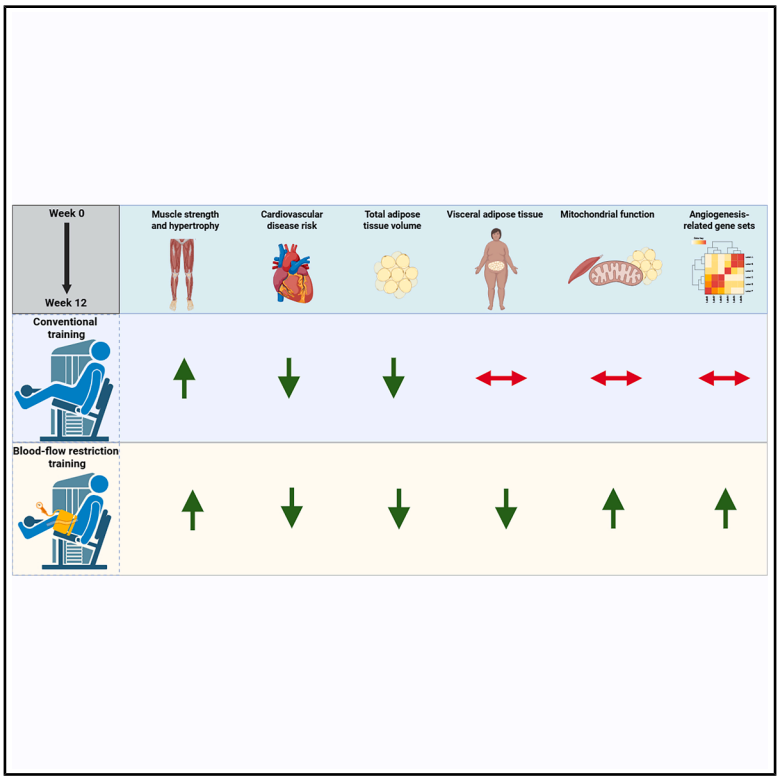
Cell Metabolism

Article

Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes

Graphical abstract



Highlights

- BFRT matches CREST in muscle strength gains despite a markedly lower load
- BFRT uniquely enhances skeletal muscle and adipose tissue oxidative capacity
- BFRT induces greater transcriptional upregulation of angiogenesis-related pathways
- BFRT exclusively reduces visceral adipose tissue volume

Authors

Nina Trinks, Sofiya Gancheva, Jennifer Pützer, ..., Patrick Schrauwen, Michael Roden, Dominik H. Pesta

Correspondence

michael.roden@ddz.de

In brief

Trinks et al. showed that blood-flow restriction training (BFRT) provides a low-load alternative to conventional resistance training (CREST), making it well suited for individuals with type 2 diabetes who have musculoskeletal limitations or reduced functional capacity because of promising effects on muscle strength, hypertrophy, mitochondrial capacity, and body composition.

Trinks et al., 2026, Cell Metabolism *38*, 1–12
April 7, 2026
https://doi.org/10.1016/j.cmet.2025.12.016

CellPress

Cell Metabolism

CellPress
OPEN ACCESS

Article

Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes

Nina Trinks,[1,2] Sofiya Gancheva,[1,2,3,4] Jennifer Pützer,[1,2] Martin Schön,[1,2,3] Maximilian Huttasch,[1,2] Kalliopi Pafili,[1,2,3] Lucia Mastrototaro,[1,2] Bedair Dewidar,[1,2] Yuliya Kupriyanova,[1,2] Christian Herder,[1,2,3] Klaus Strassburger,[2,5] Oana P. Zaharia,[1,2,3] Philip M.M. Ruppert,[6] Sander Kersten,[6,7] Joris Hoeks,[8] Sandra Trenkamp,[1,2] Vera Schrauwen-Hinderling,[1,2] Patrick Schrauwen,[1,2] Michael Roden,[1,2,3,12,*] and Dominik H. Pesta[9,10,11]

[1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[2]German Center for Diabetes Research (DZD e.V.), München-Neuherberg, Germany
[3]Department of Endocrinology and Diabetology, Medical Faculty and University Hospital, Heinrich Heine University, Düsseldorf, Germany
[4]Departamento de Nutricion, Diabetes y Metabolismo, Escuela de Medicina, Facultad de Medicina, Pontificia Universidad Catolica de Chile, Santiago, Chile
[5]Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany
[6]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA
[7]Nutrition, Metabolism and Genomics Group, Division of Human Nutrition and Health, Wageningen University, Wageningen, the Netherlands
[8]Department of Nutrition and Movement Sciences, Maastricht University, Maastricht, the Netherlands
[9]German Aerospace Center (DLR), Institute of Aerospace Medicine, 51147 Cologne, Germany
[10]Medical Faculty, University of Cologne, 50931 Cologne, Germany
[11]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), 50931 Cologne, Germany
[12]Lead contact
*Correspondence: michael.roden@ddz.de
https://doi.org/10.1016/j.cmet.2025.12.016

SUMMARY

Impaired muscle strength and mitochondrial functionality are hallmarks of type 2 diabetes (T2D). Conventional combined resistance/endurance exercise training has limited efficacy to simultaneously improve muscle function and metabolism. We examined whether low-load blood-flow restriction training (BFRT) increases both muscle strength and mitochondrial oxidative capacity in T2D. Over 12 weeks, BFRT and conventional resistance training (CREST) similarly improved muscle strength despite lower workload in BFRT. Uniquely, BFRT enhanced muscle and adipose tissue oxidative capacity and increased muscle mitochondrial content. Transcriptomic profiling revealed more pronounced changes, particularly in angiogenesis-linked pathways, upon BFRT. BFRT also preferentially led to reductions in visceral adipose tissue volume and waist circumference, whereas CREST more effectively decreased subcutaneous adipose tissue volume. Both interventions lowered resting heart rate and diastolic blood pressure. These findings position BFRT as a promising low-load exercising strategy to simultaneously improve mitochondrial oxidative capacity, muscle strength, and body composition in individuals with T2D.

INTRODUCTION

About 90% of all people with diabetes have type 2 diabetes (T2D), with skeletal muscle insulin resistance as its key feature.[1] Persons with T2D exhibit not only reduced skeletal muscle mass and strength[2–4] but also lower skeletal muscle mitochondrial density and oxidative function as well as exercise capacity.[5,6] Aging, overweight, and sedentarism are the main drivers of skeletal muscle impairments and sarcopenia in T2D.[7,8]

Both endurance and resistance exercise training are integral to the lifestyle modification recommended by international guidelines for the management of T2D and related cardiovascular disease (CVD).[9] Chronic exercise training improves insulin sensitivity and features of skeletal muscle mitochondrial function, as assessed either *in vivo* by ^{31}P magnetic resonance spectroscopy (MRS)[10,11] or *ex vivo* by high-resolution respirometry.[12–15] Nevertheless, there is an ongoing debate on the optimal and most efficient exercise program for persons with T2D. Although combined aerobic and resistance exercise training programs have demonstrated efficacy in improving mitochondrial oxidative capacity and insulin sensitivity in people with T2D,[10,16] the relative contribution of resistance training to the overall training

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

Table 1. Characteristics of the study population

	BFRT		CREST	
	Week 0	Week 12	Week 0	Week 12
N (m/f)	10 (9/1)		10 (7/3)	
Age (years)	62 (55; 68)		56 (52; 62)	
Body weight (kg)	92.9 (88.2; 110.9)	92.0 (84.6; 112.7)[a]; $p = 0.02$	85.9 (83.6; 102)	84.8 (82.5; 100.5)[a]; $p = 0.02$
BMI (kg/m^2)	31.2 (27.5; 34.2)	30.7 (27.2; 33.7)[a]; $p = 0.05$	29.6 (24.9; 33.6)	29.5 (24.3; 31.8)[a]; $p = 0.03$
WHR	1.00 (0.95; 1.02)	1.01 (0.96; 1.03)	0.90 (0.88; 0.96)[b]; $p = 0.03$	0.91 (0.90; 0.96)[b]; $p = 0.006$
Waist circumference (cm)	106 (94; 122)	104 (96; 119)	96 (94; 101)	94 (92; 96)
Blood glucose (mg/dL)	133 (116; 148)	133 (121; 145)	140 (120; 159)	139 (117; 170)
HbA_{1c} (%)	6.9 (6.4; 7.1)	6.7 (6.5; 7.1)	7.1 (6.5; 7.9)	7.0 (6.4; 7.5)
HbA_{1c} (mmol/mol)	52 (47; 54)	50 (48; 54)	54 (48; 63)	53 (47; 59)
Systolic blood pressure (mmHg)	146 (140; 151)	137 (130; 145)	147 (138; 156)	138 (127; 148)[a]; $p = 0.02$
Diastolic blood pressure (mmHg)	90 (87; 91)	79 (77; 82)[a]; $p = 0.01$	89 (76; 92)	82 (72; 86)[a]; $p = 0.004$
FRS-CHD	0.10 (0.07; 0.13)	0.07 (0.05; 0.10)	0.05 (0.01; 0.09)	0.06 (0.03; 0.08)
FRS-CVD	0.28 (0.22; 0.35)	0.22 (0.18; 0.24)	0.16 (0.14; 0.30)	0.19 (0.15; 0.21)
Heart rate (1/min)	76 (66; 81)	68 (61; 70)[a]; $p = 0.02$	67 (62; 72)	62 (53; 67)[a]; $p = 0.03$
VO_2max (mL/min/kg)	20.3 (17.0; 22.4)	22.2 (20.5; 23.1)	25.9 (22.2; 27.4)	30.0 (24.7; 30.7)[a]; $p = 0.01$
hsCRP (mg/dL)	0.16 (0.11; 0.19)	0.21 (0.11; 0.24)	0.11 (0.06; 0.35)	0.10 (0.04; 0.14)
LDL cholesterol (mg/dL)	125 (113; 139)	125 (107; 142)	110 (107; 129)	107 (93; 124)
HDL cholesterol (mg/dL)	47 (37; 49)	44 (39; 48)	46 (42; 51)	51 (44; 52)
Triglycerides (mg/dL)	103 (79; 164)	126 (76; 190)	128 (89; 203)	103 (79; 132)[a]; $p = 0.03$
NEFA (μmol/L)	476 (339; 563)	441 (365; 470)	438 (370; 530)	411 (345; 538)

Data are shown as median (1st quartile; 3rd quartile). Measurements were performed after an overnight fast. BFRT, blood flow restriction training; BMI, body mass index; CREST, conventional resistance training; FRS-CHD, Framingham risk score-coronary heart disease; FRS-CVD, Framingham risk score-cardiovascular disease; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; NEFA, non-esterified fatty acid; HbA_{1c}, glycated hemoglobin; W, week; WHR, waist-to-hip ratio.
[a]$p \leq 0.05$, pre vs. post
[b]$p \leq 0.05$, CREST vs. BFRT

load is often low, limiting increases in muscle mass.[17] Furthermore, combined training programs also demand substantial time commitment, which can discourage long-term participation among persons with diabetes. On the other hand, while resistance training can also increase insulin sensitivity along with enhanced skeletal muscle mass and strength,[18] the effects on energy and mitochondrial metabolism are largely unknown. However, conventional resistance training (CREST) with loads of ~70% of the one-repetition maximum (1-RM) is not feasible for all people with T2D[19] due to limitations in baseline muscle strength, arthropathies, or other comorbidities that make high-intensity resistance training challenging or unsafe.[9] This may be one of the reasons why only a few people follow the recommendations for physical activity participation.[20]

Interestingly, resistance training with low loads (20%–40% 1-RM) and mild blood-flow restriction via inflation cuffs may serve as an effective alternative training method by inducing local muscle hypoxia, which activates satellite cells, promotes muscle hypertrophy, and triggers mitochondrial biogenesis.[21–24] Indeed, recent evidence in healthy individuals suggests that this training modality can enhance mitochondrial metabolism, angiogenesis, and oxygen delivery.[25–27] Blood-flow restriction training (BFRT) has also been shown to improve muscle function and performance in athletic populations, making it a versatile tool across fitness levels.[28] Although BFRT seems to be similarly effective as CREST in improving muscle strength and mass as well as cardiovascular function in healthy individuals,[29] no studies have been reported employing BFRT in people with T2D.

Thus, we hypothesized that in people with T2D, BFRT could be a more effective training modality than CREST for improving skeletal muscle strength and oxidative capacity despite lower exercise intensity. Furthermore, we also assessed the effects of BFRT on adipose tissue distribution, insulin sensitivity, and CVD risk factors. To this end, we performed a randomized parallel-group trial comparing 12 weeks of supervised BFRT and CREST in individuals with T2D employing detailed metabolic phenotyping using state-of-the-art *in vivo* and *ex vivo* methods.

RESULTS

Cohort characteristics

At baseline, 20 participants with T2D had overweight, class 1 or 2 obesity, good glucometabolic control, and comparable other anthropometric and cardiometabolic parameters in both groups (Table 1). Their medication use is listed in Table S1.

BFRT and CREST are equally effective in improving muscle strength and mass

At baseline, BFRT and CREST did not differ in terms of quadriceps muscle volume, muscle cross-sectional area, or leg

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

CellPress
OPEN ACCESS

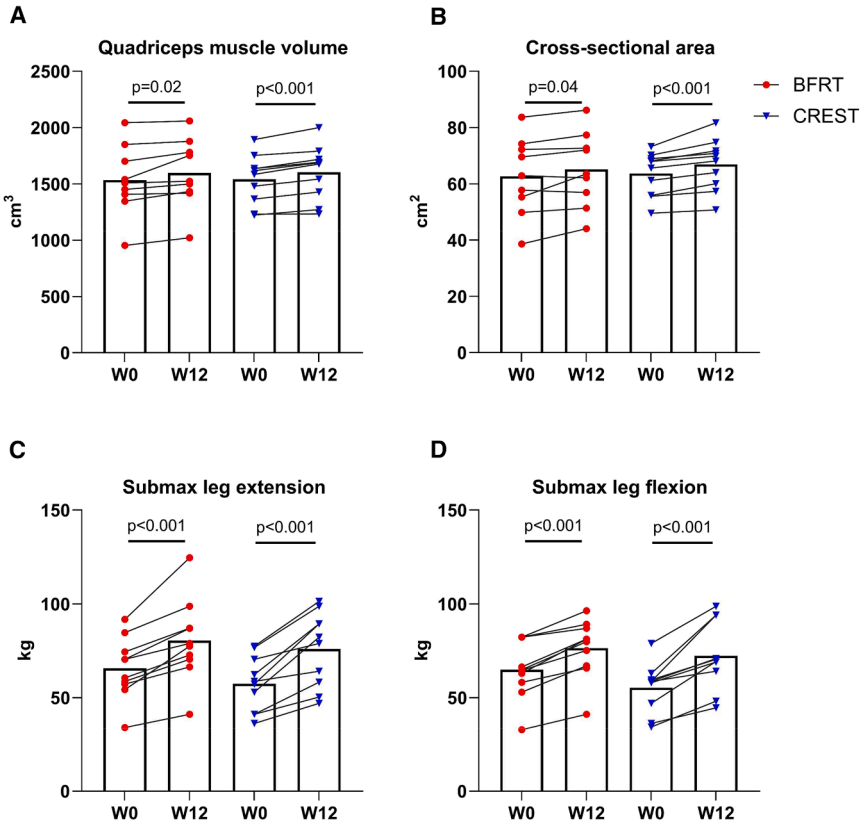


Figure 1. Muscle strength and mass after 12 weeks of resistance training

Quadriceps femoris volume (A), cross-sectional area (B), submaximal leg extension (C), and flexion strength (D) before and after 12 weeks of blood-flow restriction (BFRT) and conventional resistance training (CREST). Data are shown as means ± SD; W, week.

strength (Figure 1). Both groups exhibited comparable increases in muscle adaptations as assessed by magnetic resonance imaging (MRI) (Figures 1A and 1B) as well as functional features (Figures 1C and 1D). Quadriceps muscle volume and cross-sectional area increased by 4.3% (p = 0.02) and 4.4% (p = 0.04) in BFRT as well as by 3.4% and 5.0% (both $p < 0.001$) in CREST, respectively. Leg extension and flexion strength increased by 22.5% and by 17.6% in BFRT as well as by 32.3% and by 30.8% in CREST (all $p < 0.001$). No significant interaction (group × training) effects between groups were observed for any of these parameters.

BFRT specifically improves skeletal muscle mitochondrial oxidative capacity

At baseline, parameters of skeletal muscle mitochondrial function did not differ between groups. BFRT improved skeletal muscle mitochondrial complex I (CI)-OXPHOS capacity upon pyruvate, malate, and ADP by 52% (p = 0.003) and CI+II-OXPHOS capacity upon succinate by 58% (p = 0.002; Figures 2A and 2B), while CREST did not result in any significant changes of all mitochondrial states. Skeletal muscle citrate synthase (CS) activity, as a surrogate marker of mitochondrial content,[30] increased only after BFRT by 109% ($p < 0.001$; Figure 2C), but not after CREST (21%, p = 0.62; Figure 2C). Mitochondrial DNA (mtDNA) did not change in either group (both p = 0.08; Figure 2D).

We further investigated the sensitivity of skeletal muscle mitochondrial respiration to ADP, an intrinsic qualitative property of mitochondria, by assessing the apparent K_m for ADP. The apparent K_m for ADP showed a strong trend toward a rise in the BFRT group (49%, p = 0.06), but not in the CREST group (21%, p = 0.34), suggesting reduced ADP sensitivity following BFRT (Figure 2E). Only BFRT, but not CREST, also increased maximal ADP-stimulated respiration (V_{max}; 67%, p = 0.002 vs. 21%, p = 0.24), indicating a higher maximal respiratory capacity after BFRT (Figure 2F).

Molecular responses of skeletal muscle to BFRT and CREST

To investigate molecular mechanisms underlying the effects of BRFT and CREST in skeletal muscle, we examined factors involved in mitochondrial biogenesis, energy metabolism, and hypertrophy. BFRT, but not CREST, increased protein levels of peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1α; p = 0.01 vs. p = 0.11), AMP-activated protein kinase (AMPK) α (p = 0.02 vs. p = 0.16), and mitochondrial transcription factor A (TFAM; p = 0.05 vs. p = 0.44). Neither BFRT nor CREST affected skeletal muscle hypoxia-inducible factor 1 α (HIF-1α) (p = 0.15 vs. p = 0.88) (Figures 3A–3D) or the mechanistic target of rapamycin (mTOR) (both $p > 0.05$; Figure S1A). Protein levels of hexokinase (HXK)1 and HXK2 (Figure 3D) also increased only after BFRT (p = 0.003; p = 0.01), but not after CREST (p = 0.39; p = 0.06). The enzyme activities of HXK and phosphofructokinase (PFK) remained unchanged with training in both groups ($p > 0.05$; Figures S1B and S1C). Plasma interleukin-6 (IL-6) was not altered in both groups ($p > 0.05$; Figure S1D), whereas plasma myostatin levels increased following CREST (p = 0.01) but not BFRT (p = 0.74; Figure 3F).

To further understand the molecular adaptations to CREST and BFRT, we performed RNA sequencing (RNA-seq) analysis. Using a statistical cutoff of $p < 0.05$ and an absolute fold change of 1.5, we identified 18 differentially expressed genes (DEGs) in CREST and 110 DEGs in BFRT. Volcano plot analysis further illustrated that the transcriptional response was stronger in the BFRT group than in the CREST group (Figures 4A and 4B). We next performed KEGG gene set enrichment analysis (GSEA) for both groups and plotted corresponding NES scores for the top 20 positively enriched gene sets from the BFRT group (Figure 4C). Among these gene sets, 4 gene sets are related to angiogenesis (extracellular matrix [ECM]-receptor interaction, relaxin signaling pathway, vascular smooth muscle contraction, and platelet activation). Three out of these 4 gene sets showed stronger enrichment in the BFRT group compared with the partly nonsignificant

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

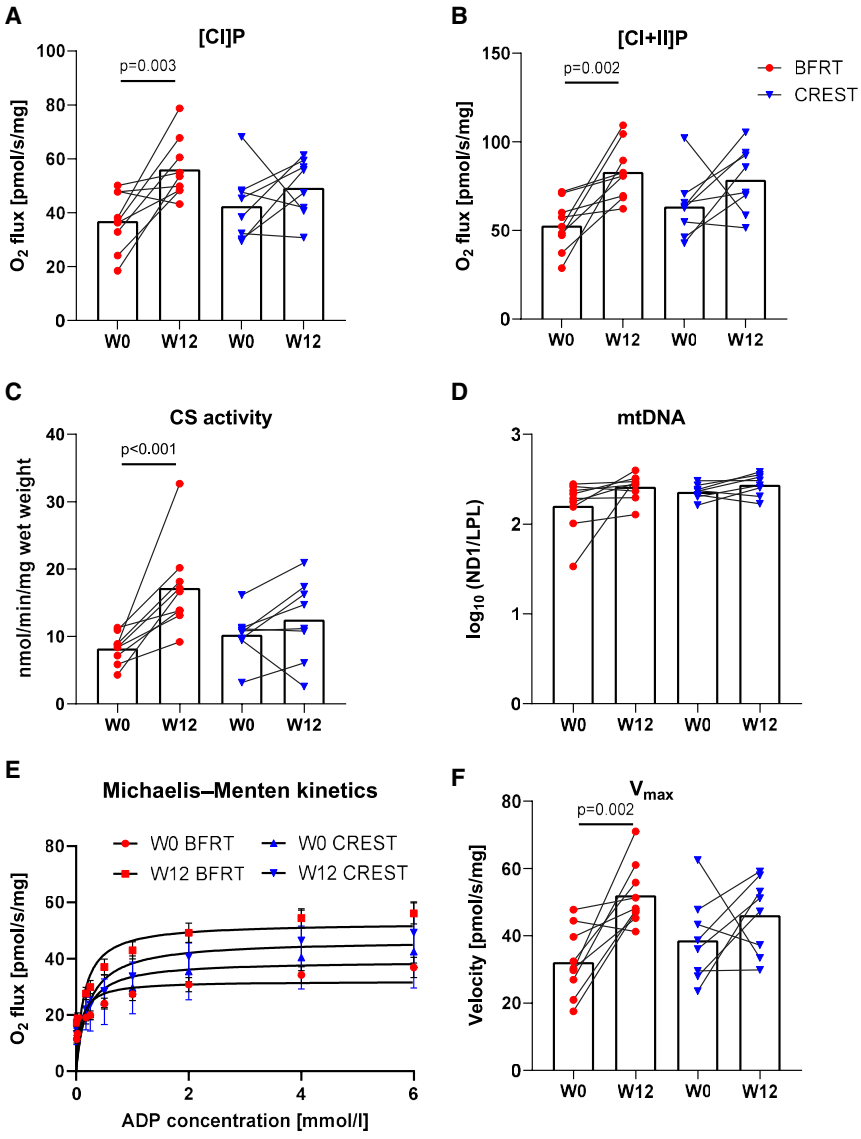


Figure 2. Skeletal muscle mitochondrial respiration and content

Complex I (CI)-OXPHOS capacity ([CI]P, A), CI+II-OXPHOS capacity ([CI+II]P, B), citrate synthase (CS) activity (C), mitochondrial DNA (mtDNA; D), and kinetics for ADP sensitivity (E) and maximal velocity (F) in skeletal muscle before and after 12 weeks of blood-flow restriction (BFRT) and conventional resistance training (CREST). Data are shown as means ± SD; W, week.

enrichment in the CREST group, suggesting that BFRT stimulates angiogenesis-related pathways to a greater extent than CREST. Consistently, when plotting the top 30 core enrichment genes from the GSEA and person-normalized heatmaps were created, these heatmaps featured many important angiogenesis-relevant genes, including endothelial growth factors (*VEGFA*, *VEGFB*, and *VEGFC*), nitric-oxide synthases (*NOS2*), and extracellular-matrix collagens (*COL1A1*, *COL1A2*, *COL3A1*, *COL4A1*, and *COL4A2*). Furthermore, the heatmaps also suggested that the transcriptional response is more consistent among BFRT participants than among the CREST participants (Figure 4D).

BFRT and CREST for 12 weeks affect neither whole-body nor adipose insulin sensitivity

Insulin sensitivity was lower in the BFRT than in the CREST group before (p = 0.01) and after the training intervention (p = 0.02; Table 2). Neither BFRT nor CREST improved insulin sensitivity, as assessed from clamp-derived M-value (mostly reflecting skeletal muscle) and from homeostatic model assessment of insulin resistance (HOMA-IR) (mostly reflecting fasting hepatic insulin resistance) ($p > 0.05$; Table 2). In line, insulin-stimulated skeletal muscle protein content of insulin receptor substrate 1 (IRS1) as well as inhibitory Ser^{1101} phosphorylation of IRS1 did not change with training in both groups ($p > 0.05$). Further, protein content and activation (Thr^{308} and SER^{473} phosphorylation) of AKT did not change after BFRT and CREST ($p > 0.05$; Figure S2). As further surrogates of insulin sensitivity, we also measured hepatic lipid content by MRS and intramuscular fat fractions by MRI. Hepatic lipid content and volume remained unchanged in both groups (all $p > 0.05$; Table 2). Intramuscular fat fraction slightly decreased in BFRT (−11.4%; p = 0.08) and CREST (−12.5%; p = 0.03).

BFRT preferentially reduces VAT volume

Whole-body fat volume (WBF), as assessed by MRI, declined in BFRT by trend (−5.1%; p = 0.07) and significantly in CREST (−4.4%; p = 0.01; Figure 5A). Of note, visceral adipose tissue (VAT) volume decreased markedly and exclusively by −13.3% ($p < 0.001$) in BFRT, but not in CREST (−3.1%; p = 0.30; Figure 5B). Conversely, subcutaneous adipose tissue (SAT) volume was reduced only in CREST by −5% (p = 0.01), but not in BFRT (−3%, p = 0.20; Figure 5C).

BFRT uniquely enhances adipose tissue mitochondrial oxidative capacity

Impaired adipose mitochondrial capacity has also been related to reduced insulin sensitivity and can be improved by exercise.[31] Both training modes did not affect adipose mitochondrial CI-OXPHOS capacity, while only BFRT increased CI+II-OXPHOS capacity by 24% (p = 0.04; Figures S3A and S3B). Also, both training modes did not affect adipose CS activity (Figure S3C), while only BFRT increased mtDNA by 4.3% (p = 0.02 vs. CREST: p = 0.31; Figure S3D).

Adipose tissue apparent K_m for ADP remained unchanged with both BFRT and CREST (Figure S3E). However, exclusively

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

CellPress
OPEN ACCESS

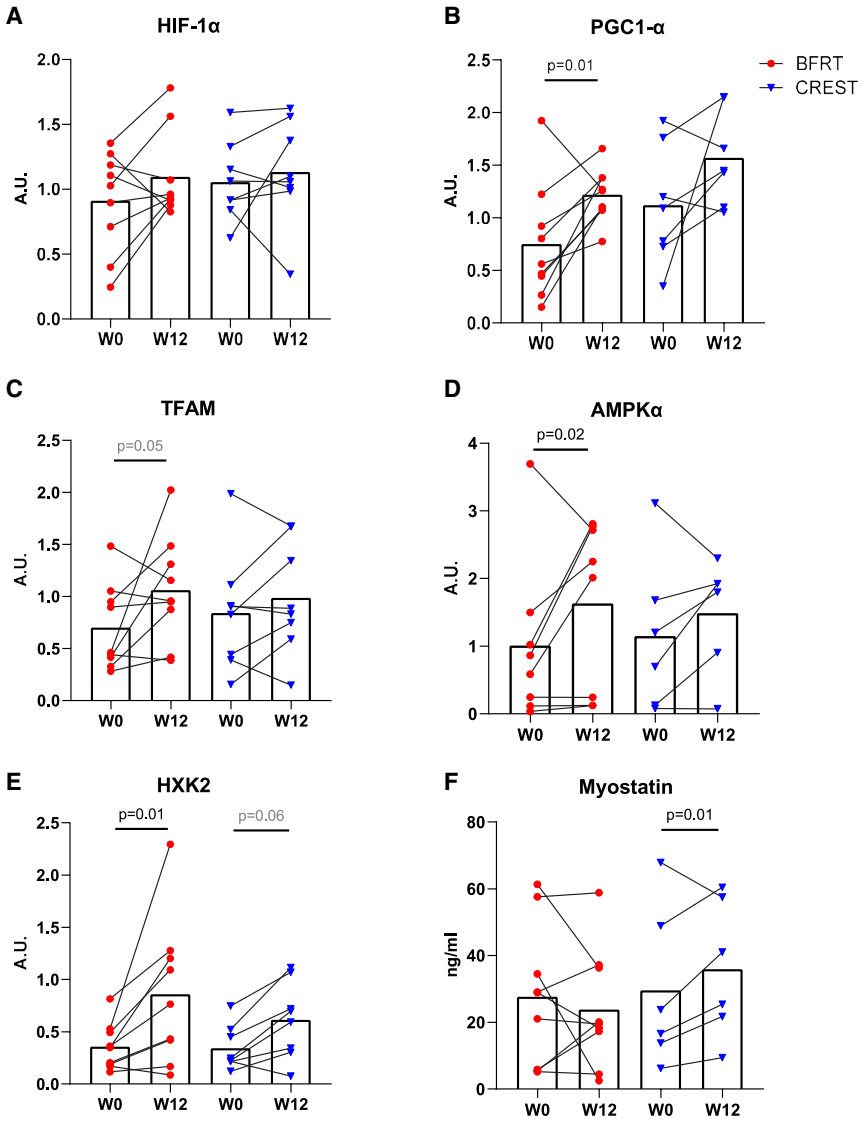


Figure 3. Molecular responses of skeletal muscle to BFRT and CREST

Hypoxia-inducible factor 1 α (HIF-1α, A), peroxisome proliferator-activated receptor gamma coactivator 1 α (PGC-1α, B), mitochondrial transcription factor A (TFAM, C), AMP-activated protein kinase (AMPK) α (D), hexokinase (HXK) 2 (E), and myostatin (F) protein levels before and after 12 weeks of blood-flow restriction (BFRT) and conventional resistance training (CREST). Data are shown as means ± SD; W, week.

BFRT increased V_{max} by 54% ($p = 0.002$), while CREST had no effect ($p = 0.23$; Figure S3F), suggesting that BFRT enhances maximal mitochondrial respiratory capacity in adipose tissue independently of ADP sensitivity.

Effects of BFRT and CREST on cardiovascular risk factors

Body mass index (BMI) decreased by about 2% in both groups (both $p < 0.05$; Table 1), but only BFRT showed a trend toward reduced waist circumference (−2%, $p = 0.07$). BFRT ($p = 0.48$) and CREST ($p = 0.89$) did not change the Framingham risk scores for CVD. Similarly, both training modes did not affect Framingham risk scores for coronary heart disease ($p = 0.12$ for BFRT and $p = 0.69$ for CREST). Resting heart rate decreased similarly by BFRT (−6.4 bpm; $p = 0.02$) and CREST (−6.8 bpm; $p = 0.03$). BFRT and CREST decreased diastolic blood pressure (−8.8 mmHg; $p = 0.01$ and −6.0 mmHg; $p = 0.004$) (Table 1). Systolic blood pressure decreased by trend upon BFRT (−8.4 mmHg; $p = 0.06$) and significantly by CREST (−8.9 mmHg; $p = 0.02$). BFRT also showed a trend for increased maximal aerobic capacity (VO_2max), which was significantly improved by CREST (+1.8 mL/min/kg; $p = 0.06$ and +2.9 mL/min/kg; $p = 0.01$). Triglycerides only improved after CREST ($p = 0.03$). Other parameters, such as high-sensitivity C-reactive protein (hsCRP), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, or non-esterified fatty acids, did not improve in both groups (all $p > 0.05$; Table 1). No significant interaction (group × training) effects between groups were observed for any of these parameters.

DISCUSSION

This study demonstrates that BFRT elicits comparable improvements in skeletal muscle strength and stimulation of muscle hypertrophy as CREST in individuals with T2D, despite markedly lower training intensity. BFRT specifically reduces VAT volume while enhancing skeletal muscle and adipose tissue mitochondrial oxidative capacity. Furthermore, BFRT had more pronounced effects on skeletal muscle molecular adaptations of metabolism and angiogenesis. Collectively, these findings demonstrate additional metabolic benefits of BFRT over CREST for individuals with T2D.

The comparable increases in muscle strength and muscle mass were observed in the face of much lower training intensity of 30% vs. 70% of the 1-RM with BFRT than with CREST. These findings extend the growing body of evidence showing comparable improvements in muscle strength and stimulation of hypertrophy with both modalities in healthy individuals,[32] older adults,[33] those with musculoskeletal conditions,[34] and people with obesity and T2D. Despite methodological differences across studies, the consistent hypertrophic and strength gains observed with BFRT highlight its clinical utility as a reduced-load yet effective resistance training strategy. Given the inverse association between muscular strength and mortality risk,[35] BFRT may be particularly valuable to prevent loss of muscle

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

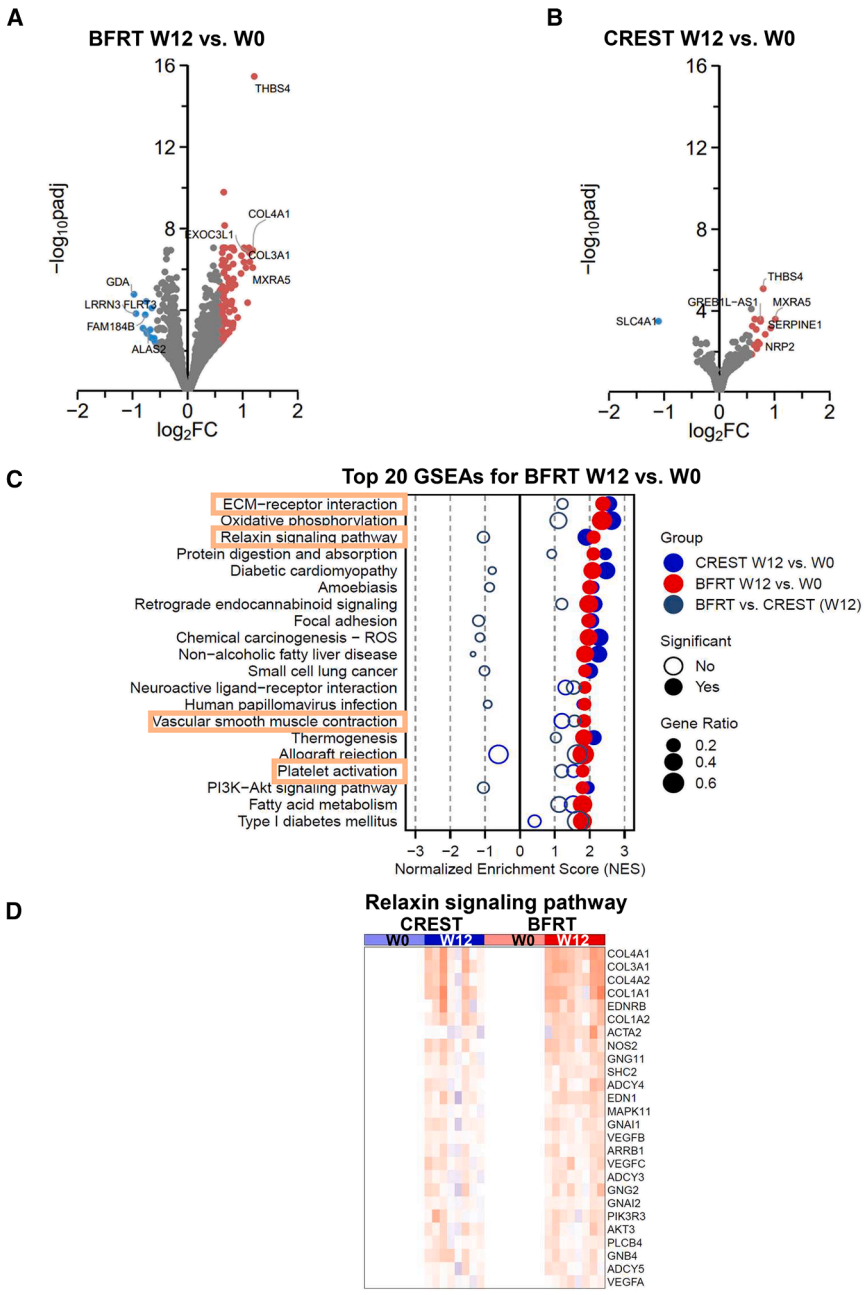


Figure 4. Transcriptional response to BFRT and CREST

(A and B) Volcano plots showing differentially expressed genes (DEGs) for each contrast, including blood-flow restriction training (BFRT) weeks 0 vs. 12 (A) and conventional resistance training (CREST) weeks 0 vs. 12 (B). Red indicates significantly upregulated, and blue indicates significantly downregulated according to adjusted $p < 0.05$, $|FC| > 1.5$. Gene set enrichment analysis (GSEA) of the top 20 positively enriched pathways in BFRT weeks 0 vs. 12.

(C and D) Dot size represents gene ratio, color denotes contrast, and significance is indicated by filled circles (adjusted $p < 0.05$; C). Heatmap of the top 30 genes contributing to core enrichment of the relaxin signaling pathway under BFRT weeks 0 vs. 12. Week 0 is set to zero (D).

mass that occurs following incretin-based or bariatric surgery-induced weight loss.[36] Although skeletal muscle mass and strength increased with exercise training, mTOR protein levels remained unchanged. In the present study, skeletal muscle biopsies were collected ~72 h after exercising, so we may have missed the peak in mTOR signaling response. Indeed, mTOR pathway activation is often transient and time-sensitive, peaking within hours post-exercise and returning to baseline within 24–48 h.[37,38] Of note, myostatin increased following CREST, but not BFRT, as previously reported for resistance training without evidence for impaired muscle adaptation.[39] Hypertrophy despite elevated myostatin levels may result from compensatory mechanisms such as increased follistatin-like related gene levels and downregulation of the activin IIB receptor, which can mitigate the inhibitory effects of myostatin.[39] Future studies should investigate if BFRT can also help to maintain muscle mass and function following weight loss and thereby—given the importance of muscle mass in energy expenditure—also help to maintain long-term body weight and prevent sarcopenia.

A key finding of our study is that skeletal muscle and adipose tissue mitochondrial oxidative capacity were only improved in BFRT, but not in CREST. So far, conventional endurance exercise has been repeatedly reported to stimulate mitochondrial biogenesis, enhance mitochondrial function, and improve insulin sensitivity in both healthy individuals[13] and those with T2D.[40] High-load resistance training has been shown to enhance mitochondrial respiratory function only in a few studies of healthy individuals.[13,41] Such high-load resistance training is unlikely to be feasible for individuals with T2D and musculoskeletal limitations. Therefore, our finding that BFRT improves both muscle strength and mitochondrial capacity at low exercise intensity is promising for diabetes prevention and treatment. This specific effect of BFRT on oxidative capacity was primarily driven by an increase in muscle mitochondrial content, as evidenced by enhanced CS activity. To investigate the molecular mechanisms underlying the distinct effects of BFRT and CREST, we examined key regulators of mitochondrial biogenesis and energy metabolism. BFRT selectively increased protein levels of HXK, PGC-1α, AMPKα, and TFAM consistent with enhanced mitochondrial signaling,[42,43] while both training modes did not affect HIF-1α. Most likely, BFRT may therefore increase mitochondrial biogenesis via increased intracellular energetic stress, activating AMPK and enhanced PGC-1α

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

Table 2. Effects of CREST and BFRT on tissue-specific insulin sensitivity and insulin signaling

	BFRT		CREST	
	Week 0	Week 12	Week 0	Week 12
M-value (mg/kg/min)	1.7 (1.3; 2.3)	2.5 (1.1; 2.8)	3.6 (3.5; 3.8)[b]; $p = 0.01$	4.0 (2.6; 7.0)[b]; $p = 0.02$
Intramuscular fat fraction (%)	5.5 (3.7; 8.3)	4.2 (3.8; 7.6)	4.6 (3.8; 5.6)	4.5 (3.2; 5.0)[a]; $p = 0.03$
HOMA-IR	2.9 (2.3; 3.7)	2.7 (2.5; 3.4)	2.1 (1.7; 2.3)[b]; $p = 0.05$	1.9 (1.5; 2.3)[b]; $p = 0.04$
HL content (%)	8.9 (6.1; 16.1)	7.3 (6.6; 11.8)	5.3 (1.8; 15.5)	4.6 (1.8; 12.0)
Liver volume (cm^3)	1,703 (1,573; 1,884)	1,664 (1,419; 1,984)	1,565 (1,477; 1,865)	1,493 (1,408; 1,982)

Data are shown as median (1st quartile; 3rd quartile); measurements were obtained under overnight fasted conditions. Expression of proteins involved in insulin signaling was analyzed by western blot. BFRT, blood flow restriction training; CREST, conventional resistance training; HOMA-IR, homeostatic model assessment of insulin resistance; HL, hepatic lipid content.
[a]$p \leq 0.05$, W0 vs. W12
[b]$p \leq 0.05$, CREST vs. BFRT

expression, as reviewed recently.[44] Interestingly, HXK enzyme activity did not change in either group following the training intervention. These results are in line with previous findings showing elevated HXK mRNA levels after exercise without a corresponding increase in HXK enzyme activity in people with T2D.[45] To further elucidate the molecular adaptations, we performed RNA-seq analysis, which revealed a markedly stronger transcriptional response in the BFRT group (110 DEGs) compared with CREST (18 DEGs). Although the overall number of DEGs was modest, GSEA highlighted several angiogenesis-related pathways—including ECM-receptor interaction, vascular smooth muscle contraction, and platelet activation—predominantly enriched in BFRT. This suggests that BFRT may more robustly stimulate vascular remodeling processes than CREST. Participant-normalized heatmaps of the top enriched genes further supported this observation, showing consistent upregulation of angiogenesis-associated genes such as VEGFA, VEGFB, NOS2, and several collagens (COL1A1, COL3A1, and COL4A1/2) in BFRT participants. These findings support the hypothesis that prolonged restricted blood flow may serve as a stimulus for transcriptional angiogenesis programs not typically activated by CREST. Notably, the more homogeneous transcriptional activation seen in BFRT may be particularly advantageous for individuals who typically exhibit blunted responses to conventional training, offering a potentially more reliable molecular stimulus in populations with reduced trainability.

Another potential explanation for the enhanced oxidative capacity of mitochondrial function with blood-flow restriction is the increased recruitment of muscle fibers due to occlusion. According to Henneman's "size principle," maximal muscle fiber recruitment—critical for muscle adaptation and neural activation—typically requires heavy training loads.[46] However, low-load BFRT creates a mild ischemic condition that promotes metabolic stress mediators, which also result in recruitment of type II fibers.[47] Such enhanced contractile activity has been shown to drive mitochondrial adaptation[48] and likely explains the greater improvements in oxidative capacity observed in the BFRT group. We refrained from normalizing skeletal muscle mitochondrial respiration to CS activity or mtDNA, as individual mitochondrial properties turn over at highly independent rates,[49] rendering these markers unreliable to reflect changes in mitochondrial content in response to exercise interventions.

We also found that only BFRT reduced ADP sensitivity—manifested as an increased K_m for ADP. Interestingly, it has previously been shown that 6 weeks of continuous and intermittent endurance training decreased ADP sensitivity in untrained individuals.[50] Likewise, 6 weeks of high-intensity interval training (HIIT) in men and women resulted in decreased ADP sensitivity and increased K_m in muscle, mirroring our findings after BFRT and suggesting that both training modalities may induce similar mitochondrial adaptations.[51] These findings suggest a metabolic adaptation toward a more oxidative phenotype, enhancing energy production for sustained muscular activity. An increased V_{max} further corroborates these mitochondrial adaptations in skeletal muscle and adipose tissue, indicating that the enhanced mitochondrial function results in a greater capacity for maximal oxidative phosphorylation, supporting sustained metabolism during prolonged physical activity.

Another clinically relevant finding of our study is the specific effect of BFRT on body composition with selective reduction in VAT volume and, consequently, a decrease in waist circumference. This is noteworthy given the strong association between VAT and triglyceride-rich lipoproteins, fatty acids, inflammation, and cardiovascular risk.[52] The mechanisms underlying this specific effect of BFRT warrant further investigation but may involve unique hormonal or metabolic responses to blood-flow restriction. Given that changes in body weight and muscle mass gains were similar between groups, the VAT volume reduction in BFRT is more likely driven by inducing a distinct physiological stress.[53] Thus, intermittent ischemia-reperfusion cycles may stimulate the release of lipolytic hormones, such as catecholamines and growth hormone, promoting fat mobilization and VAT reduction.[54] Additionally, the higher metabolic demand of BFRT, despite lower external loads, could contribute to greater lipid turnover and selective fat loss.[55] Remarkably, despite these beneficial effects of BFRT, we here found no improvements in insulin sensitivity in either group. This lack of effect of BFRT or CREST on insulin sensitivity may be explained by the relatively low gain in muscle mass in our program, which may have been insufficient to drive meaningful metabolic improvements and which likely can be explained by the short intervention duration, as it has been shown that the strongest glycemic effects typically occur after 12–16 weeks.[56]

Although participants in the BFRT group exhibited lower insulin sensitivity at baseline, this group nonetheless showed

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

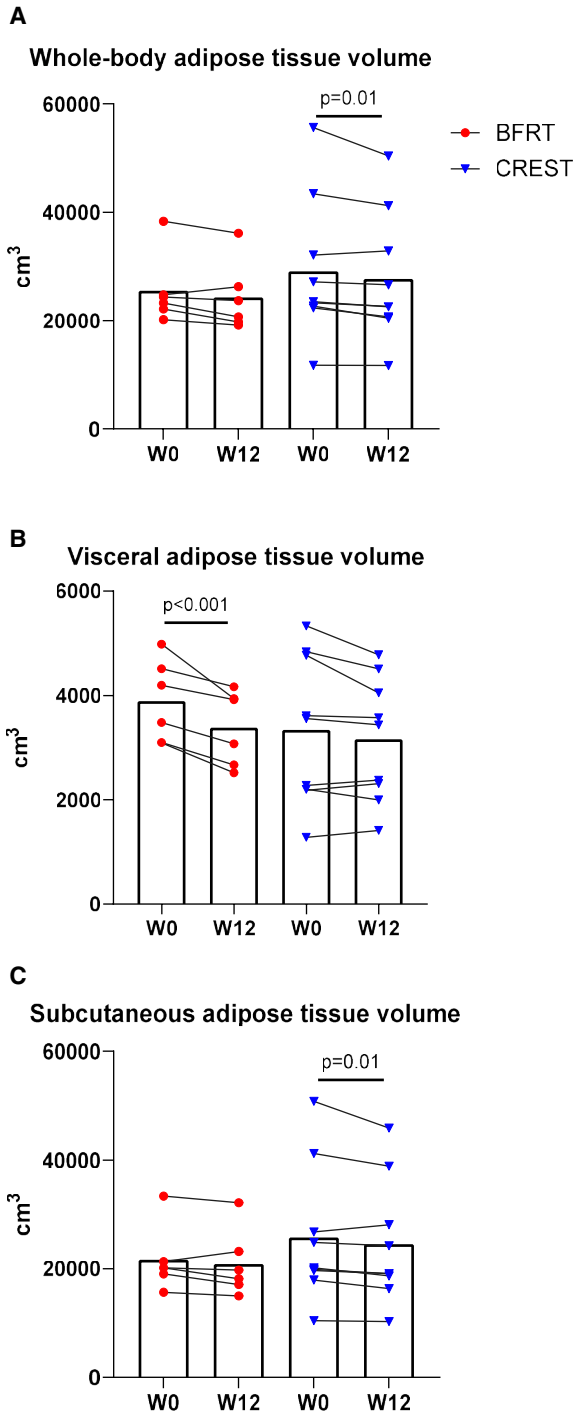


Figure 5. Changes in adipose tissue after 12 weeks of resistance training

Whole-body adiposity (A), visceral adiposity (B), and subcutaneous adiposity (C) measured by magnetic resonance imaging before and after 12 weeks of blood-flow restriction (BFRT) and conventional resistance training (CREST). Data are shown as mean ± SD; W, week.

pronounced metabolic improvements, underscoring the efficacy of BFRT even in individuals with worse insulin resistance.[57] Despite the lack of improvement in insulin sensitivity, both CREST and BFRT reduced resting heart rate and diastolic blood pressure, emphasizing their potential to improve cardiovascular health. Furthermore, both training regimens also resulted in improvements in maximal aerobic capacity, indicating that resistance training, including BFRT, can yield cardiovascular benefits typically associated with endurance training.[58] Given the present findings, future studies could investigate the effect of BFRT in individuals with diabetes-related arterial disease,[59] CVDs,[60] and other conditions, such as frailty,[29] sarcopenia,[61] and abdominal adiposity.[62] In case of beneficial results, BFRT could represent a novel strategy for prevention and treatment for those individuals. In support of this, preliminary evidence suggests that BFRT may be an efficient and safe strategy to prevent and attenuate the progression of diabetes-related complications such as neuropathy and nephropathy.[63,64]

The strengths of our study include the randomized parallel-group design, allowing for direct comparison of the controlled supervised BFRT and CREST on the different outcomes in individuals with T2D. Another strength is the comprehensive phenotyping approach, utilizing clinical and functional measurement of skeletal muscle and adipose tissue along with assessment of *in vivo* insulin sensitivity and CVD risk factors. Given its low-load nature and metabolic benefits, BFRT may represent a promising exercise strategy for individuals with T2D, pending evaluation in broader clinical contexts.

Limitations of the study

Study limitations include the relatively small sample size, which was not sufficient to perform sex-specific analyses and limits the generalizability of our findings. The small sample size was in part due to constraints imposed by the COVID-19 pandemic. Additionally, the inclusion of a predominantly European population with well-controlled T2D further limits the broader generalizability of our results. Moreover, the BFRT and CREST groups were not sex-matched so that a potential sex/gender effect cannot be excluded. Furthermore, both groups performed different numbers of repetitions per set. Also, including another group performing the identical number of sets and repetitions as the BFRT group, but without occlusion, would have allowed for a clearer attribution of effects. However, total training volume, i.e., sets × repetitions × weight, was comparable between the groups (Table S2). Also, the relatively short intervention duration may have prevented observing the full spectrum of metabolic changes, particularly regarding insulin sensitivity and glycemic control. However, the 12-week design already allowed for the detection of the early changes induced by both resistance training approaches.

In conclusion, we demonstrate that BFRT (1) elicits similar improvements in muscle strength and induces hypertrophy as CREST, despite significantly lower mechanical load; (2) specifically improves skeletal muscle and adipose tissue mitochondrial oxidative capacity accompanied by upregulation of molecular markers of mitochondrial signaling, metabolic remodeling, and angiogenic processes; and (3) selectively reduces VAT volumes, which likely translates into its cardiometabolic benefits. Given its low mechanical load, BFRT is therefore a practical and safe alternative for individuals with T2D who have musculoskeletal limitations or reduced functional capacity.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Michael Roden (michael.roden@ddz.de).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

All data and materials reported in this paper will be shared by the lead contact upon request. Source data and scans of western blots are available in Data S1. Raw RNA-seq data are available on NCBI GEO: GSE312780.

ACKNOWLEDGMENTS

The authors would like to thank all volunteers for participating in the study as well as Michelle Reina Do Fundo, Fariba Zivehe, Olga Dürrschmidt, Agnieszka Sutkowski, Monika Schulte, Petra Domass, Gunhild Heitkamp, Geronimo Heilmann, and Jan-Marc Leonhard for their excellent support. This project was supported by grants to the German Diabetes Center (DDZ) from the German Federal Ministry of Health (BMG) and the Ministry of Culture and Science of the State of North Rhine Westphalia, as well as from the German Federal Ministry for Research (BMBF), to the German Center for Diabetes Research (DZD e.V.). D.H.P. was supported by a DZD Next Grant from the German Center for Diabetes Research (DZD e.V.). M.R. and C.H. are also supported by grants from the European Community (HORIZON HLTH 2022 STAYHLTH 02 01: panel A) to the Intercept T2D consortium. M.R. is also supported by grants from the German Research Foundation (DFG; RTG/GRK 2576) and the Schmutzler Stiftung. The funding sources were not involved in the design of the study; the collection, analysis, and interpretation of data; or writing the report and did not impose any restrictions regarding the publication of the report.

AUTHOR CONTRIBUTIONS

N.T., D.H.P., M.R., and P.S. wrote the first draft of the manuscript and researched data. K.S. performed the statistical analyses and contributed to writing and editing the manuscript. N.T., S.G., J.P., M.S., M.H., K.P., L.M., B.D., Y.K., C.H., K.S., O.P.Z., S.T., V.S.-H., and D.H.P. performed experiments and collected data. P.M.M.R., S.K., and J.H. performed RNA-seq. D.H.P. and M.R. initiated the study and contributed to writing and editing the manuscript. All authors critically reviewed the manuscript. M.R. had full access to all the data in the study and guided the entire publication process. D.H.P. takes responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

M.R. received lecture fees or served on advisory boards for AstraZeneca, Echosens, Eli Lilly, Madrigal, MSD Sharp & Dohme, Novo Nordisk, Pfizer, Synlab, and Target RWE and performed investigator-initiated research with support from Boehringer Ingelheim and Sanofi-Aventis to the German Diabetes Center (DDZ).

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cmet.2025.12.016.

Received: May 30, 2025
Revised: November 7, 2025
Accepted: December 15, 2025

REFERENCES

1. Roden, M., and Shulman, G.I. (2019). The integrative biology of type 2 diabetes. Nature *576*, 51–60. https://doi.org/10.1038/s41586-019-1797-8.
2. Park, S.W., Goodpaster, B.H., Strotmeyer, E.S., de Rekeneire, N., Harris, T.B., Schwartz, A.V., Tylavsky, F.A., and Newman, A.B. (2006). Decreased muscle strength and quality in older adults with type 2 diabetes: the health, aging, and body composition study. Diabetes *55*, 1813–1818. https://doi.org/10.2337/db05-1183.
3. Sayer, A.A., Dennison, E.M., Syddall, H.E., Gilbody, H.J., Phillips, D.I.W., and Cooper, C. (2005). Type 2 diabetes, muscle strength, and impaired physical function: the tip of the iceberg? Diabetes Care *28*, 2541–2542. https://doi.org/10.2337/diacare.28.10.2541.
4. Saatmann, N., Zaharia, O.P., Strassburger, K., Pesta, D.H., Burkart, V., Szendroedi, J., Gerdes, N., Kelm, M., and Roden, M. (2022). Physical fitness and cardiovascular risk factors in novel diabetes subgroups. J. Clin. Endocrinol. Metab. *107*, 1127–1139. https://doi.org/10.1210/clinem/dgab810.
5. Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944–2950. https://doi.org/10.2337/diabetes.51.10.2944.
6. Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N. Engl. J. Med. *350*, 664–671. https://doi.org/10.1056/NEJMoa031314.
7. Kalyani, R.R., Corriere, M., and Ferrucci, L. (2014). Age-related and disease-related muscle loss: the effect of diabetes, obesity, and other diseases. Lancet Diabetes Endocrinol. *2*, 819–829. https://doi.org/10.1016/s2213-8587(14)70034-8.
8. Cohen, S., Nathan, J.A., and Goldberg, A.L. (2015). Muscle wasting in disease: molecular mechanisms and promising therapies. Nat. Rev. Drug Discov. *14*, 58–74. https://doi.org/10.1038/nrd4467.
9. Kanaley, J.A., Colberg, S.R., Corcoran, M.H., Malin, S.K., Rodriguez, N.R., Crespo, C.J., Kirwan, J.P., and Zierath, J.R. (2022). Exercise/physical activity in individuals with type 2 diabetes: a consensus statement from the American College of Sports Medicine. Med. Sci. Sports Exerc. *54*, 353–368. https://doi.org/10.1249/mss.0000000000002800.
10. Meex, R.C.R., Schrauwen-Hinderling, V.B., Moonen-Kornips, E., Schaart, G., Mensink, M., Phielix, E., van de Weijer, T., Sels, J.P., Schrauwen, P., and Hesselink, M.K.C. (2010). Restoration of muscle mitochondrial function and metabolic flexibility in type 2 diabetes by exercise training is

paralleled by increased myocellular fat storage and improved insulin sensitivity. Diabetes *59*, 572–579. https://doi.org/10.2337/db09-1322.

11. Pesta, D., Paschke, V., Hoppel, F., Kobel, C., Kremser, C., Esterhammer, R., Burtscher, M., Kemp, G.J., and Schocke, M. (2013). Different metabolic responses during incremental exercise assessed by localized 31P MRS in sprint and endurance athletes and untrained individuals. Int. J. Sports Med. *34*, 669–675. https://doi.org/10.1055/s-0032-1327648.
12. Hesselink, M.K.C., Schrauwen-Hinderling, V., and Schrauwen, P. (2016). Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus. Nat. Rev. Endocrinol. *12*, 633–645. https://doi.org/10.1038/nrendo.2016.104.
13. Pesta, D., Hoppel, F., Macek, C., Messner, H., Faulhaber, M., Kobel, C., Parson, W., Burtscher, M., Schocke, M., and Gnaiger, E. (2011). Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. Am. J. Physiol. Regul. Integr. Comp. Physiol. *301*, R1078–R1087. https://doi.org/10.1152/ajpregu.00285.2011.
14. Mastrototaro, L., Apostolopoulou, M., Hartwig, S., Strassburger, K., Lipaeva, P., Trinks, N., Karusheva, Y., Gancheva, S., Trenkamp, S., Lehr, S., et al. (2024). The role of exosomes for sustained specific cardiorespiratory and metabolic improvements in males with type 2 diabetes after detraining. EBiomedicine *110*, 105471. https://doi.org/10.1016/j.ebiom.2024.105471.
15. Schytz, C.T., Ørtenblad, N., Lundby, A.M., Jacobs, R.A., Nielsen, J., and Lundby, C. (2024). Skeletal muscle mitochondria demonstrate similar respiration per cristae surface area independent of training status and sex in healthy humans. J. Physiol. *602*, 129–151. https://doi.org/10.1113/jp285091.
16. Pataky, M.W., Heppelmann, C.J., Sevits, K.J., Asokan, A.K., Kumar, A.P., Klaus, K.A., Dasari, S., Kunz, H.E., Strub, M.D., Robinson, M.M., et al. (2025). Aerobic and resistance exercise-regulated phosphoproteome and acetylproteome modifications in human skeletal muscle. Nat. Commun. *16*, 5700. https://doi.org/10.1038/s41467-025-60049-0.
17. Balducci, S., Leonetti, F., Di Mario, U., and Fallucca, F. (2004). Is a long-term aerobic plus resistance training program feasible for and effective on metabolic profiles in type 2 diabetic patients? Diabetes Care *27*, 841–842. https://doi.org/10.2337/diacare.27.3.841.
18. Ismail, A.D., Alkhayl, F.F.A., Wilson, J., Johnston, L., Gill, J.M.R., and Gray, S.R. (2019). The effect of short-duration resistance training on insulin sensitivity and muscle adaptations in overweight men. Exp. Physiol. *104*, 540–545. https://doi.org/10.1113/ep087435.
19. Stephens, N.A., and Sparks, L.M. (2015). Resistance to the beneficial effects of exercise in type 2 diabetes: are some individuals programmed to fail? J. Clin. Endocrinol. Metab. *100*, 43–52. https://doi.org/10.1210/jc.2014-2545.
20. Plotnikoff, R.C., Taylor, L.M., Wilson, P.M., Courneya, K.S., Sigal, R.J., Birkett, N., Raine, K., and Svenson, L.W. (2006). Factors associated with physical activity in Canadian adults with diabetes. Med. Sci. Sports Exerc. *38*, 1526–1534. https://doi.org/10.1249/01.mss.0000228937.86539.95.
21. Martin, P.M., Bart, R.M., Ashley, R.L., Velasco, T., and Wise, S.R. (2022). An overview of blood flow restriction physiology and clinical considerations. Curr. Sports Med. Rep. *21*, 123–128. https://doi.org/10.1249/jsr.0000000000000948.
22. Vogt, M., Puntschart, A., Geiser, J., Zuleger, C., Billeter, R., and Hoppeler, H. (2001). Molecular adaptations in human skeletal muscle to endurance training under simulated hypoxic conditions. J. Appl. Physiol. (1985) *91*, 173–182. https://doi.org/10.1152/jappl.2001.91.1.173.
23. Farup, J., de Paoli, F., Bjerg, K., Riis, S., Ringgard, S., and Vissing, K. (2015). Blood flow restricted and traditional resistance training performed to fatigue produce equal muscle hypertrophy. Scand. J. Med. Sci. Sports *25*, 754–763. https://doi.org/10.1111/sms.12396.
24. Takarada, Y., Takazawa, H., Sato, Y., Takebayashi, S., Tanaka, Y., and Ishii, N. (2000). Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. J. Appl. Physiol. (1985) *88*, 2097–2106. https://doi.org/10.1152/jappl.2000.88.6.2097.
25. Christiansen, D., Murphy, R.M., Bangsbo, J., Stathis, C.G., and Bishop, D.J. (2018). Increased FXYD1 and PGC-1α mRNA after blood flow-restricted running is related to fibre type-specific AMPK signalling and oxidative stress in human muscle. Acta Physiol. (Oxf.) *223*, e13045. https://doi.org/10.1111/apha.13045.
26. Christiansen, D., Eibye, K., Hostrup, M., and Bangsbo, J. (2020). Training with blood flow restriction increases femoral artery diameter and thigh oxygen delivery during knee-extensor exercise in recreationally trained men. J. Physiol. *598*, 2337–2353. https://doi.org/10.1113/jp279554.
27. Larkin, K.A., Macneil, R.G., Dirain, M., Sandesara, B., Manini, T.M., and Buford, T.W. (2012). Blood flow restriction enhances post-resistance exercise angiogenic gene expression. Med. Sci. Sports Exerc. *44*, 2077–2083. https://doi.org/10.1249/MSS.0b013e3182625928.
28. Takarada, Y., Sato, Y., and Ishii, N. (2002). Effects of resistance exercise combined with vascular occlusion on muscle function in athletes. Eur. J. Appl. Physiol. *86*, 308–314. https://doi.org/10.1007/s00421-001-0561-5.
29. Saatmann, N., Zaharia, O.P., Loenneke, J.P., Roden, M., and Pesta, D.H. (2021). Effects of blood flow restriction exercise and possible applications in type 2 diabetes. Trends Endocrinol. Metab. *32*, 106–117. https://doi.org/10.1016/j.tem.2020.11.010.
30. Larsen, S., Nielsen, J., Hansen, C.N., Nielsen, L.B., Wibrand, F., Stride, N., Schroder, H.D., Boushel, R., Helge, J.W., Dela, F., et al. (2012). Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. J. Physiol. *590*, 3349–3360. https://doi.org/10.1113/jphysiol.2012.230185.
31. Mendham, A.E., Larsen, S., George, C., Adams, K., Hauksson, J., Olsson, T., Fortuin-de Smidt, M.C., Nono Nankam, P.A., Hakim, O., Goff, L.M., et al. (2020). Exercise training results in depot-specific adaptations to adipose tissue mitochondrial function. Sci. Rep. *10*, 3785. https://doi.org/10.1038/s41598-020-60286-x.
32. Ma, F., He, J., and Wang, Y. (2024). Blood flow restriction combined with resistance training on muscle strength and thickness improvement in young adults: a systematic review, meta-analysis, and meta-regression. Front. Physiol. *15*, 1379605. https://doi.org/10.3389/fphys.2024.1379605.
33. Rodrigo-Mallorca, D., Loaiza-Betancur, A.F., Monteagudo, P., Blasco-Lafarga, C., and Chulvi-Medrano, I. (2021). Resistance training with blood flow restriction compared to traditional resistance training on strength and muscle mass in non-active older adults: a systematic review and meta-analysis. Int. J. Environ. Res. Public Health *18*, 11441. https://doi.org/10.3390/ijerph182111441.
34. Jørgensen, S.L., Kierkegaard-Brøchner, S., Bohn, M.B., Høgsholt, M., Aagaard, P., and Mechlenburg, I. (2023). Effects of blood-flow restricted exercise versus conventional resistance training in musculoskeletal disorders—a systematic review and meta-analysis. BMC Sports Sci. Med. Rehabil. *15*, 141. https://doi.org/10.1186/s13102-023-00750-z.
35. Jochem, C., Leitzmann, M., Volaklis, K., Aune, D., and Strasser, B. (2019). Association between muscular strength and mortality in clinical populations: a systematic review and meta-analysis. J. Am. Med. Dir. Assoc. *20*, 1213–1223. https://doi.org/10.1016/j.jamda.2019.05.015.
36. Conte, C., Hall, K.D., and Klein, S. (2024). Is weight loss-induced muscle mass loss clinically relevant? JAMA *332*, 9–10. https://doi.org/10.1001/jama.2024.6586.
37. Dreyer, H.C., Fujita, S., Cadenas, J.G., Chinkes, D.L., Volpi, E., and Rasmussen, B.B. (2006). Resistance exercise increases AMPK activity and reduces 4E-BP1 phosphorylation and protein synthesis in human skeletal muscle. J. Physiol. *576*, 613–624. https://doi.org/10.1113/jphysiol.2006.113175.
38. Gundermann, D.M., Walker, D.K., Reidy, P.T., Borack, M.S., Dickinson, J.M., Volpi, E., and Rasmussen, B.B. (2014). Activation of mTORC1 signaling and protein synthesis in human muscle following blood flow restriction exercise is inhibited by rapamycin. Am. J. Physiol., Endocrinol. Metab. *306*, E1198–E1204. https://doi.org/10.1152/ajpendo.00600.2013.
39. Willoughby, D.S. (2004). Effects of heavy resistance training on myostatin mRNA and protein expression. Med. Sci. Sports Exerc. *36*, 574–582. https://doi.org/10.1249/01.mss.0000121952.71533.ea.

40. Freyssenet, D., Berthon, P., and Denis, C. (1996). Mitochondrial biogenesis in skeletal muscle in response to endurance exercises. Arch. Physiol. Biochem. *104*, 129–141. https://doi.org/10.1076/apab.104.2.129.12878.

41. Porter, C., Reidy, P.T., Bhattarai, N., Sidossis, L.S., and Rasmussen, B.B. (2015). Resistance exercise training alters mitochondrial function in human skeletal muscle. Med. Sci. Sports Exerc. *47*, 1922–1931. https://doi.org/10.1249/mss.0000000000000605.

42. Jäger, S., Handschin, C., St-Pierre, J., and Spiegelman, B.M. (2007). AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc. Natl. Acad. Sci. USA *104*, 12017–12022. https://doi.org/10.1073/pnas.0705070104.

43. Handschin, C., and Spiegelman, B.M. (2006). Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. Endocr. Rev. *27*, 728–735. https://doi.org/10.1210/er.2006-0037.

44. Preobrazenski, N., Islam, H., and Gurd, B.J. (2021). Molecular regulation of skeletal muscle mitochondrial biogenesis following blood flow-restricted aerobic exercise: a call to action. Eur. J. Appl. Physiol. *121*, 1835–1847. https://doi.org/10.1007/s00421-021-04669-6.

45. Cusi, K.J., Pratipanawatr, T., Koval, J., Printz, R., Ardehali, H., Granner, D.K., Defronzo, R.A., and Mandarino, L.J. (2001). Exercise increases hexokinase II mRNA, but not activity in obesity and type 2 diabetes. Metabolism *50*, 602–606. https://doi.org/10.1053/meta.2001.22568.

46. Aagaard, P., Simonsen, E.B., Andersen, J.L., Magnusson, P., and Dyhre-Poulsen, P. (2002). Increased rate of force development and neural drive of human skeletal muscle following resistance training. J. Appl. Physiol. (1985) *93*, 1318–1326. https://doi.org/10.1152/japplphysiol.00283.2002.

47. Pearson, S.J., and Hussain, S.R. (2015). A review on the mechanisms of blood-flow restriction resistance training-induced muscle hypertrophy. Sports Med. *45*, 187–200. https://doi.org/10.1007/s40279-014-0264-9.

48. Essig, D.A. (1996). Contractile activity-induced mitochondrial biogenesis in skeletal muscle. Exerc. Sport Sci. Rev. *24*, 289–319. https://doi.org/10.1249/00003677-199600240-00012.

49. Shankaran, M., King, C.L., Angel, T.E., Holmes, W.E., Li, K.W., Colangelo, M., Price, J.C., Turner, S.M., Bell, C., Hamilton, K.L., et al. (2016). Circulating protein synthesis rates reveal skeletal muscle proteome dynamics. J. Clin. Investig. *126*, 288–302. https://doi.org/10.1172/jci79639.

50. Walsh, B., Tonkonogi, M., and Sahlin, K. (2001). Effect of endurance training on oxidative and antioxidative function in human permeabilized muscle fibres. Pflugers Arch. *442*, 420–425. https://doi.org/10.1007/s004240100538.

51. Dohlmann, T.L., Hindsø, M., Dela, F., Helge, J.W., and Larsen, S. (2018). High-intensity interval training changes mitochondrial respiratory capacity differently in adipose tissue and skeletal muscle. Physiol. Rep. *6*, e13857. https://doi.org/10.14814/phy2.13857.

52. Raheem, J., Sliz, E., Shin, J., Holmes, M.V., Pike, G.B., Richer, L., Gaudet, D., Paus, T., and Pausova, Z. (2022). Visceral adiposity is associated with metabolic profiles predictive of type 2 diabetes and myocardial infarction. Commun. Med. (Lond) *2*, 81. https://doi.org/10.1038/s43856-022-00140-5.

53. Takarada, Y., Nakamura, Y., Aruga, S., Onda, T., Miyazaki, S., and Ishii, N. (2000). Rapid increase in plasma growth hormone after low-intensity resistance exercise with vascular occlusion. J. Appl. Physiol. (1985) *88*, 61–65. https://doi.org/10.1152/jappl.2000.88.1.61.

54. Ormsbee, M.J., Choi, M.D., Medlin, J.K., Geyer, G.H., Trantham, L.H., Dubis, G.S., and Hickner, R.C. (2009). Regulation of fat metabolism during resistance exercise in sedentary lean and obese men. J. Appl. Physiol. *106*, 1529–1537. https://doi.org/10.1152/japplphysiol.91485.2008.

55. Stanley, T.L., and Grinspoon, S.K. (2015). Effects of growth hormone-releasing hormone on visceral fat, metabolic, and cardiovascular indices in human studies. Growth Horm. IGF Res. *25*, 59–65. https://doi.org/10.1016/j.ghir.2014.12.005.

56. Su, W., Tao, M., Ma, L., Tang, K., Xiong, F., Dai, X., and Qin, Y. (2023). Dose-response relationships of resistance training in type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. Front. Endocrinol. *14*, 1224161. https://doi.org/10.3389/fendo.2023.1224161.

57. Apostolopoulou, M., Mastrototaro, L., Hartwig, S., Pesta, D., Straßburger, K., de Filippo, E., Jelenik, T., Karusheva, Y., Gancheva, S., Markgraf, D., et al. (2021). Metabolic responsiveness to training depends on insulin sensitivity and protein content of exosomes in insulin-resistant males. Sci. Adv. *7*, eabi9551. https://doi.org/10.1126/sciadv.abi9551.

58. Liu, Y., Lee, D.C., Li, Y., Zhu, W., Zhang, R., Sui, X., Lavie, C.J., and Blair, S.N. (2019). Associations of resistance exercise with cardiovascular disease morbidity and mortality. Med. Sci. Sports Exerc. *51*, 499–508. https://doi.org/10.1249/mss.0000000000001822.

59. Mouser, J.G., Mattocks, K.T., Buckner, S.L., Dankel, S.J., Jessee, M.B., Bell, Z.W., Abe, T., Bentley, J.P., and Loenneke, J.P. (2019). High-pressure blood flow restriction with very low load resistance training results in peripheral vascular adaptations similar to heavy resistance training. Physiol. Meas. *40*, 035003. https://doi.org/10.1088/1361-6579/ab0d2a.

60. Ma, X., Lin, X., Zhou, L., Li, W., Yi, Q., Lei, F., Tang, X., Ai, Y., Zhan, Y., Luo, H., et al. (2024). The effect of blood flow-restrictive resistance training on the risk of atherosclerotic cardiovascular disease in middle-aged patients with type 2 diabetes: a randomized controlled trial. Front. Endocrinol *15*, 1482985. https://doi.org/10.3389/fendo.2024.1482985.

61. Zhang, X.Z., Xie, W.Q., Chen, L., Xu, G.D., Wu, L., Li, Y.S., and Wu, Y.X. (2022). Blood flow restriction training for the intervention of sarcopenia: current stage and future perspective. Front. Med. *9*, 894996. https://doi.org/10.3389/fmed.2022.894996.

62. Li, S., Guo, R., Yu, T., Li, S., Han, T., and Yu, W. (2022). Effect of high-intensity interval training combined with blood flow restriction at different phases on abdominal visceral fat among obese adults: a randomized controlled trial. Int. J. Environ. Res. Public Health *19*, 11936. https://doi.org/10.3390/ijerph191911936.

63. Corrêa, H.L., Neves, R.V.P., Deus, L.A., Souza, M.K., Haro, A.S., Costa, F., Silva, V.L., Santos, C.A.R., Moraes, M.R., Simões, H.G., et al. (2021). Blood flow restriction training blunts chronic kidney disease progression in humans. Med. Sci. Sports Exerc. *53*, 249–257. https://doi.org/10.1249/mss.0000000000002465.

64. Mendonca, G.V., Mouro, M., Vila-Chã, C., and Pezarat-Correia, P. (2020). Nerve conduction during acute blood-flow restriction with and without low-intensity exercise Nerve conduction and blood-flow restriction. Sci. Rep. *10*, 7380. https://doi.org/10.1038/s41598-020-64379-5.

65. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. Bioinformatics *29*, 15–21. https://doi.org/10.1093/bioinformatics/bts635.

66. Patro, R., Duggal, G., Love, M.I., Irizarry, R.A., and Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. Nat. Methods *14*, 417–419. https://doi.org/10.1038/nmeth.4197.

67. Soneson, C., Love, M.I., and Robinson, M.D. (2015). Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. F1000Res *4*, 1521. https://doi.org/10.12688/f1000research.7563.2.

68. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. *15*, 550. https://doi.org/10.1186/s13059-014-0550-8.

69. Wu, J.Q., Mao, L.B., Liu, L.F., Li, Y.M., Wu, J., Yao, J., Zhang, F.H., Liu, T.Y., and Yuan, L. (2021). Identification of key genes and pathways of BMP-9-induced osteogenic differentiation of mesenchymal stem cells by integrated bioinformatics analysis. J. Orthop. Surg. Res. *16*, 273. https://doi.org/10.1186/s13018-021-02390-w.

70. American Diabetes Association Professional; Practice Committee (2024). 2. Diagnosis and classification of diabetes: standards of care in diabetes-2024. Diabetes Care *47*, S20–S42. https://doi.org/10.2337/dc24-S002.

71. McNair, P.J., Colvin, M., and Reid, D. (2011). Predicting maximal strength of quadriceps from submaximal performance in individuals with knee joint

osteoarthritis. Arthritis Care Res. (Hoboken) *63*, 216–222. https://doi.org/10.1002/acr.20368.

72. Patterson, S.D., Hughes, L., Warmington, S., Burr, J., Scott, B.R., Owens, J., Abe, T., Nielsen, J.L., Libardi, C.A., Laurentino, G., et al. (2019). Blood flow restriction exercise: considerations of methodology, application, and safety. Front. Physiol. *10*, 533. https://doi.org/10.3389/fphys.2019.00533.
73. Loenneke, J.P., Wilson, J.M., Wilson, G.J., Pujol, T.J., and Bemben, M.G. (2011). Potential safety issues with blood flow restriction training. Scand. J. Med. Sci. Sports *21*, 510–518. https://doi.org/10.1111/j.1600-0838.2010.01290.x.
74. Szendroedi, J., Schmid, A.I., Chmelik, M., Toth, C., Brehm, A., Krssak, M., Nowotny, P., Wolzt, M., Waldhausl, W., and Roden, M. (2007). Muscle mitochondrial ATP synthesis and glucose transport/phosphorylation in type 2 diabetes. PLoS Med. *4*, e154. https://doi.org/10.1371/journal.pmed.0040154.
75. Pesta, D., and Gnaiger, E. (2012). High-resolution respirometry: OXPHOS protocols for human cells and permeabilized fibers from small biopsies of human muscle. Methods Mol. Biol. *810*, 25–58. https://doi.org/10.1007/978-1-61779-382-0_3.
76. Pesta, D., Anadol-Schmitz, E., Sarabhai, T., Op den Kamp, Y., Gancheva, S., Trinks, N., Zaharia, O.P., Mastrototaro, L., Lyu, K., Habets, I., et al. (2025). Determinants of increased muscle insulin sensitivity of exercise-trained versus sedentary normal weight and overweight individuals. Sci. Adv. *11*, eadr8849. https://doi.org/10.1126/sciadv.adr8849.
77. Szendroedi, J., Saxena, A., Weber, K.S., Strassburger, K., Herder, C., Burkart, V., Nowotny, B., Icks, A., Kuss, O., Ziegler, D., et al. (2016). Cohort profile: the German Diabetes Study (GDS). Cardiovasc. Diabetol. *15*, 59. https://doi.org/10.1186/s12933-016-0374-9.
78. Mastrototaro, L., and Roden, M. (2021). Insulin resistance and insulin sensitizing agents. Metabolism *125*, 154892. https://doi.org/10.1016/j.metabol.2021.154892.
79. Machann, J., Thamer, C., Stefan, N., Schwenzer, N.F., Kantartzis, K., Häring, H.U., Claussen, C.D., Fritsche, A., and Schick, F. (2010). Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. Radiology *257*, 353–363. https://doi.org/10.1148/radiol.10092284.
80. Kupriyanova, Y., Zaharia, O.P., Bobrov, P., Karusheva, Y., Burkart, V., Szendroedi, J., Hwang, J.H., and Roden, M.; GDS group (2021). Early changes in hepatic energy metabolism and lipid content in recent-onset type 1 and 2 diabetes mellitus. J. Hepatol. *74*, 1028–1037. https://doi.org/10.1016/j.jhep.2020.11.030.
81. Hamilton, G., Yokoo, T., Bydder, M., Cruite, I., Schroeder, M.E., Sirlin, C.B., and Middleton, M.S. (2011). In vivo characterization of the liver fat (1)H MR spectrum. NMR Biomed. *24*, 784–790. https://doi.org/10.1002/nbm.1622.
82. Lichtenstein, T., Sprenger, A., Weiss, K., Slebocki, K., Cervantes, B., Karampinos, D., Maintz, D., Fink, G.R., Henning, T.D., and Lehmann, H.C. (2018). MRI biomarkers of proximal nerve injury in CIDP. Ann. Clin. Transl. Neurol. *5*, 19–28. https://doi.org/10.1002/acn3.502.
83. Mathur, S., Takai, K.P., Macintyre, D.L., and Reid, D. (2008). Estimation of thigh muscle mass with magnetic resonance imaging in older adults and people with chronic obstructive pulmonary disease. Phys. Ther. *88*, 219–230. https://doi.org/10.2522/ptj.20070052.
84. Pafili, K., Kahl, S., Mastrototaro, L., Strassburger, K., Pesta, D., Herder, C., Pützer, J., Dewidar, B., Hendlinger, M., Granata, C., et al. (2022). Mitochondrial respiration is decreased in visceral but not subcutaneous adipose tissue in obese individuals with fatty liver disease. J. Hepatol. *77*, 1504–1514. https://doi.org/10.1016/j.jhep.2022.08.010.
85. Ruppert, P.M.M., Güller, A.S., Rosendal, M., Stanic, N., and Kornfeld, J.-W. (2025). Dietary sulfur amino acid restriction elicits a cold-like transcriptional response in inguinal but not epididymal white adipose tissue of male mice. Preprint at bioRxiv. https://doi.org/10.1101/2025.08.06.669020.

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
IRS1	Millipore	Cat#06-248; RRID: AB_2127890
pIRS(Ser1101)	Cell Signaling Technology	Cat#2385; RRID: AB_330363
AKT	Cell Signaling Technology	Cat#9272; RRID: AB_329827
pAKT(Ser473)	Cell Signaling Technology	Cat#4060; RRID: AB_2315049
pAKT(Thr308)	Cell Signaling Technology	Cat#4056; RRID: AB_331163
TFAM	Cell Signaling Technology	Cat#7495; RRID: AB_10841294
HXK1	Cell Signaling Technology	Cat#2024; RRID: AB_2116996
HXK2	Cell Signaling Technology	Cat#2106; RRID: AB_823520
PGC	Abcam	Cat#ab191838; RRID: AB_2721267
mTOR	Cell Signaling Technology	Cat#2972; RRID: AB_2262884
AMPKα	Cell Signaling Technology	Cat#2532; RRID: AB_330331
pAMPKα(Thr172)	Cell Signaling Technology	Cat#2535; RRID: AB_331250
HIF1α	Novus Biological	Cat#NB100-134; RRID: AB_350071
PFKM	Bio-Techne	Cat#MAB7687; RRID: AB_2861389
Anti-rabbit IgG HRP-conjugated	Cell Signaling Technology	Cat#7074; RRID: AB_2099233
Anti-mouse IgG HRP-conjugated	Cell Signaling Technology	Cat#7076; RRID: AB_330924
Biological samples		
Human skeletal muscle	Human	De-identified
Human adipose tissue	Human	De-identified
Chemicals, peptides, and recombinant proteins		
4x Laemmli Sample Buffer	BioRad	Cat#1610747
Milk Powder	Roth	Cat#T145.3
Tween20	SigmaAldrich	Cat#P1379
10x Tris/Glycine Buffer	BioRad	Cat#1610734
10x Tris/Glycine/SDS	BioRad	Cat#1610732
Malate	Sigma	Cat# M1000
Pyruvate	Sigma	Cat# P2256
Succinate	Sigma	Cat# S2378
ADP	Sigma	Cat# A5285
Cytochrome c	Sigma	Cat# C7752
Saponin	Sigma	Cat# S7900-25
Digitonin	Sigma-Aldrich	Cat# D5628
Critical commercial assays		
HK activity assay kit	Abcam	Cat#ab136957
PFK activity assay kit	Abcam	Cat#ab155898
Elisa IL-6	Biovendo	Cat#RD194015200R
Elisa myostatin	Invitrogen	Cat#EH215RB
NEX Poly(A) Beads 2.0 Auto (48rxn)	Revvity	Cat#NOVA-512994
NEX Rapid Dir RNAseq Auto Kit 2.0 (48rxn)	Revvity	Cat#NOVA-5198-43
Novaseq S1 Reagent Kit v1.5 (200 cycles)	Illumina	Cat#20028318
D1000 Screentape	Agilent	Cat#5067-1582
D1000 Reagents	Agilent	Cat#5067-1583
D1000 Ladder	Agilent	Cat#5067-1586
Agilent RNA 6000 Nano Kit	Agilent	Cat#5067-1511

(*Continued on next page*)

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Qubit tubes	ThermoFisher Scientific	Cat#Q32856
Qubit 1x dsDNA High Sensitivity	ThermoFisher Scientific	Cat#Q33231
500 assay RNA high Sens.Qubit	Fisher Emergo	Cat#10320093/ Q32855
Deposited data		
RNA-seq data	This Paper	GSE312780
Western Blots	This Paper	Data S1
Raw Data	This Paper	Data S1
Oligonucleotides		
Primer for LPL forward primer: CGAGTCGTCTTTCTCCTGATGAT reverse primer: TTCTGGATTCCAATGCTTCGA	Eurofins	N/A
Primer for ND1 forward primers: CCCTAAAACCCGCCACATCT reverse primers: GAGCGATGGTGAGAGCTAAGGT	Eurofins	N/A
Recombinant DNA		
Human LPL plasmid	Origene	Cat#RC203766
Human ND1 plasmid	Origene	Cat#SC101172
Software and algorithms		
GraphPad Prism (version 10.2.3)	GraphPad	N/A
EndNoteX9	EndNote	N/A
SAS (version 9.4)	SAS	N/A
FastQC (version 0.12.1)	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/	N/A
Trim Galore! (version 0.6.10)	https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/	N/A
The R Project for Statistical Computing (version 4.5.1)	https://www.r-project.org/	N/A
STAR aligner (version 2.7.11b)	Dobin et al.[65]	https://pubmed.ncbi.nlm.nih.gov/23104886/
SALMON (version 1.10.3)	Patro et al.[66]	https://pubmed.ncbi.nlm.nih.gov/28263959/
Tximport (version 1.36.1)	Soneson et al.[67]	https://pubmed.ncbi.nlm.nih.gov/26925227/
DESeq2 (version 1.48.2)	Love et al.[68]	https://pubmed.ncbi.nlm.nih.gov/25516281/
clusterProfiler package (version 4.16.0)	Wu et al.[69]	https://pubmed.ncbi.nlm.nih.gov/33879213/

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Study participants

This study included sedentary, Caucasian women (n=4) and men (n=16) with type 2 diabetes who were recruited between October 2019 and January 2024. Diagnosis of diabetes was based on the criteria of the American Diabetes Association (Figure S4).[70] At inclusion, volunteers confirmed stable body weight over the past six months. Exclusion criteria included regular endurance or resistance training (>75-150 minutes of vigorous-intensity exercise per week) within the last 3 months, acute or chronic cardiovascular, renal, respiratory, thyroid, or liver diseases, cancer, diabetic neuropathy, muscular dystrophy, use of insulin-sensitizing, immunomodulatory medications, anticoagulants, or beta-blockers, pregnancy, alcohol consumption exceeding 20 g/day (women) or 30 g/day (men), and smoking.

Prior to inclusion, all participants provided written informed consent. The study was approved by the ethics committee of the Medical Faculty of Heinrich Heine University Düsseldorf (reference number 2019-419), registered at Clinicaltrials.gov (identifier: NCT04222231), and conducted in accordance with the Declaration of Helsinki.

METHOD DETAILS

Study design

Participants underwent a screening visit (V1) to confirm eligibility based on inclusion and exclusion criteria. Examinations included magnetic resonance imaging and anthropometric assessments. Those meeting all criteria were invited for a second visit (V2) to assess insulin sensitivity and collect biopsies of the *musculus vastus lateralis* and subcutaneous abdominal adipose tissue

(Figure S5). One participant (Resist19) had elevated hsCRP (2.95 mg/dl) at V2, so the results were excluded from the analysis. Eight days later, participants began a 12-week, three-times-weekly supervised intervention, either BFRT or CREST.

Venous blood sampling and spiroergometry were performed before the first training session (V3). Forty-eight to seventy-two hours after the final training session, the last two examination days (V4) were conducted, including spiroergometry and all measurements from V1 and V2 (Figure S4). Participants were instructed to maintain their current medication regimen; however, one participant (Resist48) increased their semaglutide dose (1 mg/5 days to 2 mg/7 days) after one week of training.

Exercise training protocol and BFRT

Prior to the start of the training, all participants underwent submaximal strength testing to determine their 1-RM and establish initial training loads. This test was repeated every 2-3 weeks to adjust the load and account for the training progress, following recognized guidelines and using established 1-RM prediction equations based on repetitions-to-fatigue.[71] Training was conducted three times per week (Mon, Wed, Fri) and included a 10-minute warm-up on a cycle ergometer (Technogym, Neu-Isenburg, Germany), followed by supervised resistance training with or without blood flow restriction (Table S2). Sessions were fully supervised by qualified instructors.

During training, arterial and venous blood flow to the exercising thigh muscles was reduced by 60% and 100%, respectively, in all BFRT participants.[72] A tourniquet cuff (Delfi Medical, Vancouver, BC, Canada) was placed around the proximal end of the working limb to reduce blood flow. Cuff inflation pressure was set based on the estimated limb occlusion pressure, the minimum pressure required to occlude arterial blood flow. The cuffs remained inflated until the final exercise set was completed. In line, the occlusion was sustained throughout the 30 sec rest period between the sets to make sure that BFRT elicits its intended effects (e.g. metabolite activation).[29] However, the cuffs were deflated between the exercises.[72] Although generally well tolerated, blood-flow restriction training carries potential risks such as venous collapse, thrombosis, and nerve compression, highlighting the importance of standardized protocols and medical supervision to ensure safe implementation. Of note, available evidence suggests that BFRT neither adversely affects blood pressure, nerve conduction velocity, blood coagulation, venous pressure or muscle integrity, nor poses greater risk of injury compared to CREST.[29,73] Upper body exercise bouts were conducted without blood flow restriction and low loads in both groups for general conditioning and to maintain muscular balance (30% of 1-RM, Table S2).

Metabolic characterization

Up to eight days before the first and ~72 hours after the last training, participants underwent a hyperinsulinemic-euglycemic clamp in the morning after an overnight fast to assess whole-body and hepatic insulin sensitivity.[74] During the 60–120 min clamp (40 mU insulin / [m^2 body surface area x min], Insuman Rapid, Sanofi-Aventis, Germany), blood glucose was maintained at ~90 mg/dl by adjusting a 20% glucose infusion (2% enriched with D-[6,6-$^2H_2$]glucose) every 5 minutes. Whole-body insulin sensitivity (M-value) was calculated as described previously,[14] and HOMA-IR was calculated as fasting plasma c-peptide (ng/ml) x fasting plasma glucose (mg/dL) / 22.5.

Tissue biopsies

Tissue biopsies were obtained from subcutaneous abdominal adipose tissue (0.5-2 g) and the *musculus vastus lateralis* (100-150 mg) under local anesthesia with 2% lidocaine before the first and at ~72 hours after the last training session. Adipose tissue was collected via a small skin incision using an aspiration needle, while muscle biopsies were taken with a Bergström needle. Samples were then cleaned of blood and connective tissue, with a fresh subsample transferred into ice-cold relaxing medium (BIOPS) for mitochondrial function analysis.[75] The remaining tissue was immediately stored in liquid nitrogen and frozen at -80°C for later analysis.

High-resolution respirometry

Ex vivo mitochondrial respiration was analyzed using high-resolution respirometry (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria), which features two closed reaction chambers, each filled with 2 ml of MIR05 at 37°C. A polarographic sensor (OroborPOS) in each chamber measures oxygen concentration and flux (oxygen consumption per second per milligram of tissue wet weight, pmol/s/mg), indicating mass-specific mitochondrial respiration. All measurements were performed in duplicates and recorded with DatLab software 7.4.0.4 (Oroboros Instruments, Innsbruck, Austria). Instrumental background oxygen flux was corrected online, accounting for sensor consumption and oxygen diffusion. Prior to measurements, sensors were calibrated at air saturation using DatLab software.

Skeletal muscle assessment

Fibers were placed in BIOPS solution in a petri dish on an ice-cold metal plate and separated with fine-tipped forceps. Permeabilization was achieved by incubating fibers in BIOPS solution with 50 μg/ml saponin at 4 °C with gentle agitation for 30 min.[75] Following two 10-min washes at 4°C in ice-cold mitochondrial respiration medium, wet weight (~2 mg) was measured using a microscale (Mettler-Toledo XP205, Columbus, OH, USA), and subsamples were added to chambers. Leak respiration (L) with CI-linked substrate (2 mM malate, 5 mM pyruvate; [CI]L, data not shown) was determined, followed by stepwise ADP titration (12.5 μM - 25 μM - 175 μM - 250 μM – 500 μM – 1 mM – 2 mM – 4 mM – 6 mM) to assess mitochondrial ADP sensitivity. OXPHOS capacity (P) with CI&CII-linked substrates (plus 10 mM succinate; [CI+II]P) was then measured. Mitochondrial membrane integrity was assessed by adding 10 μM

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

cytochrome c (samples with >10% increase in respiration were excluded; data missing from 5 participants). Chamber oxygen concentration was maintained between 250 and 400 μM to prevent oxygen diffusion limitation.

Adipose tissue assessment

Wet weight (~20 mg) was determined using a microscale (Mettler-Toledo XP205) and subsamples were transferred into the chambers. Subsequently, digitonin (5 μg/ml) was added to achieve membrane permeabilization. Leak respiration (L) with electron input through CI ([CI]L) was induced after addition of malate (2 mM) and pyruvate (5 mM). Mitochondrial ADP sensitivity was assessed by incremental titration of ADP (50 μM - 100 μM - 250 μM - 500 μM - 1 mM – 2.5 mM – 5 mM). P with convergent electron input through CI and CII ([CI+CII]P) was determined following addition of succinate (10 mM). Mitochondrial membrane integrity was assessed by adding 10 μM cytochrome c (samples with >10% increase in respiration were excluded; data missing in 5 participants).

Mitochondrial content

CS activity was determined as a surrogate measure for mitochondrial content (Sigma-Aldrich) as described previously.[30] mtDNA was extracted from 15 mg and 25 mg *musculus vastus lateralis* and subcutaneous adipose tissue, respectively using DNeasy Blood & Tissue kit (Qiagen, Düsseldorf, DE) following manufacturer's instructions. Briefly, the tissues were lysed using ATL plus buffer and proteinase K at 56°C for 2 h. Later, DNA was purified using DNeasy mini spin column and dissolved in 40 μl AE buffer. Finally, DNA concentration and purity were determined by nanoplate reader (Tecan, Männedorf, CH) and diluted to 5 ng/μl using PCR grade H_2O. Mitochondrial DNA copy number was quantified with StepOne Plus PCR system (Applied Biosystems, Foster City, USA) using primers for nuclear gene lipoprotein lipase (LPL) (forward primer: CGAGTCGTCTTTCTCCTGATGAT reverse primer: TTCTGGATTC CAATGCTTCGA) and mitochondrial gene NADH dehydrogenase subunit 1 (ND1) (forward primers: CCCTAAAACCCGCCACATCT reverse primers: GAGCGATGGTGAGAGCTAAGGT). DNA copy number for each gene was determined by comparing log-linear standard curves created using plasmid for LPL and ND1 (OriGene, Maryland, USA). Mitochondrial DNA copy number was expressed as the logarithm of mitochondrial to nuclear DNA ratio as previously described.[76] A melting curve was created to ensure primer specificity. Each sample was measured in duplicate. Inter-run calibrator was used to account for between-run differences.

Spiroergometry

Nine volunteers (11 participants could not perform an exercise test due to the COVID-19 restrictions for human studies) underwent an incremental exhaustive exercise test on a cycle ergometer (Ergometrics 900, Ergoline, Bitz, Germany),[77] with continuous monitoring of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). Parameters from breath-by-breath gas analysis are given as averaged data over 8 breaths. During the test, continuous monitoring comprised 12-lead electrocardiogram, heart rate and blood pressure measurements. Work rate was continuously increased every 15 seconds according to the workload profile, and the incremental part of the test lasted 8-12 minutes. VO_{2max} was recorded at maximal exertion and refers to the maximum oxygen uptake averaged over 8 breaths.

Laboratory analyses

Serum and plasma samples were analyzed in the biomedical laboratory as described earlier.[78] Plasma levels of interleukin-6 (Biovendor, Cat N: RD194015200R) and myostatin (Invitrogen, Cat N: EH215RB) were analyzed by ELISA according to the manufacturer's instructions with intra-assay CVs <10% for both assays. Hexokinase and phosphofructokinase activities were measured colorimetrically in samples of skeletal muscle tissue at 450 nm following the manufacturer's instructions (Abcam, Cambridge, UK).

Magnetic resonance (MR)based measurements

All MR measurements were performed after an overnight fast on a clinical 3-T MR scanner (Achieva X-series, Philips Healthcare, Best, The Netherlands). Adipose tissue analysis: whole-body, visceral and subcutaneous adiposity were quantified by whole-body MRI employing T1-weighted fast spin-echo[79] and post-processed by a trained operator using SliceOmatic v5.0 software (Tomovision, Montréal, QC, Canada). Liver lipid content and liver volume analyses: ^{1}H-MRS was performed for quantitative assessment of hepatic lipid content using single voxel stimulated echo acquisition mode (STEAM; repetition time (TR) / echo time (TE) = 4000 / 10 ms). The voxel was positioned within a homogeneous part of liver tissue, avoiding major vessels and gallbladder (volume of interest (VOI) = 25x25x25 mm^3).[80] Hepatic lipid content was quantified as the ratio of intensities of the methylene [-(CH_2)n-] peak at 1.3 ppm of liver ^{1}H spectra to the combined signal intensities of the water and methylene peaks [fat content (%) = CH_2/(water+CH_2)]. The difference in transverse relaxation times of water and fat peaks was corrected as described before.[81] Liver volume was assessed using 2-echo Dixon MRI (TR = 3.76 ms, $TE_{1/2}$ = 1.32 / 2.4 ms, slice thickness = 2 mm, no gap) and processed using SliceOmatic v5.0 software (TomoVision).

Skeletal muscle analysis

The volume of the quadriceps femoris muscle (*vastus lateralis, intermedius, medialis*, and *rectus femoris*) of the left thigh was determined using 2-echo Dixon MRI (TR = 4.1 ms, $TE_{1/2}$ = 1.45 / 2.8 ms, slice thickness = 2.5 mm, no gap) by a trained operator using Amira Software (Thermo Fisher Scientific, Berlin, Germany). Intramuscular fat fraction (muscle proton density fat fraction) and cross-sectional area of the quadriceps femoris muscle (*vastus lateralis, intermedius, medialis*, and *rectus femoris*) were determined using mDixon-Quant measurements, acquired on the transversal plane (6-echo 3D Dixon: TR / TE / ΔTE = 10 / 1.45 / 1.1 ms, slice

thickness = 5 mm, no gap). Intramuscular fat fraction was calculated as described in Lichtenstein et al.[82] The cross-sectional area was segmented using Amira Software (Thermo Fischer Scientific, Berlin, Germany) at the midthigh level.[83]

Cardiovascular risk scores

Framingham Risk Scores have been calculated as describes before.[4] In detail, the Framingham Risk Score for Coronary Heart Disease (FRS-CHD) was used to assess the 10-year risk of an individual of developing CHD. FRS-CHD was calculated based on age, sex, systolic blood pressure, total and HDL-cholesterol, smoking status and treatment for hypertension. The Framingham Risk Score for Cardiovascular Disease (FRS-CVD) estimates the 10-year risk of an individual for a cardiovascular event in regard to age, sex, systolic blood pressure, total and HDL-cholesterol, smoking status, treatment for hypertension and diabetes mellitus.

Western blotting

Protein content was evaluated by Western blotting as described previously.[57] Briefly, ~30 mg of frozen skeletal muscle was homogenized in 300 μl lysis buffer [25 mM Tris–HCl, 1 mM EDTA, 150 mM NaCl, 0.2% NP-40] supplemented with protease (complete Tablets, EASYpack, Roche Diagnostics, Basel, Switzerland) and phosphatase (PhosSTOP, EASYpack, Roche Diagnostics) inhibitors. Homogenates were disrupted in a Tissue Lyzer (3 × 1 min at 20 Hz) and centrifuged at 13,000 rpm for 15 min at 4 °C to remove insoluble material.

Protein extracts (25-30 μg) were mixed with reducing Laemmli buffer containing 2-mercaptoethanol (Bio-Rad, CA, USA), boiled for 5 min at 95 °C, and separated on 4–20% Mini-PROTEAN TGX Stain-Free Gels (Bio-Rad 199 Laboratories, California, USA), which were activated with ultraviolet radiation for 1 minute prior to immunoblotting. Proteins were transferred to polyvinylidene difluoride membranes using the Trans-Blot Turbo Transfer System (Bio-Rad) and membranes were imaged using the ChemiDoc MP and quantified using ImageLab 6.0.1 (Bio-Rad) for normalization. Membranes were blocked for 2 h at room temperature (RT) using the blocking solution (5% milk in tris-buffered saline with Tween, TBST) and then incubated overnight with primary antibodies at 4 °C. Only commercially available antibodies were used, without additional validation. Next day, membranes were washed with TBST buffer and incubated for 1 h at RT with HRP-conjugated secondary antibodies. Finally, the membranes were coated with Immobilon Western Chemiluminescent HRP substrate (Millipore, Danvers, MA, USA) and imaged with the Bio-Rad ChemiDoc MP system. Band intensities were quantified with ImageLab 6.0.1 (Bio-Rad) and expressed as arbitrary units normalized to total protein content. To account for gel-to-gel variation, an inter-run calibrator (IRC) was loaded as reference sample on each gel (Data S1).[84] As some participants refused a second skeletal muscle biopsy, the number of samples available for insulin signaling was as follows: BFRT: n=6 for IRS1; n=4 for p-IRS(Ser^{1101}); n=7 for AKT, p-AKT(Ser^{473}) and p-AKT(Thr^{308}). CREST: n=6 for IRS1; n=4 for p-IRS(Ser^{1101}); n=6 for AKT, p-AKT(Ser^{473}) and p-AKT(Thr^{308}).

RNAseq data analysis

Count tables were reconstructed from quant.sf files per sequenced sample using tximport[67] with the lengthScaledTPM option (version 1.36.1). Differential gene expression analysis was performed using DESeq2[68] (version 1.48.2) with shrinkage of fold-changes using apeglm (version 1.30.0). Genes with fewer than 10 total counts across all samples were excluded prior to normalization. The following interaction model was applied: ~ cohort + cohort:person_within_cohort + cohort:timepoint, allowing detection of main before and after effects, and their statistical interaction. Specifically, the model tested: (i) the main effect of CREST (before vs. after), (ii) the main effect of BFRT (before vs. after), and (iii) their interaction (whether the effect of before and after differs between CREST and BFRT). Genes with an adjusted p-value < 0.05 and an absolute log2FC ≥ 0.585 were considered significantly differentially expressed. Gene set enrichment analysis (GSEA) was performed using the gseKEGG() function from the clusterProfiler package[69] (version 4.16.0) with parameters: organism = "mmu", pvalueCutoff = 1, pAdjustMethod = "BH", minGSSize = 0, seed = TRUE, and eps = 0.[85]

RNA-Seq data processing

Raw FASTQ were processed using nf-core/rnaseq v3.20.0 of the nf-core collection: nextflow run nf-core/rnaseq -r 3.20.0 -profile mamba –max_cpus 32 –max_memory 180.GB –max_time 2400.h –input Samples.csv –outdir ./NF_core_results1 –fasta /Gencode_human/release_44/GRCh38.primary_assembly.genome.fa.gz –gtf /Gencode_human/release_44/gencode.v44.primary_assembly.annotation.gtf.gz –gencode true –save_reference true.

This approach automates quality control, trimming, alignment (or pseudo-alignment), quantification and reporting of RNA-seq experiments. Read quality control (QC) was assessed using FastQC (version 0.12.1) and adapter sequences were trimmed with Trim Galore! (version 0.6.10), removing reads with Phred quality scores < 20. Quality summaries across samples were compiled using MultiQC (version 1.30). Filtered reads were aligned to the Homo sapiens reference genome (GENCODE v44, GRCh38.primary_assembly) using the STAR aligner[65] (version 2.7.11b) and SALMON[66] (version 1.10.3) with default parameters implemented in nf-core/rnaseq. Duplicate reads were marked, and gene-level counts were obtained using featureCounts (release 2.0.6). Samples retained between approximately 41 and 62 million high-quality mapped reads (mapping quality > 0). All downstream statistical and differential expression analyses were performed in R (version 4.5.1) using the gene-level count matrix produced by the pipeline. All samples passed the sequencing QC. One participant was excluded from the BFRT group upon visual inspection of PCA and correlation matrices. GEO: GSE312780.

Please cite this article in press as: Trinks et al., Blood-flow restriction resistance training improves skeletal muscle mitochondrial capacity and cardiovascular risk factors in type 2 diabetes, Cell Metabolism (2025), https://doi.org/10.1016/j.cmet.2025.12.016

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed with SAS (version 9.4; SAS Institute, Cary, NC, USA). Figures were drawn using GraphPadPrism (version 10.2.3; GraphPad Software, San Diego, CA, USA). Data analysis was performed blinded to group allocation to prevent bias.

To investigate the changes from W0 to W12 and the differences in these changes between groups, we performed linear mixed models. The underlying model was specified as Outcome = visit x group, allowing separate modeling of the outcome means for each of the four visit-by-group combinations. Group membership was included as a fixed effect, and the time point of the investigation was modeled as a random effect. The variance-covariance matrices of the W0 and W12 outcomes were modeled independently for both training groups. No assumptions were made about the structure of the two 2×2 variance-covariance matrices; instead, they were estimated directly from the data. This approach helped to prevent inflation of type I and type II errors due to violations of the homoscedasticity assumption (i.e., equal variances across the four visit-by-group combinations). Interaction effects between visit and group were estimated using the corresponding least squares means (LS-means) and tested with a two-sided t-test.

For log-transformed variables, changes were expressed as relative changes, while for other variables, absolute changes were reported. Fisher's exact test was used toassess differences in sex distribution between groups. The data underlying all figures are provided in Data S1 – Source Data.

ADDITIONAL RESOURCES

Clinicaltrials.gov (Identifier number: NCT04222231): Study Details | NCT04222231 | RESIST! Blood-flow Restriction Resistance Training for Improving Insulin Sensitivity in Type 2 Diabetes | ClinicalTrials.gov