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Hu, Donghua, Ryan D. Russell, Devika Remash, Timothy Greenaway, Stephen Rattigan, Kathryn A. Squibb, Graeme Jones, et al. 2018. "Are the Metabolic Benefits of Resistance Training in Type 2 Diabetes Linked to Improvements in Adipose Tissue Microvascular Blood Flow?" *American Journal of Physiology-Endocrinology and Metabolism* 315 (6): E1242–50. <https://doi.org/10.1152/ajpendo.00234.2018>.

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2 **linked to improvements in adipose tissue microvascular blood flow?**

3

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24 conception and design of the research. D.H., R.D.R., D.R., T.G., D.P., K.A.S., R.M.R.
25 and M.A.K. performed the experiments. D.H. and M.A.K. researched data and wrote

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

30 **Running head:** Resistance training and adipose tissue blood flow

31

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38 **Key words:** Type 2 diabetes, exercise, metabolic physiology, microvascular blood
39 flow, adipose tissue

40

41 **ABSTRACT**

42 The microcirculation in adipose tissue is markedly impaired in type 2 diabetes (T2D).
43 Resistance training (RT) often increases muscle mass and promotes a favourable
44 metabolic profile in people with T2D, even in the absence of fat loss. Whether the
45 metabolic benefits of RT in T2D are linked to improvements in adipose tissue
46 microvascular blood flow is unknown. Eighteen sedentary people with T2D (7F/11M,
47 52±7 years) completed six weeks of RT. Before and after RT, overnight-fasted
48 participants had blood sampled for clinical chemistries (glucose, insulin, lipids,
49 HbA1c and pro-inflammatory markers), underwent an oral glucose challenge (OGC,
50 50g glucose x 2hr) and a DEXA scan to assess body composition. Adipose tissue
51 microvascular blood volume and flow were assessed at rest and 1hr post-OGC using
52 contrast-enhanced ultrasound. RT significantly reduced fasting blood glucose
53 (p=0.006), HbA1c (p=0.007), 2-hr glucose area under the time curve post-OGC
54 (p=0.014) and HOMA-IR (p=0.005). This was accompanied by a small reduction in
55 total body fat (p=0.002), trunk fat (p=0.023) and fasting triglyceride levels (p=0.029).
56 Lean mass (p=0.003), circulating TNF α (p=0.006) and soluble VCAM-1 (p<0.001)
57 increased post-RT. There were no significant changes in adipose tissue microvascular
58 blood volume of flow following RT, however those who did have a higher baseline
59 MBF post-RT also had lower fasting triglyceride levels (r=-0.476, p=0.045). The
60 anthropometric, glycemic and insulin sensitizing benefits of six weeks of RT in
61 people with T2D are not associated with an improvement in adipose tissue
62 microvascular responses, however there may be an adipose tissue microvascular-
63 linked benefit to fasting triglyceride levels.

64

65

66 INTRODUCTION

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

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

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

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

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

105

106

107 **METHODS**

108 The study was carried out in accordance with the Declaration of Helsinki as revised in
109 2008. The study protocol was approved by the Tasmania Health & Medical Human
110 Research Ethics Committee. Participants from this study were recruited as part of a
111 previously-published exercise study (27). However, two participants from the
112 previous study (27) were removed due to poor adipose tissue ultrasound image quality,
113 and three additional participants were recruited and underwent the 6 weeks of RT for
114 the current adipose tissue study.

115 **Screening Visit**

116 Sedentary (self-reported <30 min of moderate exercise per week) people with T2D
117 were recruited through community advertisement. On screening, participants were
118 invited to the Menzies Institute for Medical Research Clinical Centre to establish
119 eligibility by using a medical questionnaire. Participants were included in the study if
120 they were between 18 and 60 years of age, had a clinical diagnosis of T2D, and had a
121 BMI of 18 - 35 kg/m². Participants were excluded from the study if they participated
122 in any kind of resistance exercise or performed more than low-intensity walking.
123 Additional exclusion criterion included having a BMI >35 kg/m² or a personal history
124 of smoking, cardiovascular disease, stroke, myocardial infarction, uncontrolled
125 hypertension (seated brachial blood pressure >160/100 mmHg), peripheral arterial
126 disease, pulmonary disease, arthritis/muscular skeletal disease, malignancy within the
127 past five years, or severe liver disease.

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

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

138 **Clinic Visit**

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

145 **Body composition**

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

151 **Clinical Chemistries and Oral Glucose Challenge (OGC)**

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

155 challenge (OGC, 50 g glucose), plasma and serum samples were collected and sent to
156 Royal Hobart Hospital Pathology for the measurement of lipids and HbA1c. After the
157 ingestion of 50 g of glucose (GLUCO SCAN), blood samples were collected at 15, 30,
158 60, 90, and 120 min for the measurement of glucose, NEFA and insulin
159 concentrations. The blood collection tubes were immediately immersed in ice and
160 centrifuged at 2400 g for 10 min. All plasma and serum samples were frozen and
161 stored at -80°C until analysis. Plasma glucose was measured by using a YSI analyzer
162 (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was assayed by
163 using ELISA (Merckodia, Sweden). Plasma NEFA levels were determined by using an
164 enzymatic assay kit (Wako Pure Chemical Industries, Osaka, Japan).

165 **Assessment of adipose tissue microvascular blood flow**

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

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

182 Gain settings (90%), mechanical index (0.11 for continuous and 1.30 for flash),
183 compression (C=30), depth and focus were identical in pre-RT *versus* post-RT.

184 Adipose tissue within the abdomen was imaged and the region of interest drawn
185 within the adipose tissue bed that was visible as per our previous publication (15).

186 Digital image analysis was performed off-line using Qlab (Philips Medical Systems,
187 Australia). Images were background subtracted (using the 0.5 sec frame) as published
188 previously to eliminate signal from larger blood vessels and tissue *per se* (15).

189 Analysis of the data was performed identically for baseline and 1 hr after OGC.

190 Background-subtracted acoustic-intensity *versus* time was fitted to the function: $y = A$

191 $(1 - e^{-\beta(t-t_b)})$ where: y is acoustic-intensity at time t , t_b the background time, A is plateau

192 acoustic intensity (microvascular blood volume, MBV), and β is the rate constant (a

193 measure of microvascular re-filling rate). Microvascular blood flow (MBF) was

194 determined by $A \times \beta$. While the investigators performing the analysis of MBF data by

195 CEU were not blinded to the group allocation, the analysis was performed

196 independently by two investigators (D.H and D.R). Both sets of analysis produced the

197 same finding, that 6 weeks of RT did not alter microvascular response in adipose

198 tissue. Only the analysis from D.H was used for the publication.

199 **Inflammatory cytokines/markers**

200 Plasma concentrations of tumour necrosis factor alpha (TNF- α), interleukin-1 beta

201 (IL-1 β), interleukin 6 (IL-6), C-reactive protein (CRP), monocyte chemoattractant

202 protein-1 (MCP-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) were

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

205 **Resistance Training (RT) Intervention**

206 The RT programme used in this study was in accordance with recommendations from
207 the American College of Sports Medicine (ACSM) and based on previous RT studies
208 (27, 28). Exercise training was performed three days per week at the same time at a
209 local fitness centre in Hobart, Tasmania, Australia (All Aerobic Fitness). The training
210 regime was divided into a full body RT workout on Monday and Friday, with core,
211 alternative strength and stability exercises on Wednesday. The full body workout used
212 a mixture of free-weights and resistance machines. One set of each resistance exercise
213 was performed to complete muscle failure (6-15 reps) and included: leg press, lateral
214 pull-down, chest press, weighted lunges, seated row, back fly, bicep curl, incline chest
215 press, dumbbell shoulder press, leg extension, leg curl, dips, lateral shoulder raise,
216 triceps extension, dumbbell deadlift, and push-ups respectively. Core, alternative
217 strength, and stability exercise workouts used a range of resistance-focused
218 techniques including, but not limited to, dumbbell sit-ups, dynamic medicine ball
219 movements, weighted farmer's walk, and a series of floor exercises, including leg-lifts,
220 3-way plank position, burpees, and exercise ball movements. Workouts were
221 continually monitored and modified to match increased strength and fitness with load
222 progression.

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

227 **Statistics**

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

237

238 **RESULTS**

239 **Characteristics of subjects before and after RT**

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

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

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

259 **Adipose tissue MBF responses to OGC before and after RT**

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

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

264 **Effect of RT on circulating pro-inflammatory markers**

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

269 **Correlates of Adipose Tissue MBV and MBF**

270 Adipose MBV and MBF were correlated with measures that were significantly
271 improved following RT. Changes in fasting blood glucose, glucose AUC during the
272 OGC, HbA1c, insulin AUC during the OGC, NEFA AUC during the OGC, TNF α ,
273 sVCVAM-1 levels, HOMA IR and truncal adiposity did not correlate with changes in
274 the microcirculation (MBV and MBF) in adipose tissue following RT (Table 2).
275 However, there was a negative correlation ($r=-0.476$, $p=0.045$) between changes in
276 baseline adipose MBF and changes in fasting triglyceride levels following RT (Table
277 2 and Figure 4). However, this relationship is influenced by one individual with
278 unusually elevated triglyceride level and lower baseline MBF following RT. The
279 correlation is no longer significant ($r= -0.377$, $p=0.131$) if this data point is removed
280 from the analysis.

281 **DISCUSSION**

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

291 There are very few studies that have investigated the effects of chronic exercise
292 interventions on human adipose tissue blood flow. To date, most studies on human
293 adipose tissue blood flow, such as those of Frayn and colleagues (10-12), have used
294 $^{133}\text{Xenon}$ washout which measures the disappearance of the isotope injected into
295 adipose tissue, where faster disappearance reflects higher blood flow in adipose tissue.
296 Using this technique it has been reported that adipose tissue blood flow is higher in
297 trained *versus* sedentary healthy individuals (29, 30). Adipose tissue blood flow
298 (using microspheres) has also been reported to be markedly higher in subcutaneous,
299 mesenteric, parametrial and retroperitoneal fat depots of trained *versus* untrained rats
300 (8). Given this finding, it would be reasonable to assume that exercise training
301 interventions would likewise increase adipose tissue blood flow, however the
302 evidence so far is not clear. Sixteen weeks of endurance exercise training in young
303 healthy lean men improves aerobic capacity by ~25%, but does not improve body
304 composition (fat mass or lean muscle mass) or resting or epinephrine stimulated
305 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

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

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

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

402

403 **ACKNOWLEDGEMENTS**

404 This work was supported by the American College of Sports Medicine (2014 Visiting
405 Scholar Award), the virtual Tasmanian Academic Health Science Precinct, and the
406 Royal Hobart Hospital Research Foundation.

407

408 **DISCLOSURE**

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

410

411

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529

530

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 \pm 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 \pm 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-1 β (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

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

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

561

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/m²)	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

562

563 **Table 1: Characteristics of study participants before and after RT.** Data

564 expressed as Mean ± SD (n=18). Student's t-test (or Signed Rank Test if data not

565 normally distributed) was used to determine differences. ACEi (angiotensin

566 converting enzyme inhibitor), ARB (angiotensin receptor blocker), DPP4 (dipeptidyl

567 peptidase 4), GLP-1 RA (glucagon-like peptide-1 receptor agonist), HDL (high

568 density lipoprotein), LDL (low density lipoprotein), NEFA (non-esterified fatty acid),

569 SGLT2 (sodium-glucose cotransporter 2).

570

571

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

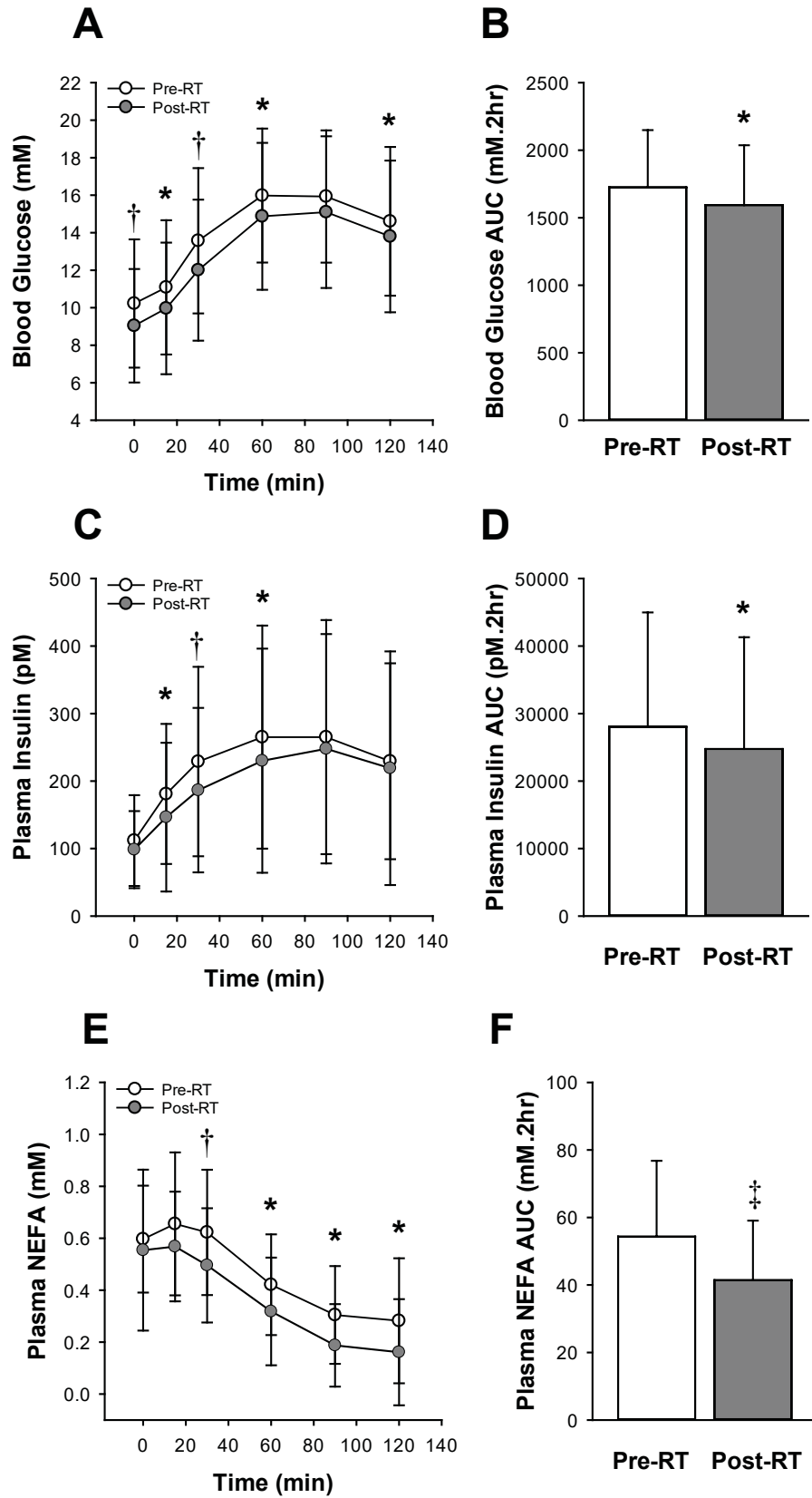


Figure 1

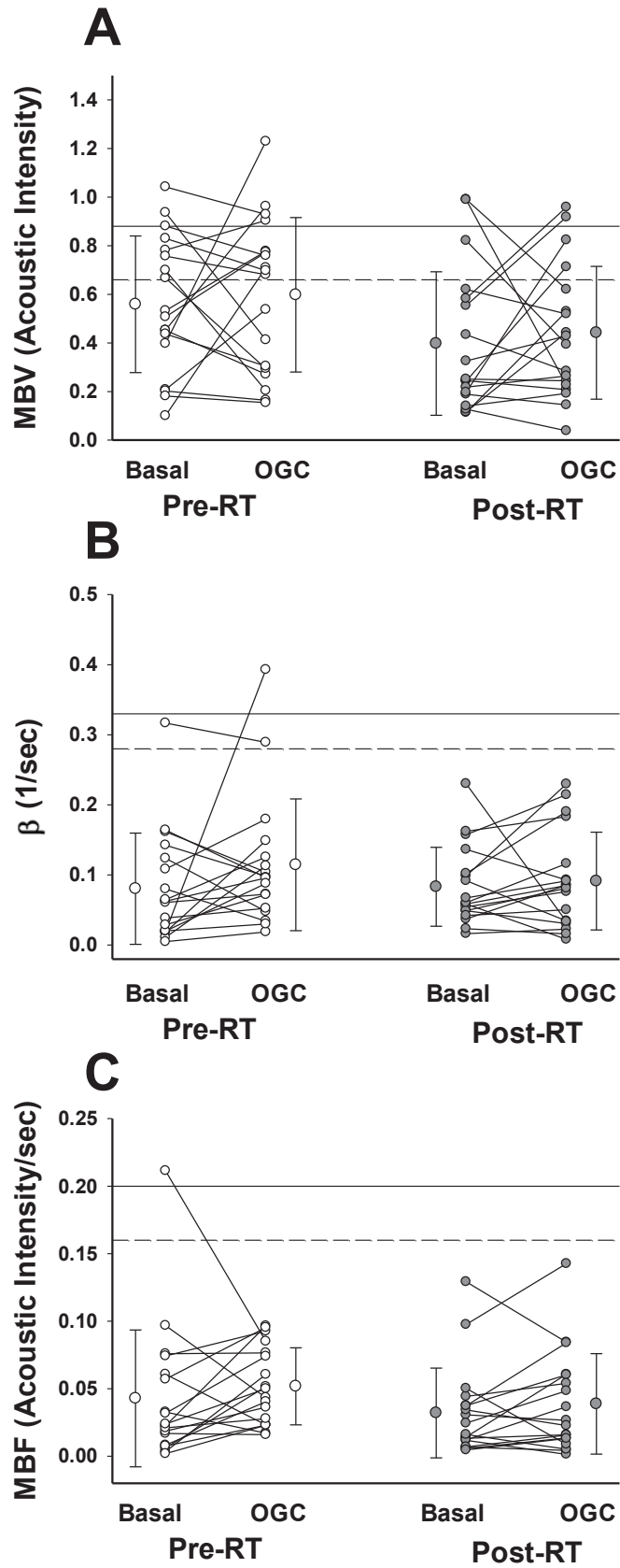


Figure 2

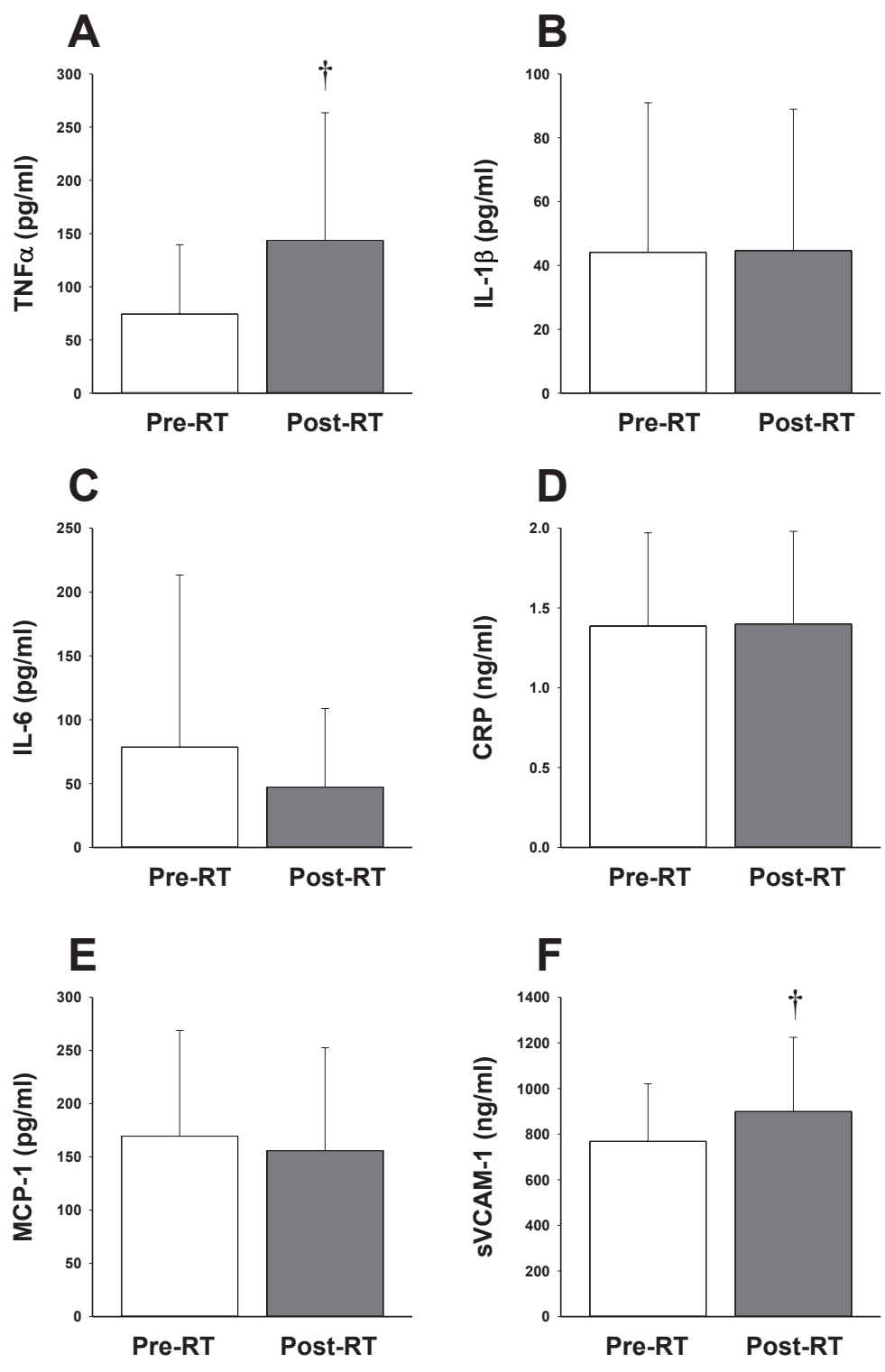


Figure 3

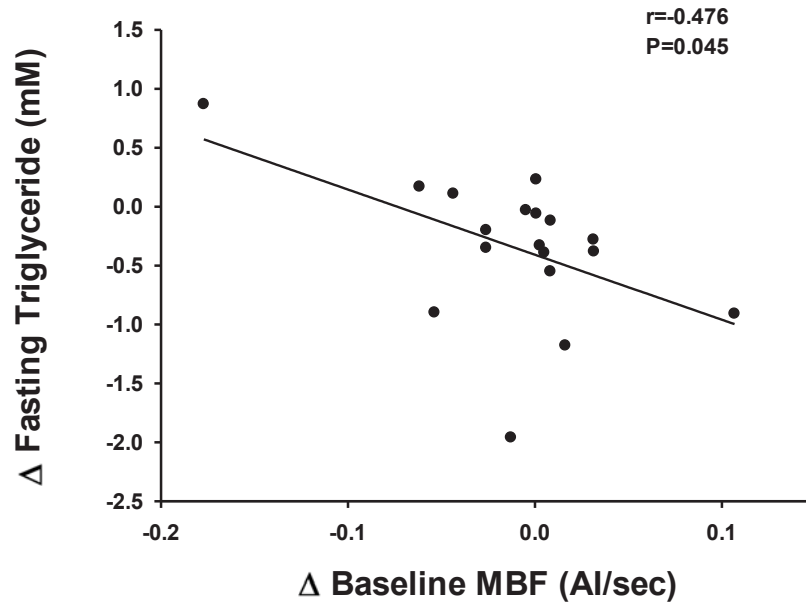


Figure 4