insulin (pM)
Time (min)
D
Plasma Insulin AUC (pM.2hr)
E
Plasma NEFA (mM)
Time (min)
F
Plasma NEFA AUC (mM.2hr)

Figure 1

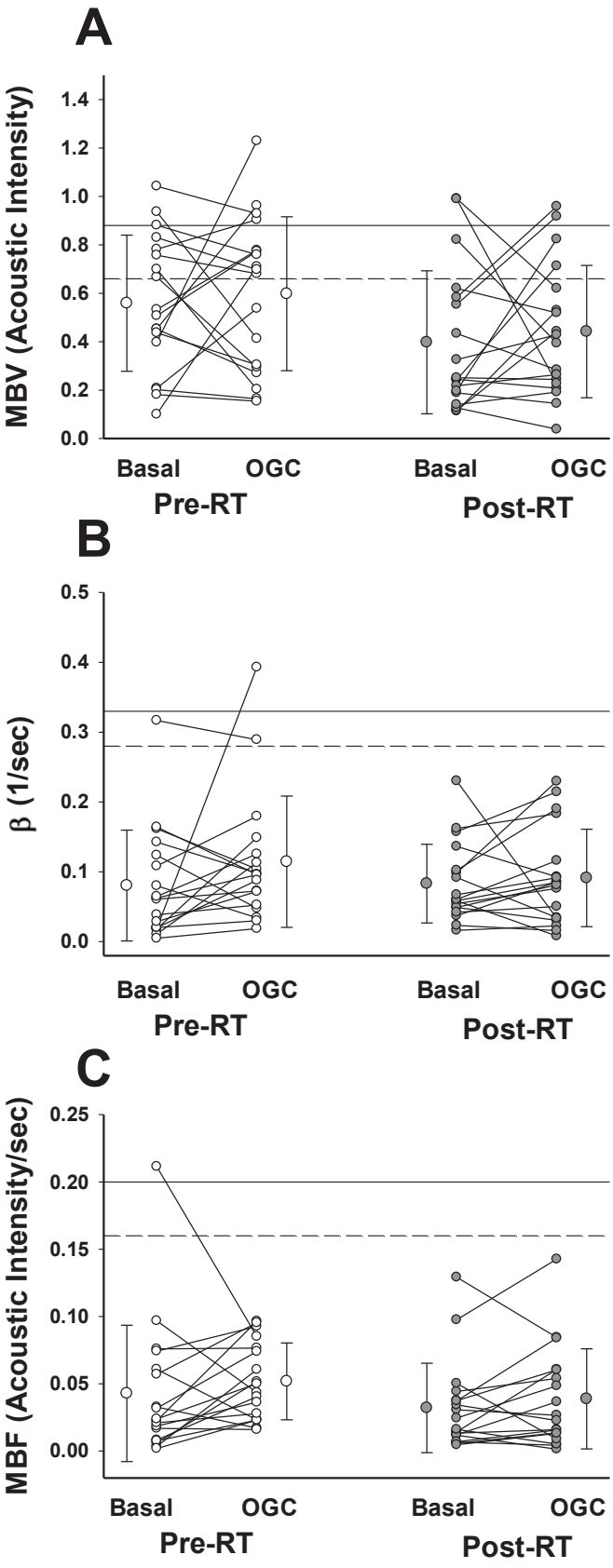

A
MBV (Acoustic Intensity)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Basal
OGC
Basal
OGC
Pre-RT
Post-RT
B
β (1/sec)
0.5
0.4
0.3
0.2
0.1
0.0
Basal
OGC
Basal
OGC
Pre-RT
Post-RT
C
MBF (Acoustic Intensity/sec)
0.25
0.20
0.15
0.10
0.05
0.00
Basal
OGC
Basal
OGC
Pre-RT
Post-RT

Figure 2

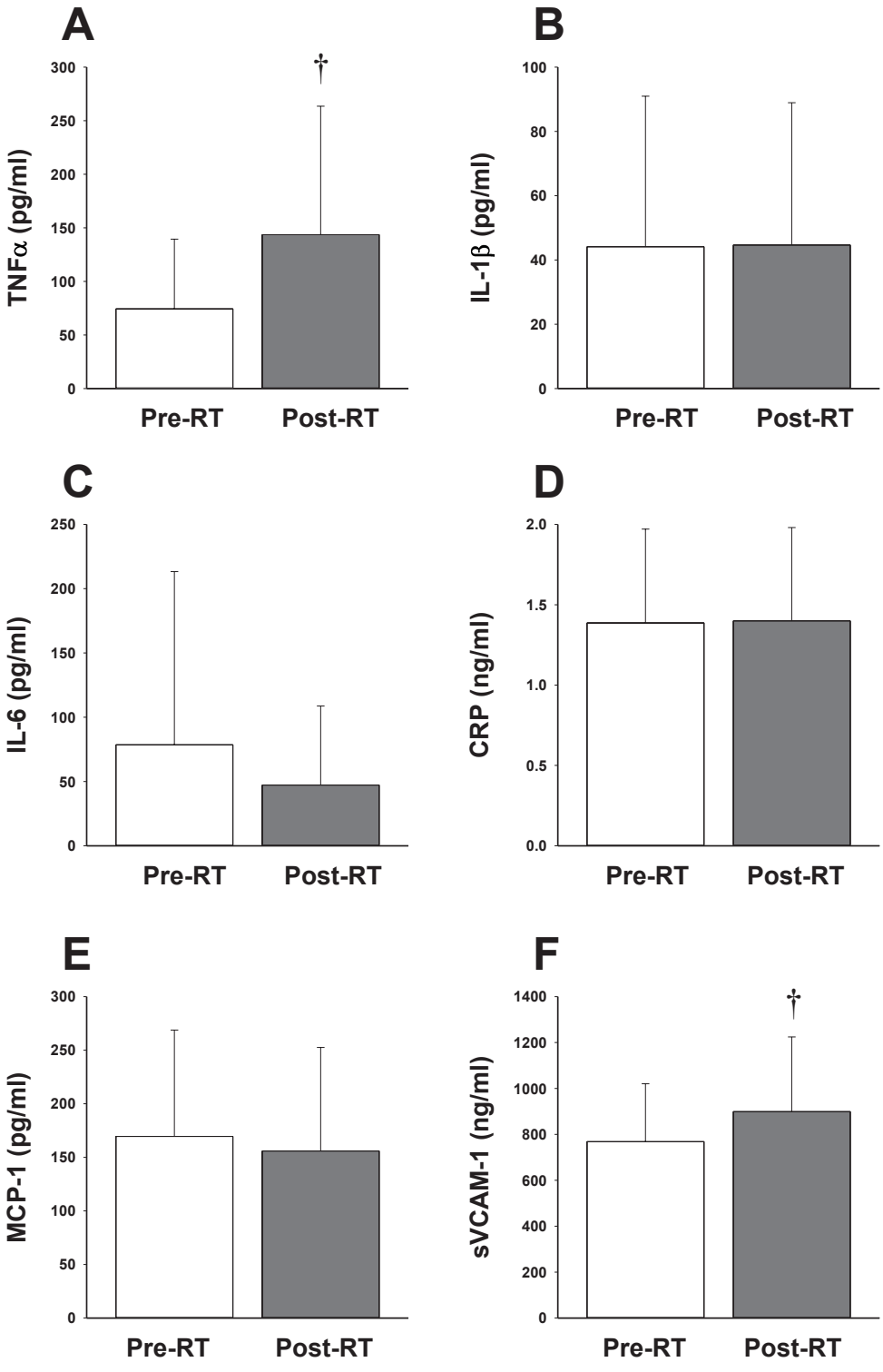
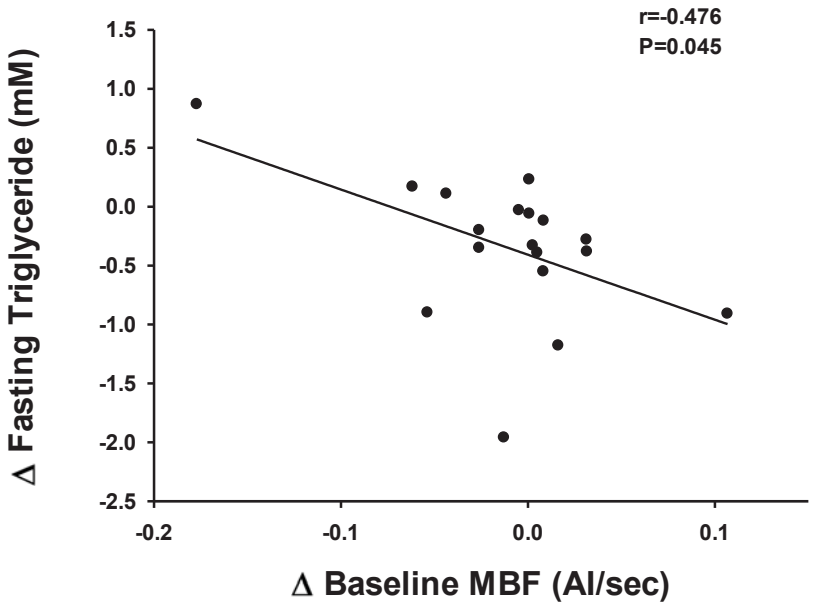

A
TNFα (pg/ml)
300
250
200
150
100
50
0
†
Pre-RT
Post-RT
B
IL-1β (pg/ml)
100
80
60
40
20
0
Pre-RT
Post-RT
C
IL-6 (pg/ml)
250
200
150
100
50
0
Pre-RT
Post-RT
D
CRP (ng/ml)
2.0
1.5
1.0
0.5
0.0
Pre-RT
Post-RT
E
MCP-1 (pg/ml)
300
250
200
150
100
50
0
Pre-RT
Post-RT
F
sVCAM-1 (ng/ml)
1400
1200
1000
800
600
400
200
0
†
Pre-RT
Post-RT

Figure 3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1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-0.2
-0.1
0.0
0.1
Δ Fasting Triglyceride (mM)
Δ Baseline MBF (Al/sec)

Figure 4