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ORIGINAL ARTICLE

Effects of acute aerobic, resistance and combined exercises on 24-h glucose variability and skeletal muscle signalling responses in type 1 diabetics

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Abstract

Purpose To compare the effect of high-intensity aerobic (AER), resistance (RES), and combined (COMB: RES + AER) exercise, on interstitial glucose (IG) variability and skeletal muscle signalling pathways in type 1 diabetes (T1D).

Methods T1D participants (6 M/6F) wore a flash glucose monitoring system in four randomized sessions: one control (CONT), and one AER, RES and COMB (40 min each). Mean amplitude of glycemic excursions (MAGE), standard deviation (SD) and coefficient variation (CV) of IG were used to compare the 24 h post-exercise IG variability. Blood and muscle samples were collected to compare exercise-induced systemic and muscle signalling responses related to metabolic, growth and inflammatory adaptations.

Results Both RES and COMB decreased the 24 h MAGE compared to CONT; additionally, COMB decreased the 24 h SD and CV. In the 6–12 h post-exercise, all exercise modalities reduced the IG CV while SD decreased only after COMB. Both AER and COMB stimulated the *PGC-1α* mRNA expression and promoted the splicing of *IGF-1Ea* variant, while Akt and p38MAPK phosphorylation increased only after RES and COMB. Additionally, COMB enhanced eEF2 activation and RES increased *myogenin* and *MRF4* mRNA expression. Blood lactate and glycerol levels and muscle *IL-6*, *TNF-α*, and *MCP-1* mRNAs increased after all exercise sessions, while serum CK and LDH level did not change.

Conclusion COMB is more effective in reducing IG fluctuations compared to single-mode AER or RES exercise. Moreover, COMB simultaneously activates muscle signalling pathways involved in substrate metabolism and anabolic adaptations, which can help to improve glycaemic control and maintain muscle health in T1D.

Keywords Exercise modalities · Glucose variability · Hypoglycaemia · Skeletal muscle signalling · Type 1 diabetes

Abbreviations

AER	Aerobic
Akt	AKT serine/threonine kinase 1
AMPK	Protein kinase AMP-activated catalytic subunit alpha 1
ANOVA	Analysis of variance

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ATP	Adenosine triphosphate
B2M	Beta-2-microglobulin
BMI	Body mass index
CK	Creatine kinase
COMB	Combined
CONT	Control
CV	Coefficient of variation
DEXA	Dual-energy X-ray absorptiometry
eEF2	Eukaryotic translation elongation factor 2
ERK1/2	Extracellular signal-regulated protein kinases 1 and 2
FGM	Flash interstitial glucose monitoring
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GLUT4	Solute carrier family 2, facilitated glucose transporter member 4
GPAQ	Global Physical Activity Questionnaire
HbA1c	Glycated hemoglobin
HR	Heart rate
HRR	Heart rate reserve
IG	Interstitial glucose
IGF-1	Insulin-like growth factor 1
IGF1R	Insulin-like growth factor 1 Receptor
IL-6	Interleukin-6
LDH	Lactate dehydrogenase
MAGE	Mean amplitude of glycemic excursions
MCP-1	Monocyte chemoattractant protein 1/C–C motif chemokine 2
MRF4	Myogenic regulatory factor 4/Myogenic factor 6
mRNA	Messenger ribonucleic acid
mTOR	Mechanistic target of rapamycin kinase
Myf5	Myogenic factor 5
MyoD	Myogenic differentiation 1
p38MAPK	P38 map kinase
PGC-1α	Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
PI3K	Phosphatidylinositol-4,5-bisphosphate 3-kinase
1RM	One-repetition maximum
RES	Resistance
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation
T1D	Type 1 diabetes
TNF-α	Tumor necrosis factor-alpha

Introduction

The maintenance of optimal glycaemic control is an essential goal for people living with type 1 diabetes (T1D). However, keeping glycaemic levels within a narrow range is extremely difficult in T1D and even in the case of tightly regulated insulin therapy, long-term complications such as microvascular diseases, retinopathy, neuropathy, and cardiovascular diseases, invariably arise as a direct/indirect result of T1D (Brownlee 2005; McCarthy et al. 2016).

Exercise and physical activity, in conjunction with adequate dietary care, can prevent/regress the development of diabetes-related complications (Colberg et al. 2016). In a recent study comprising nearly 2000 T1D adults, the most physically active individuals exhibited better HbA1c levels, fewer diabetes-related microvascular complications, reduced dyslipidemia, and decreased risk of developing severe hypoglycaemia than those who were less physically active (Bohn et al. 2015).

Nevertheless, glycaemic management may be challenging for people living with T1D, both during and post-exercise, and exercise-induced hypoglycaemia or hyperglycaemia can impact both immediate and long-term health in adults with T1D (Riddell et al. 2017). For instance, large fluctuations in blood/interstitial glucose (IG) concentration, reported as the coefficient of glucose variation (CV), are detrimental and associated with higher cardiovascular risk (Gorst et al. 2015). Moreover, most individuals living with T1D identify exercise as a risk factor for hypoglycaemia even several hours afterwards (Riddell et al. 2017).

Several factors influence the glucose response to exercise in T1D, including the type of insulin, mode of delivery, the glycaemic concentration before exercise, the consumption of a pre-exercise meal, and the exercise modality performed, e.g., aerobic (AER) or resistance (RES) exercise (Minnock et al. 2019). A common assumption is that moderate-intensity AER exercise causes a blood glucose reduction in most individuals with T1D and may increase the risk of experiencing hypoglycaemia (Riddell and Perkins 2009). In contrast, compared with the AER exercise, only a few studies have investigated the effects of RES exercise on glucose concentration in T1D, demonstrating that RES exercise may result in a more stable glucose level than AER exercise (Yardley et al. 2013; Reddy et al. 2019). Moreover, the combination of RES and AER exercise in the same session (COMB) is associated with improved glucose stability, although only if the RES exercise is performed before AER exercise (Yardley et al. 2012). Interestingly, in people living with type 2 diabetes, the combination of aerobic and resistance training was associated with better improvements in HbA_{1c} levels and in general fitness compared with either resistance or aerobic training alone (Church et al. 2010). To our knowledge, the mechanisms underlying the greater glycaemic variability observed after AER compared to RES exercise are mostly unknown. The higher lactate production during RES, with respect to AER exercises, has been proposed as a stimulus of gluconeogenesis that could potentially attenuate declines in blood glucose in people living with T1D (Yardley et al. 2013). Moreover, in healthy participants performing COMB exercise, starting with RES exercise

raises blood concentrations of free fatty acids and glycerol hence reducing the reliance on carbohydrates as a fuel source during the following AER exercise (Goto et al. 2007). Although not specifically addressed so far in T1D, different exercise modalities also induce the activation of distinct skeletal muscle signalling pathways, which orchestrate the adaptive response to exercise and could directly affect glucose homeostasis (Röhling et al. 2016). For example, AER exercise determines an immediate fluctuation in intracellular calcium, ATP, and redox potential levels activating the 'metabolic/stress' kinases such as p38MAPK, ERK1/2, and AMPK (Coffey and Hawley 2007). This is succeeded by an increase of GLUT4 translocation and upregulation in the levels of transcription factors such as PGC-1α, which is associated with increased mitochondrial biogenesis and improved oxidative capacity of skeletal muscle (Jäger et al. 2007). On the other hand, RES exercises stimulate the signalling responsible for skeletal muscle growth, which mainly converges on PI3K/Akt/mTOR signalling pathway and hence its effectors that control protein synthesis such as eukaryotic elongation factor 2 (eEF2) and insulin-like growth factor 1 (IGF-1) (Egerman and Glass 2014; Rose et al. 2005).

Moreover, many of these components have additional roles in the regulation of glucose uptake and glycogen synthesis (Röhling et al. 2016). For example, the deletion of muscle IGF-1 and IGF-1R impaired glucose clearance both at rest and during exercise (O'Neill et al. 2015; Vassilakos et al. 2019). Thus, although these studies were conducted on rodents and therefore are not directly transferable to humans growing evidence suggests an important role of PI3K/Akt/mTOR signalling pathway in exercise-induced glucose uptake (Röhling et al. 2016).

Notably, although COMB exercise has been suggested as the best exercise approach to minimize glycaemic fluctuations (Yardley et al. 2013), no attention has been given to the potential interference between AER- and RES-induced muscle signalling pathways, which in turn can compromise the post-exercise training adaptations (Coffey and Hawley 2007). Furthermore, structural, functional, and metabolic alterations in the skeletal muscle of individuals with T1D (i.e., diabetic myopathy), evident even before the appearance of other diabetic complications, have been recently reported in a number of studies (Krause et al. 2011; Monaco et al. 2017). Despite this recent evidence, the effect of T1D on exercise capacity, muscle damage, and inflammatory response are still largely unknown (Krause et al. 2011).

The aim of this study was to compare the effects of a single bout of high-intensity AER, RES, and COMB exercises on blood glucose homeostasis during the 24-h following exercise in people living with T1D. Furthermore, blood samples and muscle biopsies were collected before and after each exercise session to compare the metabolic alteration and exercise-induced muscle changes in gene expression and signalling pathways related to muscle metabolism, growth and inflammation.

We hypothesized that COMB exercise would elicit the best response both in terms of 24-h glycemic control and skeletal muscle signalling in people living with T1D.

Methods

Participant information and baseline evaluations

The present study was conducted in 2017 and all data collection and exercise testing took place in the Human Physiology Laboratory and the High-Performance Gym at the local University Institute for Sport and Health (ISH) at the University College Dublin, Ireland. T1D Participants were recruited from study advertisements placed in the local University Hospital and shared with Diabetes support groups in the area. Inclusion criteria were: age between 18–45 years, no contraindications to exercise, T1D diagnosed for at least 12 months, BMI < 30 kg m^{-2}, and non-smokers. Twelve T1D participants (6 males; age 31.8 ± 5.3 years; BMI 27.5 ± 2.5 kg m^{-2}; duration of diabetes 13.7 ± 8.5 years; HbA1c 65 ± 11 mmol mol^{-1}) with good glycaemic control and without known diabetic comorbidities were enrolled. Participants gave their written, informed consent to participate in the study, which was approved by the local University Human Research Ethics Committee (Application ID-LS-16-67-Minnock-DeVito). Participants were not habitual exercisers and were classified as inactive following completion of the Global Physical Activity Questionnaire (GPAQ). Ten of the participants were using multiple daily injections of insulin, while the remaining two continuous subcutaneous insulin infusion with an insulin pump. All-female participants ($n = 6$) were using oral hormonal contraceptives and provided information about their menstrual cycle to avoid being tested during menstruation. Body composition was assessed by dual-energy X-ray absorptiometry (DEXA) (Lunar iDXA, GE Healthcare, Buckinghamshire, United Kingdom). DEXA results reported a total body fat percentage of 28.5 ± 9.8 (fat mass: 22.6 ± 8.4 kg; lean body mass: 53.9 ± 11.3 kg; trunk fat mass: 10.9 ± 4.1 kg; trunk lean body mass 25.8 ± 5.1 kg). Following the DEXA scan, since participants had been lying down for approximately 20 min during the scan, the resting heart rate (HR) was recorded (Omron M2, USA). A total of 3 h values were taken to provide an average resting value to establish the target HR zone of AER sessions (set at 80% of HR reserve (HRR)) using the Karvonen Formula (target HRR = [(max HR − resting HR) × 0.8] + resting HR) (Karvonen et al. 1957). Muscle strength was assessed by evaluating the one-repetition maximum (1RM) for each of the six-RES exercises that were to be used during the exercise intervention (lateral pull-down,

triceps extension, biceps curl, knee extension, squat press, leg press).

Study design

Participants attended one preliminary visit, followed by one control session (CONT) and three randomized experimental exercise sessions separated by a 1-week washout period (Fig. 1). Each participant was assigned the same 50-min time slot to attend between 06:00 am and 12:00 noon, across all intervention sessions. Participants were unaware of which intervention they would partake in until they arrived (except for the last session). In order, to guarantee a normal hydration status, participants were invited to drink at least 400 ml of water in the 60-min period preceding the start of each experimental session (Sawka et al. 2007). During CONT session, each participant sat in a semi-recumbent position on a treatment table for 40 min, ensuring to avoid physical activity during this time. On the day of the AER intervention, participants were fitted with an HR monitor (Polar H7, USA) and then positioned on an electromagnetic cycle ergometer (Lode Excalibur Sport, Netherlands). Participants cycled for 5-min to warm-up, maintaining a cadence of 50 revolutions per minute (rpm) and a power of 50 watts and 80 watts for female and male participants, respectively. Following the warm-up, they were asked to increase to 80 rpm and to maintain this pace for the remaining 30 min. The resistance on the cycle ergometer was adjusted (if required) to ensure participants remained in the desired target HR intensity zone (i.e. at 80% of HRR) with verbal support provided when necessary. Since the monitoring of intensity was based on the measure of HR we decided to use water ad libitum to

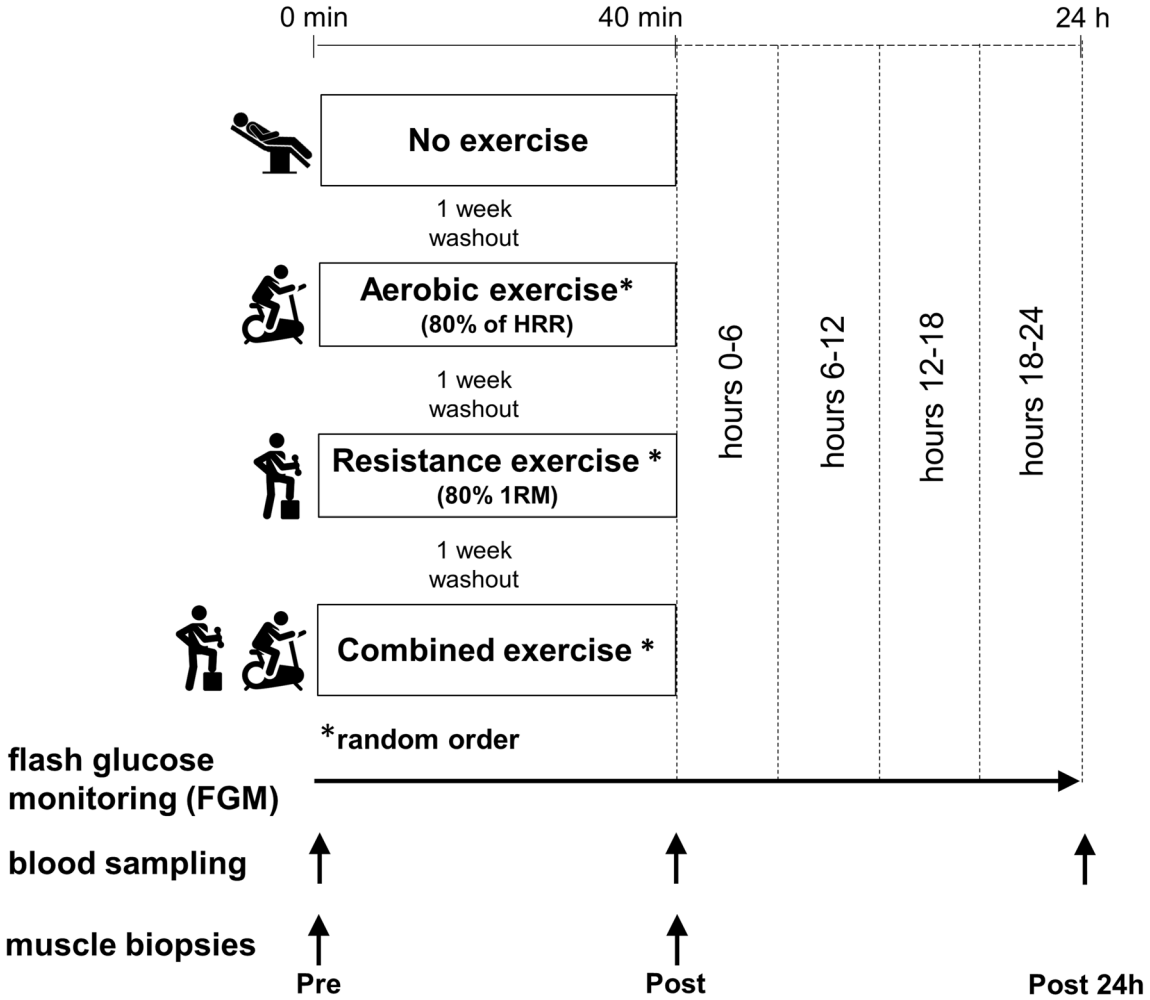

Fig. 1 Participants underwent four testing sessions separated by one week each. No-exercise control session (CONT) was performed first and was followed by three different high-intensity exercise sessions performed in random order: aerobic (AER; 80% of HRR), resistance (RES; 80% of 1RM) or combined resistance and aerobic, exercise (COMB; 20 min each). The interstitial glucose was recorded over a 24 h period by a flash interstitial glucose monitoring (FGM) system. Venous blood and muscle samples (*vastus lateralis*) were also taken at the time points indicated in the figure

minimise the fluctuations of HR due to blood plasma shifting. In any case, this corresponded to a minimum of 200 ml and a maximum of 500 ml of water consumed during exercise by each participant. In addition, the laboratory constant conditions and the relatively short duration of the sessions (40 min including warm up and cool down phases) should have not represented a particular striking condition (Coyle and González-Alonso 2001; Montain and Coyle 1992). At minute 35 of the intervention, the bike resistance was gradually reduced in conjunction with rpm to provide a cool-down period. The intervention ceased once participants completed the 40-min total exercise.

For the RES session, the warm-up consisted of 5 min RES exercises executed at 30% 1RM, 6–8 reps per machine (~ 45 s), moving directly from one machine to the next (allowing for approximately 5–10 s transfer time between each machine) for (1) lateral pull-down, (2) biceps curl, (3) knee extension, (4) squat press, (5) triceps extension, (6) leg press. Following the warm-up, the exercise continued for 30 min, where two complete circuits of the 6 exercises performed at 80% 1RM, each lasting about 900 s including rest periods, were executed. Each exercise was executed 8/10 within 60 s followed by a 90 s rest period and transfer time to the next machine (i.e. within 150 s in total). The RES session ended with a 5 min cool down period spent exercising at 30% 1RM the same protocol used for the warm-up period.

In the COMB session, the RES and AER components were modelled directly on the single RES and AER interventions. Participants performed RES exercise before AER exercise (Yardley et al. 2012). The COMB exercise commenced with a warm-up consisting of approximately 2.5 min of RES exercises executed at 30% 1RM, 20 s per machine, following the same progression as the RES intervention. Following the warm-up, the exercise continued for 15 min, where one circuit of six exercises was executed at 80% 1RM. Completion of all six exercises (1 circuit), including rest periods between exercises, lasted 900 s. The session ended with a 2.5 min cool down period spent exercising at 30% 1RM using the same protocol for the warm-up period as mentioned above. Participants next moved directly to the AER component of the COMB exercise. Participants cycled for 2.5 min to warm-up in accordance with the AER intervention above. Following the warm-up, participants increased to 80 rpm and to maintain this pace for the remaining 15 min. At minute 37.5 of the COMB intervention, the bike resistance was gradually reduced in conjunction with rpm to provide a cool-down period. The COMB intervention ceased once participants completed the 40-min total exercise.

Participants were not fasting during the sessions and were issued with a food diary to log meals and snacks in the 24 h before and following each session. To ensure consistency throughout the study, these diaries were then used to replicate meal-type and time each week. Retrospective analysis of the food diary revealed that each participant replicated their meal composition and there were no statistically significant differences in total carbohydrate intake and daily insulin doses between sessions (Online resource 1). Participants were instructed to refrain from exercise, caffeine, and alcohol for the 24 h before and following each intervention day.

Blood samples

Venous blood samples were taken from an antecubital vein in heparin-coated Vacutainer™ tubes using standard aseptic techniques. Blood samples collection occurred at the onset of the intervention (Pre), immediately after intervention (Post), and 24 h post (Post 24 h) (Fig. 1). Participants were rested for approximately 15 min prior to the first sample collection and 5 min post exercise for the second sample collection. On exercise day both samples were collected by 2 single venepunctures. Samples were immediately centrifuged (at 1000×*g* for 15 min at 4° C), after which serum was removed and stored at − 80° C for further analysis. Lactate and glycerol were measured using the Randox Daytona benchtop analyzer (RX Daytona+, United Kingdom). Serum creatine kinase (CK) and lactate dehydrogenase (LDH) activity were measured by a standardized commercially available colourimetric enzymatic assay (BioVision, Vinci-Biochem, Italy).

Interstitial glucose data collection and evaluation

Each participant received two FreeStyle Libre flash interstitial glucose monitoring (FGM) system (Abbot Diabetes Care, Alameda, California, USA) and an FGM reader. Participants were instructed to apply the FGM patch to their left arm and have an active FGM patch on the day before each intervention session. Each participant registered their personal FGM reader, and instructions were provided on how to record the FGM data and to create data reports. IG was recorded every 15 min. The IG data was then downloaded to provide IG data from pre-intervention (time 0) until 24 h post-intervention (Fig. 1). Once applied and activated, FGM systems lasted for 14 days before cessation, and for this reason, participants retained the FGM system for its full lifecycle. IG variability was evaluated using the following four indices: mean amplitude of glycaemic excursions (MAGE), glucose variance (VAR), glucose coefficient of variance (CV), and glucose standard deviation (SD) (Hill et al. 2011; Zaccardi et al. 2008). MAGE analysis was performed on the IG data collected during the entire 24 h intervention period, and its calculation was based on the differences between consecutive points, considering those who were higher than 1 SD than the previous point (Hill et al. 2011). The analysis

using VAR, CV, and SD instead was performed for both the entire 24 h period and fractioning it into four 6-h periods.

Muscle sampling

Muscle biopsies were collected from the muscle *vastus lateralis* Pre and Post each test session (Fig. 1) using a 14-gauge semi-automatic spring-loaded biopsy system with a compatible coaxial introducer needle (Medax Srl Unipersonale; San Possidonio, Italy). Briefly, after local anaesthesia with 3 mL of 2% lidocaine, the skin, previously cleansed with an antiseptic solution, was punctured with the insertion cannula. The biopsy needle inserted through the cannula, and after the activation of a trigger button, a small muscle piece remained inside the needle. The muscle sample was then removed from the biopsy needle, immediately frozen in liquid nitrogen, and stored at -80° C until further analyses.

RNA extraction, cDNA synthesis, and qRT-PCR

Without thawing, all muscle tissues were weighed (≤ 30 mg), placed directly into the QIAzol Lysis Buffer (Qiagen, Milan, Italy), and ruptured using a Polytron homogenizer (KINEMATICA AG, Switzerland). Total RNA was extracted and purified using the miRNeasy® Mini Kit (Qiagen, Milan, Italy) according to the manufacturer's instructions. The amount and quality of RNA were assessed with DU-640 UV Spectrophotometer (Beckman Coulter, United Kingdom), and the complementary DNA was synthesized from 1 µg of total RNA using Omniscript RT (Qiagen, Milan, Italy) and random hexamers. Subsequently, quantitative RT-PCR was performed with 2 µl of cDNA and 300 nM of each primer in an Applied Biosystems StepOnePlus™ Real-Time PCR System using SYBR Select Master Mix (Applied Biosystems, Monza, Italy). The mRNA expression of the target genes was normalized to the geometric mean of *GAPDH* and *B2M* reference genes. The RT-PCR conditions were: 50° C for 2 min, 95° C for 2 min followed by 40 cycles of three-steps at 95° C for 15 s, 60° C for 15 s and 72° C for 30 s. The specificity of the amplification products was confirmed by examining thermal denaturation plots and by sample separation in a 4% DNA agarose gel. The genes of interest and the sequence of the specific primer used in real-time RT-PCR quantification are listed in Supplementary Data Table 2.

Western blotting

Total protein extracts were obtained from the organic phase following the QIAzol protocol. The protein content of muscle tissues was determined according to Bradford's method using bovine albumin as the standard (Bradford 1976). Multi-strip western blotting protocol was performed as previously described (Aksamitiene et al. 2007). Briefly, proteins from each participant (40 µg per lane) were separated on 10% SDS polyacrylamide gel, and the gel cut into two strips with the following protein ranges: one between 34 and 72 kDa and another between 72 and 180 kDa. Four strips of polyacrylamide gels containing protein of the same molecular weight range from different participants were then assembled and electroblotted onto a single nitrocellulose membrane sheet (0.2 µm pore size) (Bio-Rad Laboratories Inc., Hercules, CA, USA) for 1.5 h at 100 V. The blots were probed with the following primary antibodies diluted 1:2000: anti-phospho-Akt (Ser473) (Cell Signaling Technology Cat# 9271, RRID:AB_329825), anti-phospho-p38MAPK (Thr180/Tyr182) (Cell Signaling Technology Cat# 9211, RRID:AB_331641), anti-phospho-p44/42 (ERK1/2) (Cell Signaling Technology Cat# 9101, RRID:AB_331646), anti-phospho eEF2 (T 56) (Cell Signaling Technology Cat# 2331, RRID:AB_10015204) and anti-phospho AMPKalpha (T172) (Cell Signaling Technology Cat# 2535, RRID:AB_331250). Anti-GAPDH MAb (Thermo Fisher Scientific Cat# AM4300, RRID: AB_2536381) was used to normalize protein expression since there was a positive correlation between western blot quantification obtained with GAPDH as a loading control or total Akt ($r = 0.61$, $p < 0.001$), ERK1/2 ($r = 0.80$, $p < 0.001$) and total p38MAPK ($r = 0.88$, $p < 0.001$). Protein bands were detected using a horseradish peroxidase-conjugated secondary antibody (Bio-Rad, Italy). The blots were then treated with enhanced chemiluminescence reagents (Clarity ECL Western Blot Substrate kits, Bio-Rad, Italy), and the immunoreactive bands were detected and quantified using Fluor-S® MAX MultiImaging System (Bio-Rad, Italy) equipped with QuantityOne software.

Statistical analyses

The statistical analyses were performed with SPSS (IBM SPSS Statistics for Windows, Version 20.0, IBM Corp.) and GraphPad Prism (v6.0; IBM Corp.). Descriptive data are reported as mean ± SD. Data were checked for normality of distribution using the Shapiro–Wilk test. The data that were not normally distributed (i.e., phospho p38/GAPDH, phospho eEF2/GAPDH and *IL-6* and *MCP-1* relative mRNA levels) were log10 transformed. A one-way ANOVA with Tukey's post hoc test was applied to study the 40 min intervention and the 24 h post-intervention IG responses as MAGE, VAR, CV, glucose SD, and blood parameters. A two-way (time × intervention) repeated measures analysis of variance (ANOVA), with Bonferroni post hoc tests adjusting for multiple comparisons, was instead used to detect differences between all the remaining variables. For pairwise comparison of variables mean difference (MD), 95% confidence interval (CI), p value, and Cohen's effect size (ES)

were determined. For all analyses, statistical significance was accepted at $p < 0.05$.

Results

Interstitial glucose levels

The analysis of IG data showed a trend of more events of post-exercise late-onset hypoglycaemia (glycaemic level < 3.9 mmol L^{-1}) after AER exercise (CONT = 2/12; AER = 7/12; RES = 0/12; COMB = 2/12). Moreover, AER exercise slightly increased the average duration of hypoglycaemic events (CONT = 44 ± 8 min; AER = 62 ± 29 min; RES = 0 min; COMB = 33 ± 18 min; $p = 0.098$) while the mean overnight IG values did not changed (CONT 9.8 ± 1.2 mmol L^{-1}, AER 8.2 ± 1.3 mmol L^{-1}, RES 9.2 ± 0.8 mmol L^{-1} and COMB 8.9 ± 0.8 mmol L^{-1}; $p = 0.747$). All hypoglycaemic events occurred during nocturnal hours. Mean 24 h post-exercise IG profiles for the four experimental sessions are shown in Fig. 2.

Interstitial glucose variability indices

Both RES and COMB exercises, but not AER, reduced the 24 h MAGE compared to CONT (Fig. 3a). Concerning the other IG indexes, glucose SD (Fig. 3b) and CV (Fig. 3c) were reduced only after the COMB session respect to CONT. No differences were recorded in glucose variance over the 24 h period among all exercise sessions (Fig. 3d). The 6 h post-exercise period analysis showed a reduction of glucose CV 6 h to 12 h after all exercise sessions (Fig. 4a) and a decreased glucose SD 6 h to 12 h after COMB session compared to CONT (Fig. 4b). The glucose variance over 6 h periods did not vary among all different sessions (Fig. 4c).

Blood parameters

Serum levels of blood lactate, glycerol, CK, and LDH are reported in Table 1. Lactate and glycerol levels were elevated immediately post-exercise compared to baseline. Exercise sessions did not influence both CK and LDH levels.

Exercise-induced skeletal muscle signalling pathways

The AER session did not modify the phosphorylation level of Akt, p38MAPK and eEF2, while both RES and COMB exercise increased Akt (RES: MD = 0.35, 95% CI 0.39–0.67, $p = 0.031$, ES = 0.71; COMB: MD = 0.62, 95% CI 0.19–1.05, $p = 0.009$, ES = 0.91) (Fig. 5a) and p38MAPK (RES: MD = 1.37, 95% CI 0.69–2.05, $p = 0.015$, ES = 0.83; COMB: MD = 1.06, 95% CI 0.30–1.83, $p = 0.049$, ES = 0.64) (Fig. 5b) phosphorylation. Moreover, phosphorylation of eEF2 decreased only after COMB exercise (COMB: MD = − 0.36, 95% − 0.60 CI to − 0.122, $p = 0.002$, ES = -1.14) (Fig. 5d). ERK1/2 (Fig. 5c) and AMPK (Fig. 5e) phosphorylation did not change after the testing sessions.

Exercise-induced changes in muscle mRNA expression

The expression of *PGC-1α* increased after AER and COMB sessions (AER: MD = 899.59, 95% CI 517.42–1281.77,

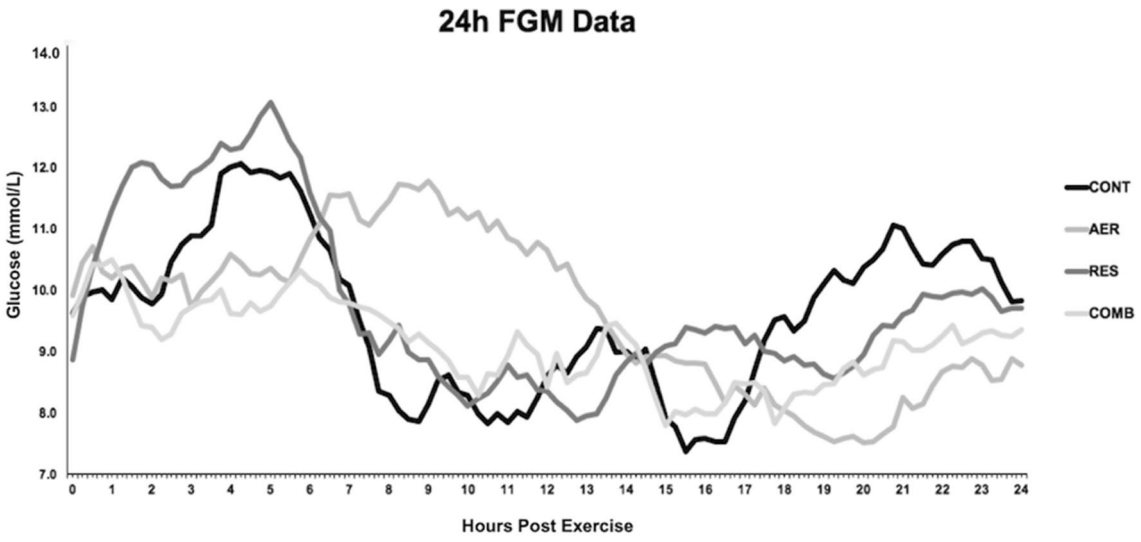

Fig. 2 Mean glucose as measured by a flash interstitial glucose monitoring (FGM) system from pre-intervention (time 0) until 24 h post-intervention

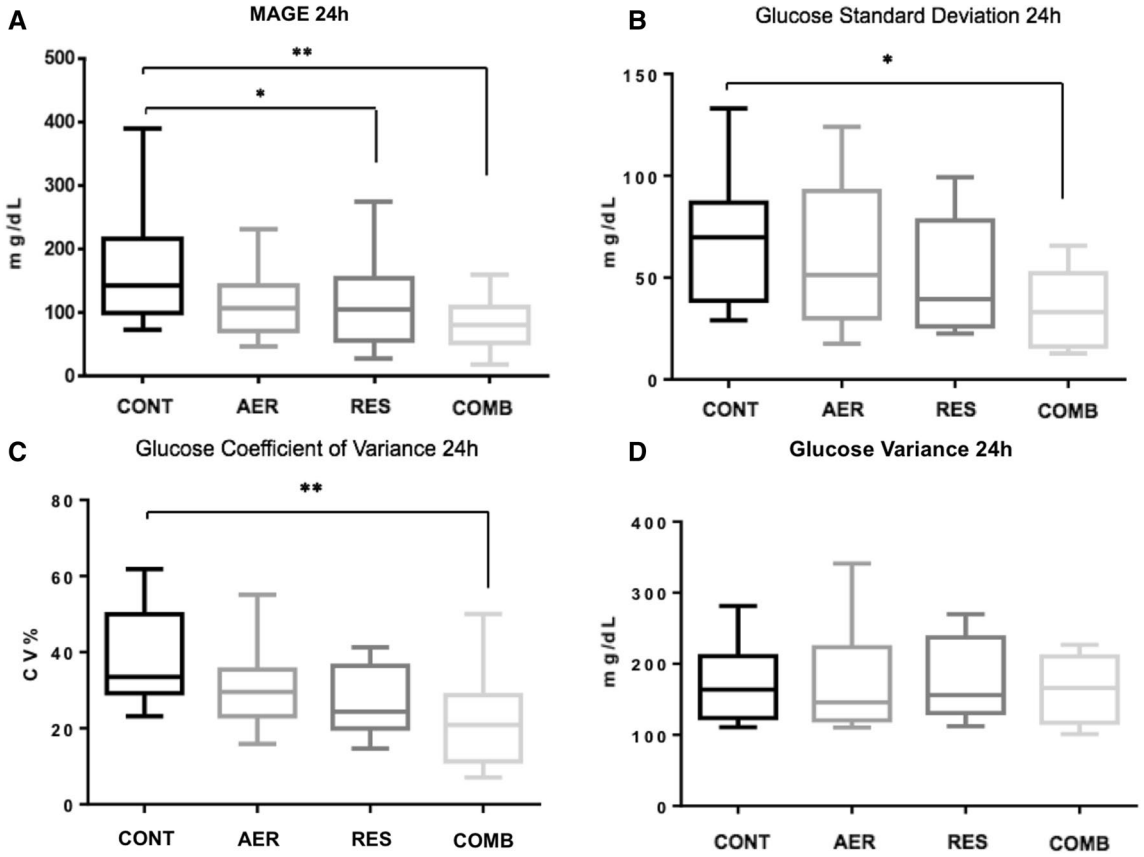

Fig. 3 The 24 h interstitial glucose profile was used to calculate the mean amplitude of glycaemic excursions (MAGE) (**a**), glucose standard deviation (SD) (**b**), glucose coefficient of variance (CV) (**c**), and glucose variance (VAR) (**d**). Statistical differences were determined by one-way ANOVA.*$p<0.05$, **$p<0.01$, ***$p<0.001$

$p=0.001$, ES = 1.50; COMB: MD = 586.29, 95% CI 133.27–1039.31, $p=0.016$, ES = 0.82) (Fig. 6A), while the mRNA quantity of *myogenin* (RES: MD = 0.39, 95% CI 0.15–0.63, $p=0.005$, ES = 1.02) (Fig. 6d) and *MRF4* (RES: MD = 2785.58, 95% CI 1101.68–4469.48, $p=0.004$, ES = 1.05) (Fig. 6e) increased only after the RES exercise session. The expression of *GLUT4* (Fig. 6b), *MyoD* (Fig. 6c) and *Myf5* (Fig. 6f) mRNAs did not change after the testing sessions. The absolute *IGF-1* isoforms mRNA quantity between the different sessions was also analysed, as previously described (Annibalini et al. 2016). As expected, muscle *IGF-1Ea* was much more expressed compared to both *IGF-1Eb* and *IGF-1Ec* isoforms, while the *IGF-1Ec* isoform mRNA was barely detectable (Fig. 7a–d). We did not observe significant modulations in any of the *IGF-1* isoforms after the different exercise sessions. However, it should be noted that the expression of *IGF-1Ea* mRNA showed a trend toward an increase after AER and COMB exercises while the *IGF-1Eb* mRNA level showed an opposite trend. Thus, the AER and COMB exercise altered the steady-state of *IGF-1* splice isoform, increasing the *IGF-1Ea/IGF-1Eb* ratio (AER: MD = 1.40, 95% CI 0.74–2.05, $p=0.001$, ES = 1.36; COMB: MD = 1.86, 95% CI 0.13–3.60, $p=0.037$, ES = 0.68) (Fig. 7d). Finally, the analysis of inflammation-related genes showed an increase of post-exercise *IL-6* (AER: MD = 2.25, 95% CI 1.29–3.21, $p=0.001$, ES = 2.16; RES: MD = 3.01, 95% CI 0.52–5.49, $p=0.002$, ES = 1.18; COMB: MD = 1.78, 95% CI 0.59–2.97, $p=0.005$, ES = 1.02) (Fig. 8A), *TNF-α* (AER: MD = 8.55, 95% CI 4.63–12.47, $p=0.001$, ES = 1.39; RES: MD = 8.81, 95% CI 3.43–14.21, $p=0.004$, ES = 1.04; COMB: MD = 8.15, 95% CI 2.14–14.16, $p=0.012$, ES = 0.86) (Fig. 8b) and *MCP-1* (AER: MD = 25.96, 95% CI − 2.36–54.27, $p=0.063$, ES = 0.60; RES: MD = 25.78, 95% CI − 9.10 to 60.67, $p=0.022$, ES = 0.77; COMB: MD = 39.54, 95% CI − 10.76 to 89.84, $p=0.065$, ES = 0.59) (Fig. 8c) mRNA levels after all exercise sessions.

Discussion

This study compared the effect of AER, RES, and COMB exercise modalities on 24 h glucose variability and skeletal muscle signalling pathways regulation in individuals with T1D. It was found that both RES and COMB exercises, but

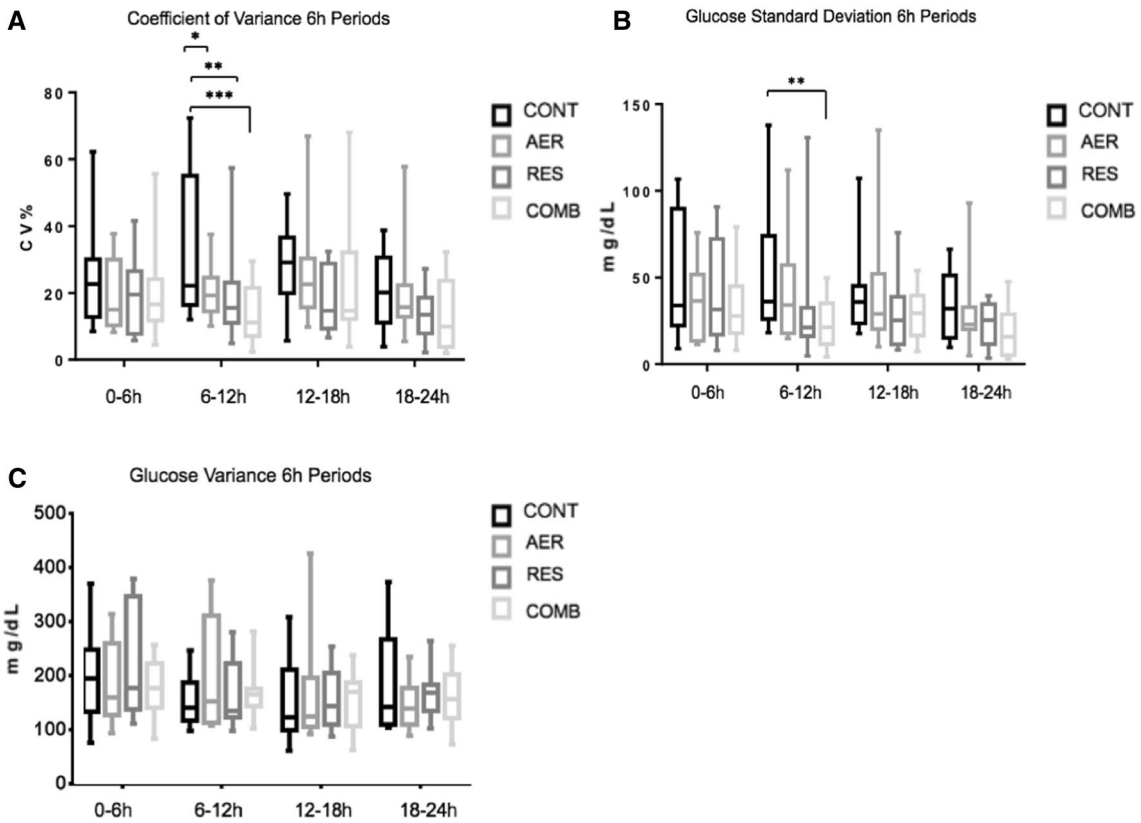

Fig. 4 Glucose coefficient of variance (CV) (**a**), glucose standard deviation (SD) (**b**), and Glucose variance (VAR) (**c**) were calculated in the 6-h time frame of interstitial glucose values to obtain the measures according to the specific period of the day. Statistical differences were determined by one-way ANOVA. $*p<0.05$, $**p<0.01$, $***p<0.001$

not AER, reduced the 24 h MAGE. Additionally, COMB exercise decreased the 24 h post-exercise SD and CV. In the 6–12 h post-exercise, all exercise sessions reduced the CV of IG while SD decreased only after COMB session. We did not observe severe events of hypoglycaemia requiring hospitalization during and after the exercise sessions; however, AER slightly, but not significantly, increased the number and duration of nocturnal hypoglycaemic events compared to the other two sessions. The relatively low rate of hypoglycaemia found in the present study was likely because the exercise sessions took place in the morning instead of the afternoon (Gomez et al. 2015). Furthermore, the real-time availability of IG data, provided by the FGM, may have contributed to the low incidence of hypoglycaemic events reported in the present study (Mancini et al. 2018). Accordingly, almost all hypoglycaemic events of the CONT and AER sessions occurred at night. In contrast, the self-initiated adjustments in insulin administration or carbohydrate intake during the day appeared to be sufficient to prevent most of the hypoglycaemic events (see Supplementary Data Table 1).

The mechanisms behind the reduction of glucose excursions after all the exercise modalities and mainly after RES and COMB are largely unknown. In the present study, blood lactate was elevated following all exercise interventions when compared to baseline. This response is consistent with other studies in T1D where elevated lactate after exercise was proposed as a stimulus of gluconeogenesis that could potentially attenuate declines in blood glucose (Turner et al. 2016; Yardley et al. 2013) or as a glucose-competing agent, reducing the glucose uptake by the muscle during exercise (Farinha et al. 2017). Moreover, we documented a strong increase of glycerol immediately after each exercise session, suggesting that the high availability of fat for fuel during exercise might have also contributed to the stabilization of exercise-associated glycaemia during the early phase (4–12 h) of post-exercise period (Chatzinikolaou et al. 2008). However, since lactate and glycerol level was comparable across exercise interventions they were unlikely to be responsible for the better 24 h glucose control found after RES and COMB than AER session. Thus, further research on exercise-related hormones potentially able to stabilize blood glucose such as cortisol, growth hormone (GH) and catecholamines in individuals with T1D is warranted. The analysis of GH/IGF-1 axis response after different exercise modalities will be particularly relevant for patients with T1D since GH resistance with low IGF-1 is frequently seen in

Table 1 Blood lactate, glycerol, CK and LDH levels

Variable	Control group (CONT)			Aerobic session (AER)			Resistance session (RES)			Combined session (COMB)		
	Pre	Post	Post 24	Pre	Post	Post 24	Pre	Post	Post 24	Pre	Post	Post 24
Blood Lactate (mmol L^{-1})	1.4 ± 0.9	1.3 ± 0.8	1.44 ± 1.16	1.7 ± 0.8	4.3 ± 2.0*	1.55 ± 1.27	1.1 ± 0.6	7.5 ± 2.9*	1.35 ± 0.9	1.3 ± 1.2	5.2 ± 2.0*	1.24 ± 0.73
Glycerol (µmol L^{-1})	62.2 ± 45.4	60.3 ± 46.3	73.0 ± 38.59	62.3 ± 48.0	135.7 ± 89.3**	57.67 ± 26.31	53.5 ± 25.9	114.2 ± 56.9**	53.67 ± 29.21	52.9 ± 26.6	121. 6 ± 45.9**	45.83 ± 21.55
CK (mU m^{-1})	256.8 ± 145.7	254.1 ± 139.1	223.0 ± 111.1	244.6 ± 143.5	263.0 ± 143.2	278.7 ± 194.4	211.9 ± 81.2	341.6 ± 102.4	202.2 ± 74.9	180.3 ± 73.1	288.7 ± 130.3	181.3 ± 94.1
LDH (mU mL^{-1})	111.3 ± 22.4	114.7 ± 36.2	113.7 ± 36.2	107.5 ± 23.5	131.2 ± 31.3	119.6 ± 33.8	110.5 ± 22.1	116.7 ± 21.1	113.7 ± 20.4	111.2 ± 18.9	112.9 ± 26.6	111.8 ± 24.5

Values are mean ± SD. Statistical differences were determined by two-way repeated ANOVA

*$p < 0.05$ and **$p < 0.001$ significantly different compared to pre-exercise (Pre)

these patients (Nambam and Schatz 2018). Noteworthy, we also found distinct skeletal muscle molecular responses to the different exercise modalities, which might also explain part of the IG variation observed after diverse exercise modes. In particular, AER exercise, but not RES exercise, increased the expression of *PGC-1α*, a key regulator of mitochondrial biogenesis and the oxidative capacity of skeletal muscle (Jäger et al. 2007). Recent evidence also supports a role for PGC-1α in the synthesis of genes involved in the regulation of glycogen storage and lactate metabolism, contributing to the maintenance of whole-body glucose homeostasis (Summermatter et al. 2013). AER exercise also increased the *IGF-1Ea/IGF-1Eb* ratio and hence promoted splicing of the *IGF-1Ea* variant. Although the functional roles of the different *IGF-1* splice variants are still unclear (Annibalini et al. 2016), overexpression of muscle-specific *IGF-1Ea* sustains muscle hypertrophy but also metabolic homeostasis, increasing carbohydrate use and insulin sensitivity both at rest and during exercise (O'Neill et al. 2015; Vassilakos et al. 2019). Intriguingly, we found an increase of *PGC-1α* mRNA and *IGF-1Ea/IGF-1Eb* ratio not only after AER but also after the COMB sessions, which is notable since this occurred despite the completion of 50% less AER exercise volume than in the single-mode AER trial. These data add support to the notion that COMB exercise appears not to interfere with AER and RES signalling compared with single-mode exercise, at least within the early post-exercise recovery period (Coffey and Hawley 2007). However, future studies which include additional muscle tissue samplings beyond immediately post-exercise, and which consider the order of AER and RES in the COMB session (Fyfe et al.2014) are needed to clarify this point. No other signalling pathways involved in the exercise-induced metabolic adaptation (i.e. AMPK, p38MAPK, ERK1/2 and Akt phosphorylation and *GLUT4* mRNA level) were modified by AER exercise. On the other hand, both RES and COMB exercise stimulated the Akt and p38MAPK phosphorylation. Additionally, COMB enhanced eEF2 dephosphorylation (i.e. activation) and RES increased the *myogenin*, and *MRF4* mRNA expression. The activation of these signalling pathways after RES and COMB exercises are consistent with other works conducted on healthy people and were likely related to increased transcriptional and protein synthesis activation (Egerman and Glass 2014; Rose et al. 2005). Moreover, both Akt and p38MAPK signalling pathways play a crucial role in acute substrate metabolism during recovery from acute exercise, including insulin-independent muscle glucose transport and glycogen synthesis (Coffey and Hawley 2007; Röhling et al. 2016). Although still correlative, the Akt and p38MAPK activation found after RES and COMB might have also contributed to the exercise-induced improvement of glucose homeostasis. Notably, although AER and RES exercise stimulated almost distinct activation of specific molecular signalling

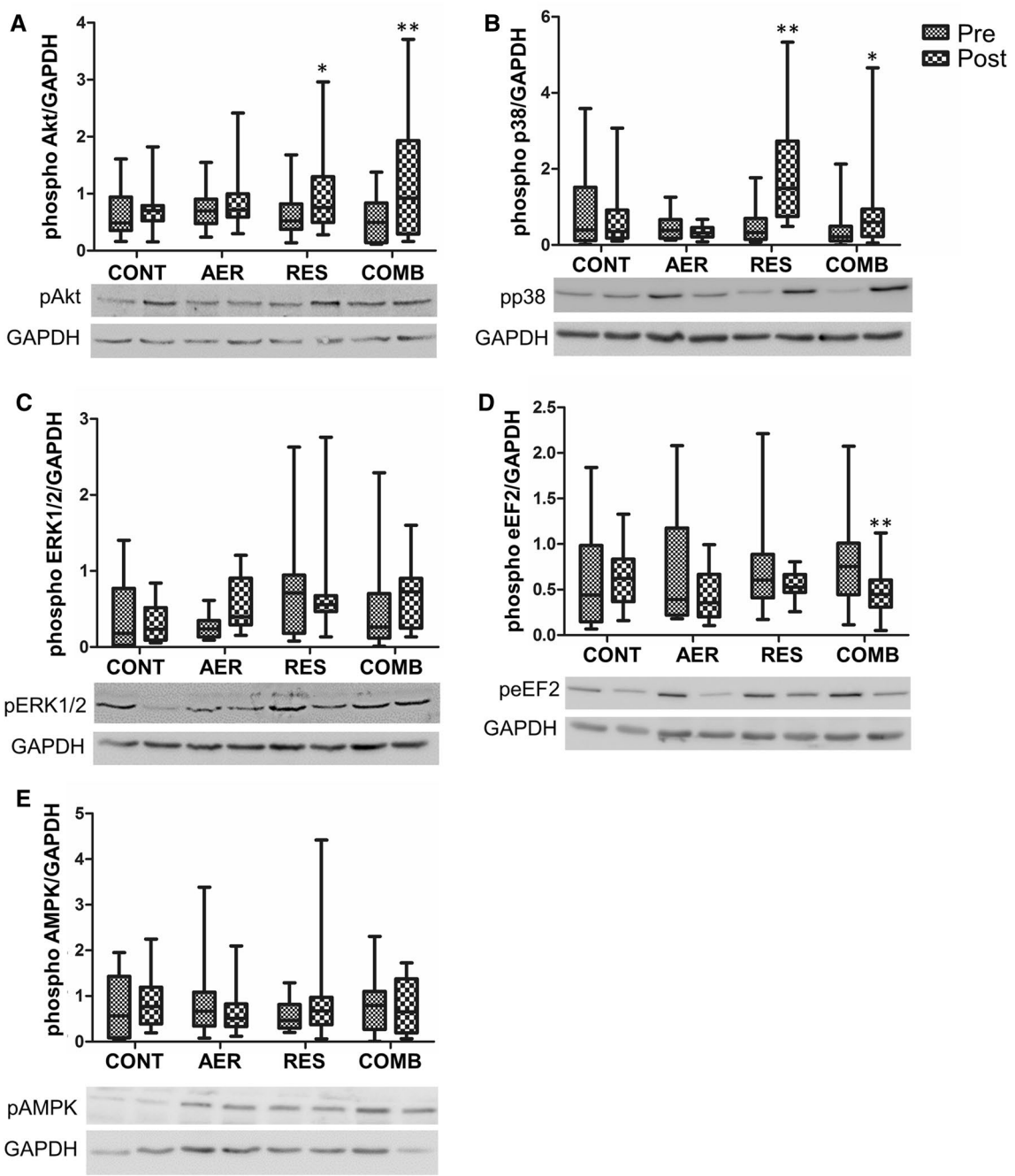

Fig. 5 Representative western blot images and box-plot of the densitometry analyses for phospho Akt (**a**), p38MAPK (**b**), ERK1/2 (**c**), eEF2 (**d**), and AMPK (**e**). GAPDH was used as loading controls. Statistical differences were determined by two-way repeated ANOVA. *$p < 0.05$, ** $p < 0.01$

pathways, we observed few differences in post-exercise signalling responses between COMB exercise and single-mode AER or RES exercise. This finding is particularly relevant for individuals living with T1D since emerging evidence shows that diabetes might impact various aspects of muscle quality, including structural and functional derangements of mitochondria, a down-regulation of myogenic factors and a higher susceptibility to exercise-induced muscle damage (Krause et al. 2011; Monaco et al. 2017). In this regard, the present study highlights that COMB exercise not only improved glycaemic control but concurrently activates molecular response directly involved in substrate metabolism and anabolic adaptations, which may have implications to maintain/improve muscle health in T1D.

Notably, none of the exercise modalities used in this study increased the serum CK and LDH levels, ruling out severe

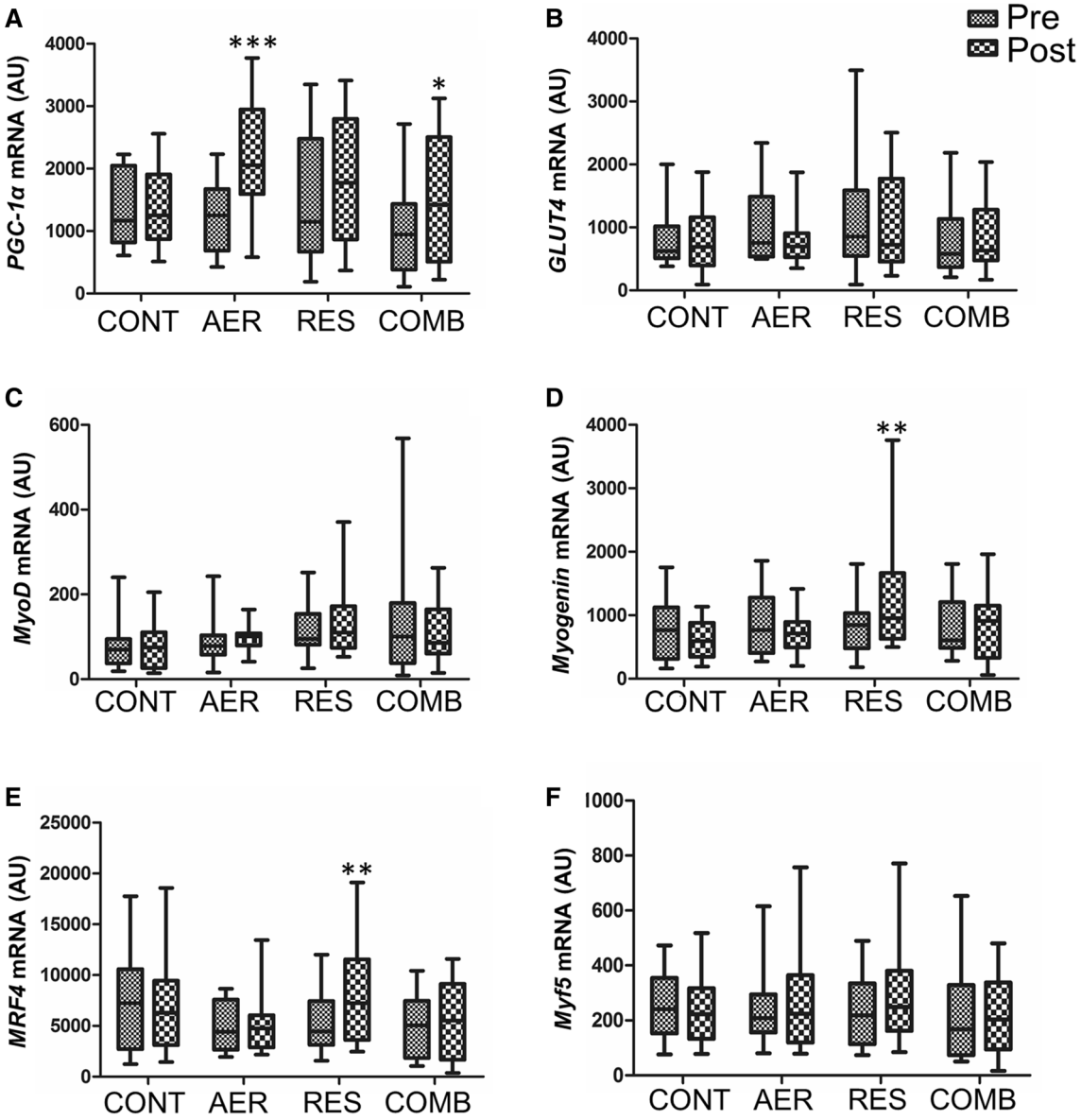

Fig. 6 Box plot for muscle mRNA expression levels of *PGC-1α* (**a**), *GLUT4* (**b**), *MyoD* (**c**), *myogenin* (**d**), *MRF4* (**e**), *Myf5* (**f**) measured by real-time PCR. The geometric mean of *GAPDH* and *B2M* mRNA levels used as the internal reference. Statistical differences were determined by two-way repeated ANOVA. $*p < 0.05$; $***p < 0.001$

exercise-induced muscle damage. Nevertheless, we found a marked induction of inflammation-related genes *IL-6*, *TNF-α*, and *MCP-1* in skeletal muscle after all exercise sessions. Particularly interesting is the marked increase of *IL-6* mRNA after all exercise sessions since this myokine acts both in a paracrine manner to regulate muscle growth and in a hormone-like fashion to support exercise-induced glucose disposal (Turner et al. 2014). In this regard, one potential limitation to study exercise-induced changes of inflammation is the variability of gene expression that may have been associated with the biopsy procedure itself (Friedmann-Bette et al. 2011). In the present study, however, a no-exercise session (i.e., CONT) was included, and by randomizing the order of each session, we have considered this potential bias. Thus, it remains for future studies to determine whether the increase of inflammatory gene expression found in our study reflects the transient and coordinated physiological inflammatory response associated with exercise (Coffey and Hawley 2007) or higher susceptibility to exercise-induced muscle microtrauma and inflammation in people with T1D (Krause et al. 2011).

We acknowledge some limitations to this study, which include its small sample size ($n = 12$) and the single biopsy post-exercise analysis, which limits the observation of

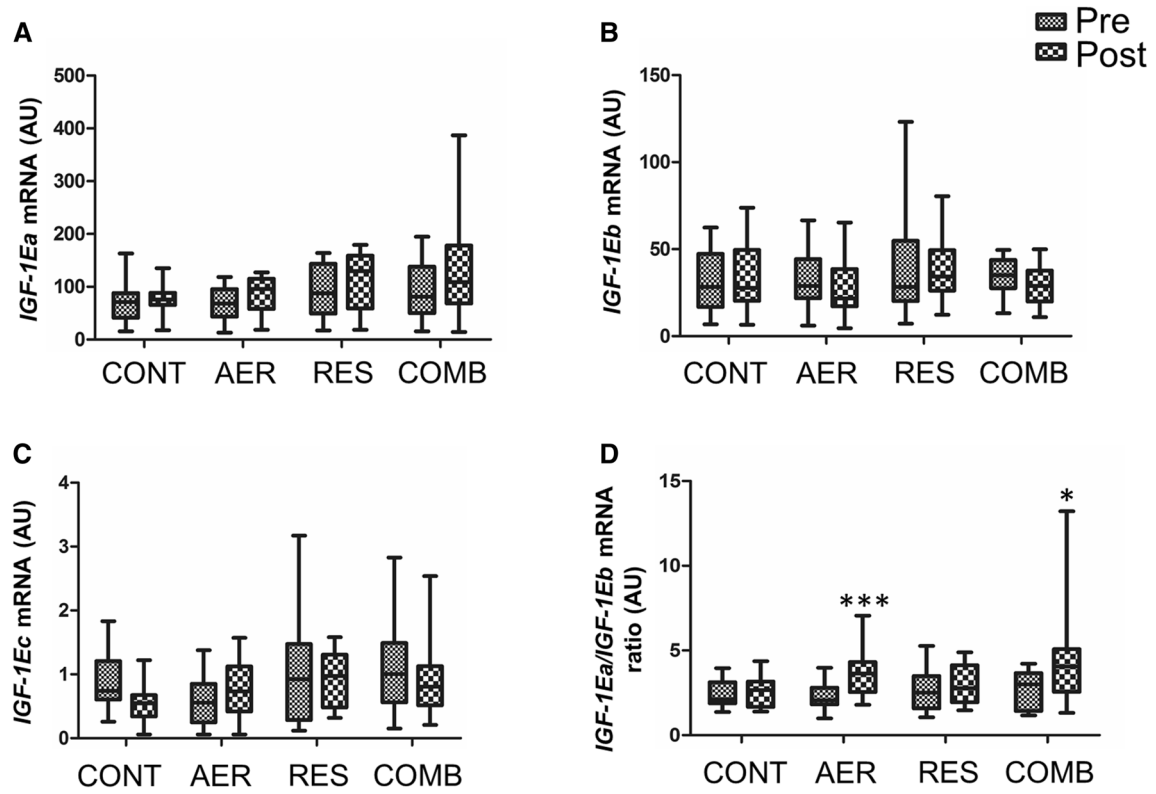

Fig. 7 Box plot for muscle mRNA expression levels of *IGF-1Ea* (**a**), *IGF-1Eb* (**b**), *IGF-1Ec* (**c**) and *IGF-1Ea/IGF-1Eb* ratio (**d**) measured by real-time PCR. The geometric mean of *GAPDH* and *B2M* mRNA levels used as the internal reference. Statistical differences were determined by two-way repeated ANOVA. $*p < 0.05$; $***p < 0.001$

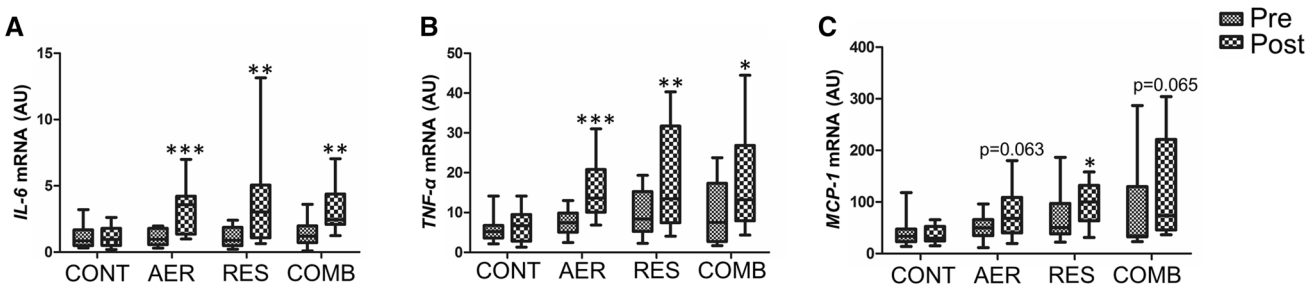

Fig. 8 Box plot for muscle mRNA expression levels of *IL-6* (**a**), *TNF-α* (**b**), and *MCP-1* (**c**) genes measured by real-time PCR. The geometric mean of *GAPDH* and *B2M* mRNA levels used as the internal reference. Statistical differences were determined by two-way repeated ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

acute molecular markers that may have peaked before or after this time point (e.g. *GLUT4* mRNA and AMPK phosphorylation). Another noted limitation to the present study was our inability to account for reporting bias and compliance in their dietary log as well as the temporal distance between meals and exercise. Moreover, although we controlled for the duration and intensity of exercise, we did not control for the differences in energy expenditure and muscle mass involvement between the different exercise modalities. One of the main strengths of this study is its randomized design; furthermore, the study group has a perfect balance of male and female participants, and no dropout had occurred. Despite the limitations, this study provides novel aspects for this important research field and poses new noteworthy aspects to consider when prescribing exercise to people living with T1D.

Conclusions

In summary, this study provides new insights into the 24 h glucose variability and the associated molecular events in skeletal muscle of people with T1D in response to AER, RES, or COMB exercise. We demonstrated that RES and COMB are more effective than AER exercise in reducing 6–12 h post-exercise and 24 h IG variability indices. AER was also associated with a slight increase of nocturnal hypoglycaemic events. All exercise modalities increased post-exercise serum lactate and glycerol levels, which might be, at least partially, involved in the exercise-induced glucose stabilization. RES and AER exercise increased muscle signalling related to muscular growth/remodelling and oxidative metabolism, respectively, while both of them might provide beneficial effects on glucose homeostasis. We also found that combining different stimuli in the same exercise session (i.e., COMB exercise) and halving the volume of the single-mode AER and RES protocols, did not compromise the skeletal muscle metabolic and anabolic responses to exercise. While future studies are required to support this proposed line, our results suggested that a training program based on COMB exercise might represent an attractive strategy to improve glucose homeostasis and muscle health in people with T1D.

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Author contributions DM and GDV conceived and designed the study. DM, GA and GDV analysed the data and drafted the manuscript. DM, CWLR, MK and GDV designed, prescribed and supervised the exercise sessions and contributed to the drafting of the manuscript. DM, SC, RS, GV and GDV collected, processed and stored the biological samples. GA, SC, RS, GV and EB performed the RT-PCR experiments and western blotting. CWLR, MK, VS, EB, and GDV provided overall direction to the project and revised the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest Innovation Zed Ltd, are a medical technology company based in Dublin, Ireland developing technologies for self-injection devices and declares no conflict of interest or self-gain from this study. This study has no other potential conflicts of interest relevant to this study.

Ethical approval This study was approved by the Human Research Ethics Committee of University College Dublin (Application ID-LS-16-67-Minnock-DeVito).

Consent to participate All participants gave their written, informed consent to participate in the study prior to its commencement.

Consent for publication All participants gave their written, informed consent for publication.

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