# Moderate intensity exercise in hypoxia increases IGF-1 bioavailability and serum irisin in individuals with type 1 diabetes

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### Abstract

**Aim:** This study aimed to determine the effect of moderate intensity continuous exercise (Ex) and hypoxia (Hyp) on serum brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1) and its binding protein-3 (IGFBP-3), irisin and cytokines levels in patients with type 1 diabetes (T1D).

**Methods:** A total of 14 individuals with T1D (age: $28.7 \pm 7.3$ years) and 14 healthy adults (age: $27.1 \pm 3.9$ years) performed 40-min continuous Ex at moderate intensity (50% lactate threshold) on a cycle ergometer in normoxia (Nor) and Hyp ($FiO_2 = 15.1\%$) Biochemical factors, glucose concentrations and physiological variables were measured at rest, immediately and up to 24 h after both Ex protocols.

**Results:** Patients with T1D had significantly lower pre-Ex serum concentrations of BDNF ($p < 0.05$, $p < 0.01$), and total IGF-1 ($p < 0.001$, $p < 0.05$) and significantly higher irisin levels ($p < 0.05$, $p < 0.01$) in Nor and Hyp, compared with healthy subjects. Ex significantly increased in T1D group serum BDNF (in Nor only $p < 0.05$) and total IGF-1 levels in Nor and Hyp ($p < 0.001$ and $p < 0.01$, respectively). Immediately after Ex in Hyp, freeIGF-1 ($p < 0.05$) and irisin levels ($p < 0.001$) were significantly higher compared with the levels induced by Ex alone. Free IGF-1 and irisin serum levels remained elevated in 24 h post-Ex in Hyp. In T1D, significant blood glucose (BG) decrease was observed immediately after Ex in Hyp ($p < 0.001$) and in 24 h recovery ($p < 0.001$) compared with pre-Ex level.

**Conclusion:** The study results suggest that moderate intensity continuous Ex has beneficial effect on BDNF and IGF-1 levels. Ex in hypoxic conditions may be more effective in increasing availability of IGF-1. The alterations in the post-Ex irisin levels and IGF-1 system may be contributing to more effective glycaemia control in patients with T1D.

*Keywords:* diabetes, exercise, hypoxia, myokines

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## Introduction

Type 1 diabetes (T1D) is an autoimmune disease where targeted destruction of pancreatic β-cells results in insulin (INS) deficiency and, consequently, hyperglycaemia. Longstanding hyperglycaemia leads to the accumulation of advanced glycation end-products and uncontrolled oxidative stress,[1] which have a detrimental effect on the mitochondria function and amino acids metabolism in myocytes and neurons of the peripheral and central nervous system.[2–4] Hence, chronically elevated blood glucose (BG) is a major risk factor for cognitive and neuromuscular dysfunction.[5,6] Patients with T1D are often found with muscle atrophy and reduced muscle strength, neuropathy, cognitive and behavioural problems.[6,7] Therefore, maintaining BG and haemoglobin A1c (HbA1c) near the recommended level (preprandial BG 80–130 mg/dl, postprandial BG < 180 mg/dl and HbA1c < 7.0%) is a major

goal of the therapy and long-term management of the disease.[8,9] Sufficient INS therapy and regular physical activity (PA) and exercise (Ex) are recommended as an integral part of the treatment and prevention of diabetes-related complications in T1D.[9–11]

During Ex, contracting skeletal muscles cause an increase in glucose transport into the cell, *via* the INS-independent pathway, enhanced synthesis of glucose transporters (i.e. GLUT-4) and increased INS sensitivity, all of which can decrease INS requirements in diabetes mellitus treatment.[3,4,12] Furthermore, skeletal muscle produces and releases myokines that exhibit autocrine, paracrine or endocrine effects.[13,14]

Irisin is a recently identified myokine that increases energy expenditure through promoting mitochondrial biogenesis or metabolic gene expression in skeletal muscle,[15,16] and plays a key regulatory role in browning of white adipose tissue.[17] Irisin has also been shown to stimulate muscle growth-related genes in healthy humans,[18] which would be of importance for patients with T1D suffering with muscle atrophy and reduced muscle strength. Faienza *et al.* observed that elevated irisin levels were associated with better glycaemic control and bone health in children with T1D.[19] Espes *et al.* found a negative correlation between irisin and INS requirements in women with T1D.[20]

Insulin-like growth factor (IGF-1) has been identified as a myokine that controls muscle growth and plays an essential role in maintaining adequate functioning of the neuromuscular system.[2,21–23] IGF-1 and its binding proteins contribute to the considerable strength gain and bone metabolism reported in patients with T1D.[24] Since IGF-1 is an important autocrine/paracrine factor, its reduced expression and serum concentration in patients with T1D have been implicated in progressive neuropathy and muscle atrophy.[2,6]

With its important role in maturation, neuronal repair and plasticity of the central nervous system, brain-derived neurotrophic factor (BDNF) is a crucial neurotrophic factor also secreted by contracting skeletal muscles.[25,26] Studies have shown that Ex stimulates expression of BDNF and its receptors in the brain,[27,28] and increases serum and plasma BDNF levels in healthy individuals.[29,30] Low plasma BDNF levels and an inverse correlation between BDNF and fasting glucose were observed in patients with type 2 diabetes (T2D).[31] Therefore, this myokine may be a good predictor of metabolic risk and impaired neuromuscular function.[31]

It is now widely accepted that Ex and PA should be an integral part of the treatment for T1D,[9,12] although Ex and PA may lead to the development of glycaemic disorders, such as hypo- or hyperglycaemia.[1] In order to increase the therapeutic value of Ex and PA, studies have been conducted to assess the metabolic effects of inhaled air with reduced oxygen concentration, hence inducing hypoxia (Hyp) to facilitate improved glucose control.

The effectiveness of interval Hyp, Hyp training and Hyp and single Ex in increasing cardiorespiratory, metabolic and glycaemic control, in healthy individuals and patients with diabetes has been demonstrated in recent studies.[32–34] The studies have shown that Hyp stimulates glucose transport in INS-resistant human skeletal muscles and, when combined with Ex, improves INS sensitivity in T2D and prevents vascular complications in T1D.[34–36] Hypoxic Ex training significantly increased serum irisin concentrations in diet-induced obese rats.[37] A study by Hall *et al.* demonstrated that 40 min of continuous Ex at moderate intensity (50% lactate threshold: LAT), as well as high intensity (120% LAT) intermittent (4 × 5 min intermittent with 5 min rest) Ex during Hyp, improved glycaemia and had beneficial effects on the concentrations of selected markers of vascular function in patients with T1D.[34] Furthermore, Ex with Hyp contributed to reducing serum levels of proinflammatory cytokine tumour necrosis factor alpha (TNF-α),[34,38] and increased the activity of erythrocytes NO synthase levels in patients with T1D,[38] potentially leading to beneficial changes in skeletal muscle oxygen capacity. These benefits were also observed in animal studies, where the exposure of rats with streptozotcin-induced T1D to hypobaric Hyp increased capillarity, reduced tissue injury and helped maintain glucose homeostasis.[39,40] Although Ex and PA are widely recommended in T1D treatment,[9,10,12] the fear of hypoglycaemia often stops patients with T1D from participating regularly.[41] More scientific evidence for the role of Ex in preventing diabetes-related muscle atrophy and neuropathy is needed to encourage patients to be regularly physically active. Therefore, the aim of this study was to assess the effects of continuous Ex performed in hypoxic

**Table 1.** Anthropometric characteristics and physiological variables before (rest) and in response to graded Ex test (max) of T1D and CG.

| Variable | T1D n = 14 | | | CG n = 14 | | | p | 95% CI for differences | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Min–Max | Mean | SD | Min–Max | | Mean differences | Lower bound | Upper Bound |
| Age (years) | 28.7 | 7.3 | 18.0–43.0 | 27.1 | 3.9 | 21.0–45.0 | 0.070 | 1.6 | 1.5 | 3.2 |
| BMI (kg/m²) | 24.1 | 3.2 | 18.1–29.9 | 23.2 | 2.4 | 19.7–27.9 | 0.300 | 0.9 | −0.9 | 2.7 |
| WHR | 0.9 | 0.1 | 0.8–1.0 | 0.8 | 0.1 | 0.7–0.9 | 0.010 | 0.1 | 0.0 | 0.1 |
| FFM (kg) | 62.3 | 12.7 | 41.6–84.8 | 58.5 | 8.4 | 43.4–73.7 | 0.400 | 3.7 | −5.3 | 12.7 |
| SMM | 35.1 | 7.7 | 22.7–48.4 | 33.1 | 5.1 | 23.8–42.0 | 0.400 | 2.0 | −3.4 | 7.4 |
| TBW (%) | 45.7 | 9.3 | 30.6–62.3 | 42.9 | 6.2 | 31.9–54.0 | 0.300 | 2.8 | −3.8 | 9.4 |
| INS (μIU/ml) | 5.4 | 2.3 | 3.6–7.9 | 10.6 | 3.9 | 5.2–16.8 | 0.000 | −5.2 | −7.6 | −2.9 |
| BG (mg/dl) | 148.9 | 19.9 | 128–168 | 87.2 | 8.5 | 70.0–108 | 0.000 | 61.7 | 47.1 | 76.4 |
| SBPrest (mmHg) | 120.6 | 14.0 | 100–142 | 115.1 | 15.1 | 103–128 | 0.300 | 5.5 | −5.2 | 16.2 |
| DBPrest (mmHg) | 73.9 | 7.1 | 70.0–90.0 | 68.2 | 7.7 | 50.0–80.0 | 0.030 | 5.7 | 0.4 | 11.1 |
| HRrest (b/min) | 83.0 | 9.0 | 76.0–96.0 | 84.0 | 14.0 | 72.0–98.0 | 0.800 | −1.1 | −14.6 | 12.4 |
| VO₂max (ml/kg/min) | 41.4 | 10.9 | 31.0–58.0 | 46.6 | 9.3 | 32.0–69.0 | 0.200 | −5.3 | −13.6 | 3.0 |

BG, postprandial blood glucose; BMI, body mass index; CG, control group; CI, confidence interval; DBP, diastolic blood pressure; Ex, exercise; FFM, free fat mass; HR, heart rate; INS, postprandial total insulin; Max, maximum; Min, minimum; SBP, systolic blood pressure; SD, standard deviation; SMM, skeletal muscle mass; T1D, type 1 diabetes; TBW, total body water; VO₂max, maximal oxygen uptake; WHR, waist-hip ratio.

conditions on serum levels of selected myokines and proinflammatory cytokines involved in regulating neuromuscular function in patients with T1D.

## Materials and methods

### Subjects

A total of 14 (8 males and 6 females) patients with T1D treated at the Diabetes Clinic of the Silesian Centre, who fulfilled the following criteria were selected in this study: with confirmed T1D (ADA criteria),[9] no evidence of diabetic complications, no personal history of other metabolic or cardiovascular diseases, no acute infection 1 week prior to the study. Moreover, only patients with at least a good Ex tolerance (maximal oxygen uptake VO₂max > 31 ml/kg/min for females and VO₂max > 37 ml/kg/min for males) were included in this study (Table 1).[42] The mean blood haemoglobin A1c (HbA1c) level was 7.2 ± 0.6%, the minimum and maximum were 6.1% and 8.2%, respectively. Two patients

had HbA1c > 7.5% and one HbA1c > 8.0%. The patients had been suffering from the disease for 12.0 ± 6.0 years (Table 1). In the study group, 50% of the patients were using continuous subcutaneous INS infusion (CSII) (rapid-acting INS: Humalog, NovoRapid or Apidra) and the other half multiple daily INS injections (MDII) with long-acting (Lantus or Levemir) and rapid-acting (NovoRapid) INS. The maximum glucose-lowering effect of all three rapid-acting types of INS occurs between 1 and 3 h and INS effect lasts 3–5 h. For the long-acting INS, the peak effect is observed after 6–14 h (Levemir only, with Lantus having no peak effect) and duration of action is between 16 and 20 (Levemir) and 20–24 h (Lantus).[43] The control group (CG) consisted of 14 healthy individuals (9 males and 5 females), without impaired glucose tolerance (Table 1). For the entire duration of the experiment, all patients were asked to consume the same balanced diet. No caffeine, antioxidants supplements or alcohol were permitted 48 h before and during the experiment. A few days prior to the study the subjects were asked to refrain from PA/Ex. All

patients performed self-monitoring of BG levels under glycaemia control and recorded results of glycaemia measurements, INS dosages and consumption of digestible carbohydrates. All individuals were instructed to arrive at the laboratory at least 1.5 h after breakfast. The patients were asked to have the pre-Ex glycaemia in the range of 100–160 mg/dl. Patients using MDII were advised to reduce their pre-Ex meal INS bolus (short-acting INS) by about 50% and those using CSII to reduce basal INS infusion also by about 50% 3 h before the Ex test and maintain it for 2 h post-Ex.[9,41] This advice was based on the peak and duration of action of the rapid-acting INS.[43] The T1D patients were advised to monitor their glycaemia more frequently after the completion of Ex to adjust INS dose and carbohydrate intake. The assessment of the digestible carbohydrate intake was calculated with dedicated software (Dietus, B.U.I. InFit. Warsaw, Poland). Body composition of all participants was evaluated using a model In Body220 analyser (Biospace Inc., Seoul, Korea).

All individuals were informed about the aim of the research, the possibility of refusal of the participation and provided written informed consent. The study was approved by The Jerzy Kukuczka Academy of Physical Education Bioethical Committee (Ethics Committee decision KBN3/2016) and conducted in accordance with the Declaration of Helsinki of the World Medical Association.

*Exercise protocol*
The study protocol consisted of two stages and four visits to the laboratory. During the first stage, all participants were subjected to an incremental Ex test in normoxia (Nor) and, after 7 days, in normobaric Hyp. At baseline, the cycling ramp protocol was used to measure individual aerobic performance (maximal oxygen uptake, $VO_2max$) and lactate threshold (LAT).[42] Based on the values achieved by each individual LAT load (Watt), the relative Ex intensities (50% LAT with a cadence maintained in the range of 60–70 rpm) were calculated for both oxygen conditions. The incremental test started with a 3-min warm-up, then intensity was increased by 30 W every 3 min up to volitional exhaustion (maximal Ex intensity) (Table 2).

Before and during both Ex protocols, heart rate (HR) was continuously monitored (PE-3000 Sport-Tester, Polar Inc. Finland). Blood pressure [systolic blood pressure(SBP)/diastolic blood pressure (DBP)] was measured in duplicate with a sphygmomanometer (HEM-907 XL, Omron Corporation, Kyoto, Japan) before and immediately after Ex. Pulmonary ventilation (VE), oxygen uptake ($VO_2$) and carbon dioxide output ($VCO_2$) were measured continuously from the sixth minute before the start and throughout each stage of the Ex test using the Ergospirometr Metalyzer 3B-2R (Cortex, Leipzig, Germany). Criteria for the termination of $VO_2$ max included: voluntary exhaustion, respiratory ratio equal to or exceeding 1.15 ($RER \geqslant 1.15$), a $VO_2$ plateau and blood lactate concentration $\geqslant 8.0$ mmol/l.

In the second stage of the study, the subjects participated in the following: 40 min continuous Ex of a moderate intensity ($60.0 \pm 5.0\%$ $VO_2max$), which was equivalent to 50.0% of LAT and 65.0% of maximal HR (HRmax). The Ex tests were performed randomly in Nor and Hyp. Hypoxic conditions were characterized by a fractional concentration of inspired oxygen ($FiO_2$) of 15.1% and atmospheric pressure (p) of 990 hPa. Produced Hyp was equivalent to that at an altitude of 2500 m. Normoxic conditions were characterized by $FiO_2$ of 20.9% and $p = 990$ hPa. All Ex sessions were conducted in an environmentally controlled chamber using an electromagnetically braked cycle ergometer (Lode B.V., Groningen, The Netherlands). The Altitude Trainer Hypoxico System (HYP-123 Hypoxic Generator, LOWOXYGEN Technology GmbH, Berlin, Germany) was used to produce the hypoxic environment.

*Biochemical analyses*
Samples of venous blood were collected after 15 min of rest before Ex test (rest), immediately after continuous Ex test (max) and during post-workout recovery (24 h after the end of the test) in Hyp and Nor. During each trial, blood samples from the antecubital vein were collected to serum separator tubes. All serum samples were left to clot at room temperature for 30 min and centrifuged for 15 min at $1000 \times g$. Obtained serum was aliquoted into Eppendorf and kept frozen at $-80°C$ (for a period not longer than 8 months, without repeated freezing) until the analyses for concentration of BDNF, IGF-1, IGFBP-3, INS, irisin, transforming growth factor beta (TGF-β), tumour necrosis factor alpha (TNF-α) and interleukins (Il-6 and Il-1β). Capillary blood was

collected from a fingertip for the determination of BG and lactate (LA) concentration. Prior to the start of Ex test, the concentrations of HbA1c were analysed by Ames DCA-2000™ Immunoassay Analyser in patients with T1D.

Serum concentrations of BDNF were analysed using immunoassays (BDNF Ultrasensitive, Human; ELISA Kit, Immuniq, Poland). Intra- and inter-assay coefficients of variation for BDNF were <8% and <10%, respectively, with sensitivity 0.06 ng/ml. Serum concentrations of IGF-1 and IGFBP-3 were analysed by an immunoradiometric method (DIAsource, Belgium). Intra- and inter-assay coefficients of variation for IGF-1 were <6% and <8%, respectively, with sensitivity 7.8 ng/ml. The molar ratio of IGF-1/IGFBP-3 was evaluated as [IGF-1 (ng/ml) $\times$ 0.130]/[IGFBP-3 (ng/ml) $\times$ 0.036], considered as a parameter determining the concentration of biologically active free IGF-1 fraction.

Serum concentrations of total INS were analysed by an immunoradiometric method (DIAsource, Ottignies-Louvain-la-Neuve, Belgium). The intra- and inter-assay variability was less than 5% and 10%, respectively, with sensitivity at 1 µIU/ml. Serum irisin concentration was measured using an enzyme-linked immunosorbent assay (Irisin Human ELISA, Biovendor, Czech Republic). The intra- and inter-assay variations were both <6.8% and <9.8%, respectively, with sensitivity 1 ng/ml.

Serum levels of TGF-β were measured by enzyme-linked immunosorbent assay ELISA kit (BlueGene Biotech, China) and TNF-α by immunoassays (DIAsource, Belgium). Serum levels of IL-6 and IL-1β levels were measured using human IL-6 and IL-1β high-sensitivity ELISA kit (Diacone, Besançon, France). Intra- and inter-assay coefficients of variation for IL-6 were <4% and <6%, respectively, with sensitivity 0.7 pg/ml. Intra- and inter-assay coefficients of variation for IL-1β were <5.1% and <8.6%, respectively, with sensitivity 0.3 pg/ml. BG concentration was measured by enzymatic method (glucose dehydrogenase), and BG differences before and after both Ex tests were calculated (BG decline: ΔBG) (Glucose 201+, HemoCue, Ängelholm, Sweden).

Pulse oxygen saturation (SatO$_2$) (pulse oximeter) and partial pressure of oxygen (pO$_2$) were measured in arterialized capillary blood from the fingertip (RapidLab 348, Bayer Diagnostics, Leverkusen, Germany) (Table 2).

Biochemical analyses were performed in our certified laboratory fulfilling the requirements of PN EN-ISO 9001:2009 (certificate No. 129/2015) according to instructions provided by the manufacturers of laboratory tests used in this study.

*Statistical analysis*
Shapiro-Wilk and Levene's tests were used in order to verify the normality, homogeneity and sphericity of the samples' data variances, respectively. The magnitudes of differences between results of pre-test and post-test were expressed as a standardised mean difference (Cohen effect sizes). Descriptive statistics were calculated and the results presented as means (x) $\pm$ standard deviations (SD). One-way analysis of variance (ANOVA) was used to compare anthropometric and physiological variables between the studied groups. The remaining data were analysed by means of repeated measures ANOVA followed by the Student-Newman-Keuls test when appropriate. The group (T1D group *versus* CG) was the grouping factor. The repetition factors included: the Ex test (pre- *versus* post-Ex, 24 h post-Ex), the Ex conditions (Nor *versus* Hyp) and the pre-Ex BG levels. In the T1D group, ANOVA was used to analyse the interactions between the type of INS treatment (CSII *versus* MDII) and the Ex test and Ex conditions. Spearman's rank correlation coefficients were used to examine the degree of correlation between biochemical variables. The significance of the intergroup differences was verified with the *post hoc* Bonferroni test. All analyses were performed using the Statistica *versus* 12 statistical software package (StatSoft, Tulsa, OK, USA). Statistical significance was set at $p < 0.05$.

## Results

*Participants' characteristics and BG monitoring*
The analysis of somatic indices did not show significant differences in body mass index (BMI) and body composition variables between patients with T1D and the healthy subjects. Patients with diabetes had higher WHR ($p < 0.01$), higher BG but lower INS levels compared with healthy controls. All subjects were characterised with normal arterial blood pressure and met aerobic fitness criteria (Table 1). In our study group there was a

**Table 2.** Physiological variables before (rest), after Ex (max) incremental VO$_2$max tests in Hyp and Nor in both groups (mean; SD).

| Variable | T1D $n = 14$ | | | CG $n = 14$ | | |
|---|---|---|---|---|---|---|
| | Hyp | Nor | *p* | Hyp | Nor | *p* |
| VO$_2$rest (ml/kg/min) | 7.1 (0.5) | 7.3 (0.6) | 0.999 | 7.1 (0.4) | 7.4 (0.5) | 0.999 |
| VO$_2$max (ml/kg/min) | 43.9 (2.4) | 41.4 (10.9) | 0.868 | 46.4 (2.1) | 46.6 (9.3) | 0.999 |
| VE (l/min) | 120.8 (7.0) | 101.6 (7.5) | 0.007 | 122.0 (6.0) | 102.3 (6.2) | 0.007 |
| HRmax (b/min) | 178.0 (10.0) | 179.0 (12.0) | 0.999 | 181.0 (12.0) | 183.0 (11.0) | 0.999 |
| LAT (Watt) | 146.0 (8.2) | 153.7 (10.4) | 0.112 | 147.1 (7.6) | 156.0 (9.4) | 0.296 |
| LAmax (mmol/l) | 9.4 (0.8) | 9.5 (0.7) | 0.894 | 9.3 (0.4) | 9.4 (0.5) | 0.990 |
| SatO$_2$ (%) | 90.6 (4.0) | 97.0 (3.0) | 0.027 | 92.0 (0.8) | 96.8 (1.7) | 0.079 |
| pO$_2$max (mmHg) | 62.8 (1.8) | 87.0 (2.0) | 0.012 | 60.2 (2.0) | 87.5 (2.0) | 0.011 |

CG, control group; HR, heart rate; Hyp, hypoxia; LA, blood lactate concentration; LAT, lactate threshold; Nor, normoxia; pO$_2$, partial pressure of oxygen in blood; SatO$_2$, oxyhaemoglobin saturation; SD, standard deviation; T1D, type 1 diabetes; VE, lung minute ventilation; VO$_2$, oxygen consumption; VO$_2$max, maximal oxygen uptake.

non-significant tendency to age difference ($p = 0.07$) between T1D and healthy subjects. VO$_2$max and LAT did not show significant differences between the groups or in response to Ex test in normoxic and hypoxic conditions. Statistical analysis of changes in physiological variables after (max) Ex in Nor and Hyp showed significant differences in maximal VE, SatO$_2$ and pO$_2$ in blood (Table 2).

The statistical analysis did not reveal significant differences between postprandial BG before continuous Ex in Nor and Hyp in T1D group; however, a tendency to higher BG levels was observed before Hyp Ex ($p = 0.052$) (Table 3). The patients were requested to have pre-Ex glycaemia in the range of 100–160 mg/dl; however, the mean pre-Ex BG levels achieved higher values in four patients before Nor and in five patients before Hyp. In T1D, significant BG decrease was observed immediately after Ex in Hyp ($p < 0.001$) and in 24h recovery ($p < 0.001$) compared with pre-Ex level (Figure 1). A higher BGΔ was observed in response to Ex in Hyp compared with Nor (66% *versus* 35% respectively; $p < 0.01$).

ANOVA revealed a significant main effect of the Ex and oxygen conditions on BG concentration (F = 8.8, $p < 0.02$) in the T1D group but not in CG (F = 0.5, $p = 0.58$). In the T1D group, the ANOVA result was not significant in response to interaction between glycaemia, Ex and oxygen

conditions on BG level (F = 1.1, $p = 0.35$) as well as the combined effect of type of treatment (CSII *versus* MDII), Ex and BG (F = 3.4, $p = 0.08$).

Significantly lower INS levels were observed in patients with T1D before Ex in Hyp and Nor ($p < 0.05$ and $p < 0.01$, respectively) and in response to both Ex tests ($p < 0.05$ and $p < 0.05$, respectively) compared with CG. No significant effect of oxygen conditions and Ex on total INS levels were observed in T1D (F = 0.18, $p = 0.8$). In patients with T1D, there were no significant differences between daily INS dosage, INS dosage at breakfast and carbohydrate (CHO) intake (Table 3).

### Myokines response to exercises in different oxygen conditions

ANOVA revealed a significant main effects of diabetes on pre-Ex serum concentration of irisin (F = 7.7, $p < 0.01$) and an interaction between diabetes, Ex and oxygen conditions (F = 8.8, $p < 0.02$) on serum irisin levels. A significantly higher serum concentration of irisin before Ex in Nor and Hyp ($p < 0.5$, $p < 0.01$, respectively) and after 24h recovery ($p < 0.001$, $p < 0.01$, respectively) was observed in the T1D group compared with the CG. In T1D, significantly higher serum levels of irisin in response to Ex in Hyp (immediately after Ex) compared with Nor were observed ($p < 0.001$). Ex- and Hyp-induced irisin levels remained elevated 24h post-Ex
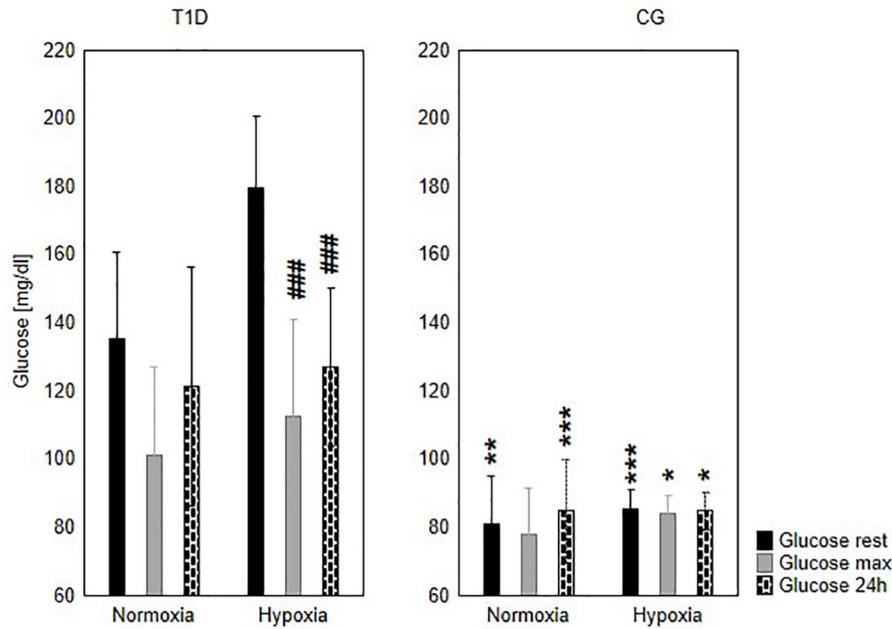
**Figure 1.** BG concentrations at rest, immediately after (max) and in response to 24-h recovery (24 h) of continuous Ex in Hyp and Nor in patients with T1D and healthy CG.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate statistically significant differences between T1D and CG; #$p < 0.05$, ###$p < 0.001$ indicate statistically significant differences between pre- and post-Ex.

BG, blood glucose; CG, control group; Ex, exercise; Hyp, hypoxia; Nor, normoxia; T1D, type 1 diabetes.

(Figure 2). In Nor, irisin levels tended to decrease immediately after Ex ($p = 0.060$) and were significantly higher after the 24 h recovery compared with pre-Ex levels in the T1D group ($p < 0.001$) (Figure 2). No significant interactions between type of treatment, Ex and Ex conditions were observed in regards to serum irisin levels (F = 0.4, $p = 0.69$). Similarly, no effects of pre-Ex glycaemia on these factors were revealed (F = 0.9, $p = 0.43$).

Significantly lower pre-Ex serum concentrations of BDNF ($p < 0.05$, $p < 0.01$) and IGF-1 ($p < 0.001$, $p < 0.05$) in Nor and Hyp, respectively, were observed in T1D compared with healthy subjects. ANOVA revealed a significant effect of the group (F = 25.4, $p < 0.001$) and Ex (F = 8.1, $p < 0.01$) on serum BDNF levels. Serum BDNF levels significantly increased in Nor Ex ($p < 0.05$) and tended to be higher in Hyp Ex ($p = 0.08$) compared with rest values in T1D (Figure 3). No significant interactions between Ex and Ex conditions (F = 0.2, $p = 0.81$) and glycaemia (F = 1.9, $p = 0.17$) and these factors and the type of treatment (F = 0.5, $p < 0.59$) in regards to serum BDNF levels in T1D group were observed.

ANOVA revealed a significant effect of T1D on pre-Ex total IGF-1 levels (F = 17.1, $p < 0.001$) and a combined effect of Ex and diabetes (F = 10.4, $p < 000$) was also found. Ex significantly increased total IGF-1 levels in Nor ($p < 0.001$) and Hyp ($p < 0.01$) but only in T1D group (Figure 4). ANOVA revealed significant interaction between diabetes, Ex and oxygen conditions (F = 3.9 $p = 0.05$). Moreover, significant effect of type of INS treatment (pump *versus* injection) was observed on IGF-1 (F = 10.0 $p < 0.04$) and a combined effect of INS treatment and Ex on IGF-1 levels (F = 6.4, $p < 0.01$) with a tendency to higher IGF-1 levels in patients using MDII ($p = 0.11$). In the T1D group, Ex-induced IGFBP-3 levels were significantly lower in Hyp compared with Nor ($7193.8 \pm 2621.9$ *versus* $11864.2 \pm 2173.6 \mu mol/l$, respectively; $p < 0.001$). ANOVA analysis revealed an interaction between the oxygen conditions and diabetes on post-Ex freeIGF-1 levels (F = 5.8, $p < 0.05$). Significantly higher freeIGF-1 levels were revealed immediately after Ex in Hyp compared with Nor in T1D group ($p < 0.05$). Ex in Hyp and Nor significantly decreased freeIGF-1 in CG ($p < 0.01$) (Figure 5).

**Table 3.** BG, INS and CHO intake at breakfast and the day of the Ex test in Hyp and Nor.

| Variable | T1D n = 14 | | | CG n = 14 | | |
|---|---|---|---|---|---|---|
| | Hyp | Nor | p | Hyp | Nor | p |
| BG rest (mg/dl) | 179.7 (28.0) | 135.4 (26.3) | 0.052 | 85.6 (8.9) | 81.0 (23.1) | 0.530 |
| BG max (mg/dl) | 113.7 (38.7) | 101.4 (36.3) | 0.430 | 83.9 (8.7) | 78.3 (22.3) | 0.380 |
| INS rest (µIU/ml) | 6.4 (2.2) | 6.2 (4.8) | 0.924 | 10.4 (4.1) | 11.2 (3.8) | 0.660 |
| INS max (µIU/ml) | 6.0 (2.2) | 5.6 (3.5) | 0.722 | 8.9 (3.5) | 9.8 (3.6) | 0.460 |
| INS DB MDII (Us) | 4.9 (3.0) | 5.3 (3.5) | 0.870 | – | – | – |
| INS DD (MDII, CSII) (Us) | 32.1 (12.7) | 32.8 (14.5) | 0.999 | – | – | – |
| CHO breakfast (g) | 61.5 (31.7) | 63.4 (44.9) | 0.995 | 95.9 (33.2) | 97.9 (30.5) | 0.735 |
| CHO daily (g) | 185.7 (75.1) | 187.8 (96.8) | 0.999 | 347.9 (136.8) | 340.9 (135.5) | 0.999 |

BG, postprandial blood glucose; CG, control group; CHO, carbohydrates; CSII, patients using continuous subcutaneous insulin infusion; Hyp, hypoxia; INS, serum level of total insulin; before (rest) and immediately after Ex (max); INS DB, insulin dosage at breakfast; INS DD, insulin daily dosage; MDII, multiple daily insulin injections; Nor, normoxia.
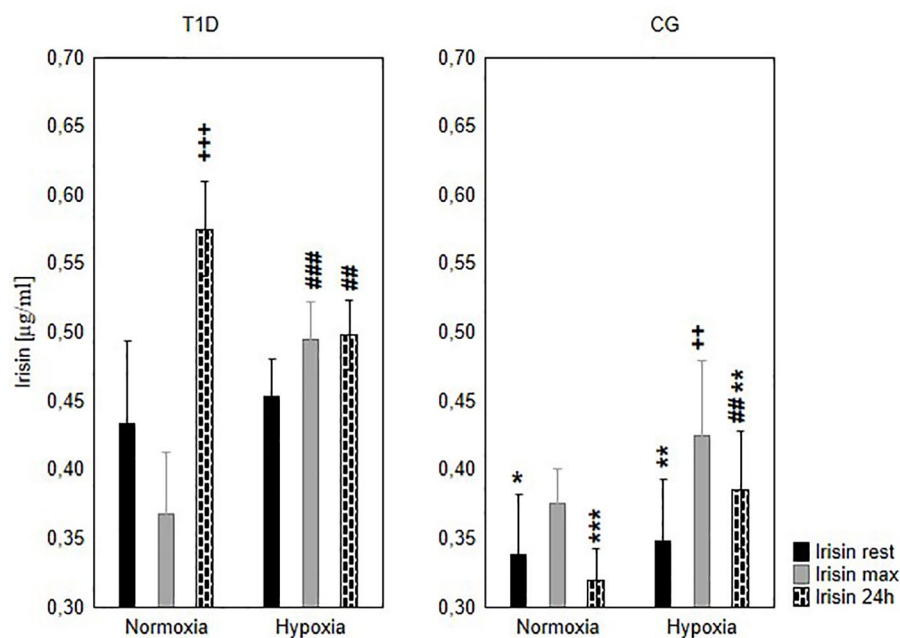


**Figure 2.** Irisin concentrations at rest, immediately after (max) and in response to 24-h recovery of continuous Ex in Hyp and Nor in patients with T1D and healthy CG.
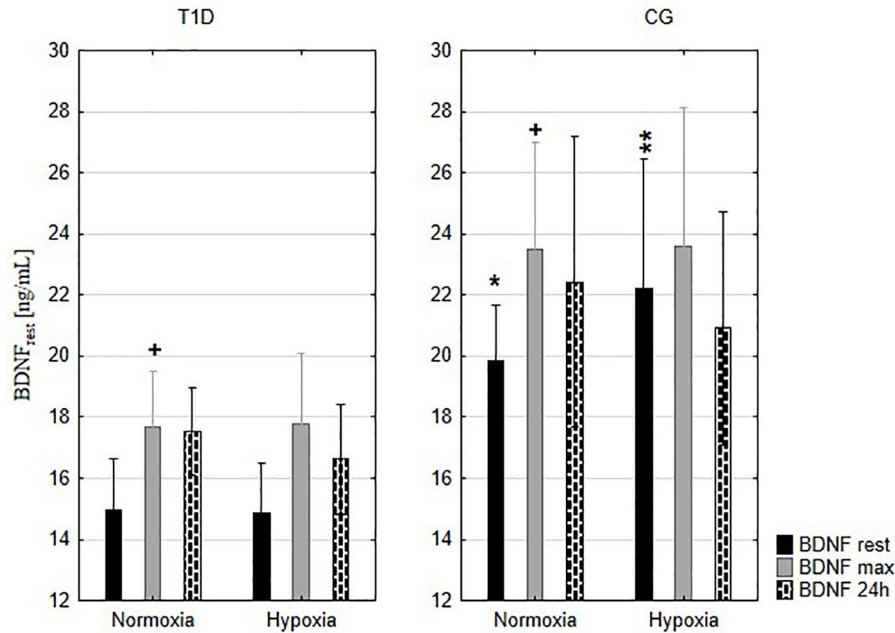+p < 0.05 indicate statistically significant differences between pre- and post-Ex; *p < 0.05, **p < 0.01, ***p < 0.001 indicate statistically significant differences between T1D and CG; #p < 0.05, ###p < 0.001 indicate statistically significant differences between Hyp and Nor.
CG, control group; Ex, exercise; Hyp, hypoxia; Nor, normoxia; T1D, type 1 diabetes.

Significant interaction effects for the group, Ex and oxygen conditions (F = 8.1  p < 0.001) on TGF-β serum concentration were found. Pre-Ex and post-Ex concentration of TGF-β did not differ between both groups. TGF-β increased significantly in response to Ex in Hyp in CG
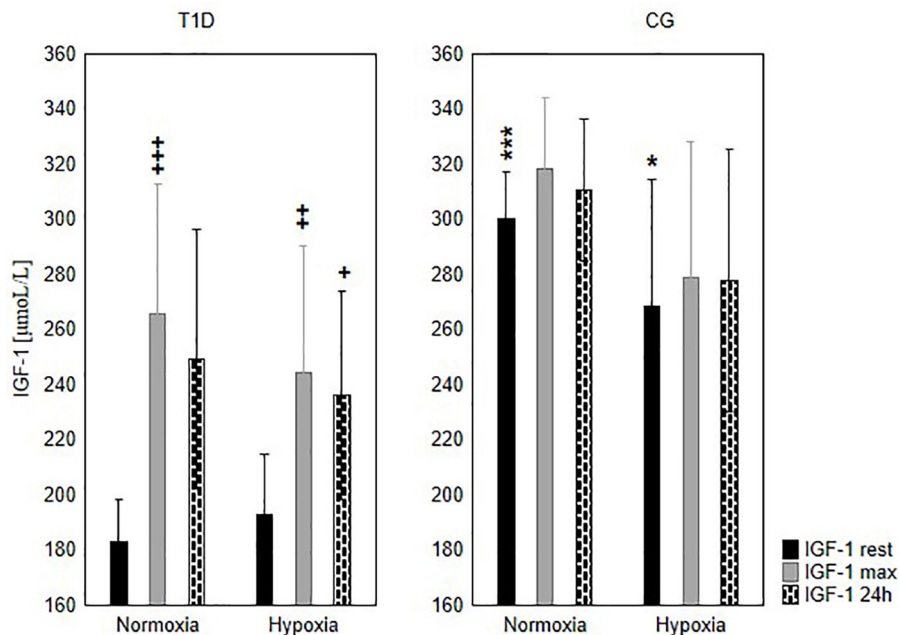
**Figure 3.** BDNF concentrations at rest, immediately after (max) and in response to 24-h recovery (of continuous Ex in Hyp and Nor in patients with T1D and healthy CG.
[+]$p < 0.05$ indicate statistically significant differences between pre- and post-Ex; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate statistically significant differences between T1D and CG; [#]$p < 0.05$, [###]$p < 0.001$ indicate statistically significant differences between Hyp and Nor.
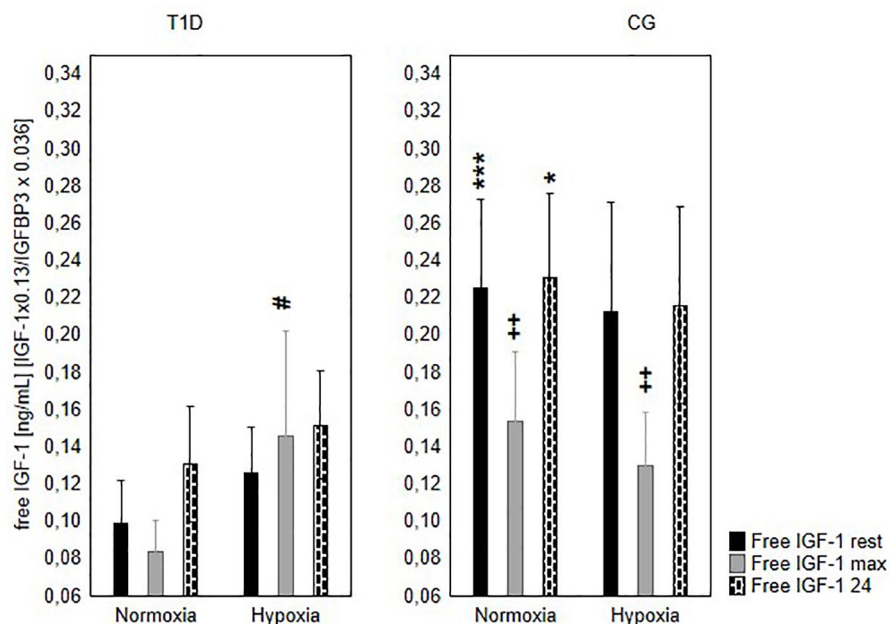BDNF, brain-derived neurotrophic factor; CG, control group; Ex, exercise; Hyp, hypoxia; Nor, normoxia; T1D, type 1 diabetes.



**Figure 4.** IGF-1 concentrations at rest, immediately after (max) and in response to 24-h recovery of continuous Ex in Hyp and Nor in patients with T1D and healthy CG.
[+]$p < 0.05$ indicate statistically significant differences between pre- and post-Ex; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate statistically significant differences between T1D and CG; [#]$p < 0.05$, [###]$p < 0.001$ indicate statistically significant differences between Hyp and Nor.
CG, control group; Ex, exercise; Hyp, hypoxia; IGF-1, insulin-like growth factor-1; Nor, normoxia; T1D, type 1 diabetes.

**Figure 5.** IGF-1/IGFBP-3 concentrations at rest, immediately after (max) and in response to 24-h recovery of continuous Ex in Hyp and Nor in patients with T1D and healthy CG.

+$p < 0.05$ indicate statistically significant differences between pre- and post-Ex; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ indicate statistically significant differences between T1D and CG; #$p < 0.05$, ###$p < 0.001$ indicate statistically significant differences between Hyp and Nor.

CG, control group; Ex, exercise; Hyp, hypoxia; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin like growth factor binding protein; Nor, normoxia; T1D, type 1 diabetes.

**Table 4.** Cytokine concentrations before (rest), after Ex (max) and 24 h recovery in Hyp and Nor in both groups.

| Variable | Hyp | | Nor | | p | 95% CI for differences | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | Mean differences | Lower bound | Upper bound |
| T1D group | | | | | | | | |
| TGF-$\beta_{rest}$ (pg/ml) | 145.4 | 32.6 | 140.5 | 25.9 | 0.710 | 4.9 | −22.9 | 32.7 |
| TGF-$\beta_{max}$ (pg/ml) | 156.7 | 42.4 | 167.9 | 28.1 | 0.510 | −11.1 | −46.9 | 24.7 |
| TGF-$\beta_{24h}$ (pg/ml) | 131.7 | 24.1 | 148.5 | 31.8 | 0.110 | −16.7 | −38.3 | 4.7 |
| TNF-$\alpha_{rest}$ (pg/ml) | 30.0 | 5.9 | 34.6 | 14.3 | 0.160 | −5.6 | −13.8 | 2.6 |
| TNF-$\alpha_{max}$ (pg/ml) | 35.7 | 19.4 | 35.5 | 10.0 | 0.960 | 0.2 | −10.2 | 10.7 |
| TNF-$\alpha_{24h}$ (pg/ml) | 69.6 | 52.9 | 43.0 | 15.0 | 0.080 | 26.5 | −4.2 | 57.3 |
| IL-6$_{rest}$ (pg/ml) | 1.1 | 0.8 | 1.2 | 0.7 | 0.550 | −0.5 | −0.3 | 0.1 |
| IL-6$_{max}$ (pg/ml) | 1.1 | 0.7 | 1.4 | 0.6 | 0.170 | −0.30 | −0.75 | 0.15 |
| IL-6$_{24h}$ (pg/ml) | 1.1 | 0.3 | 1.2 | 0.4 | 0.650 | −0.6 | −0.3 | 0.2 |
| IL-1$\beta_{rest}$ (pg/ml) | −0.2 | 0.9 | −0.2 | 0.9 | 0.980 | −0.001 | −0.21 | 0.21 |
| IL-1$\beta_{max}$ (pg/ml) | 1.3 | 1.4 | 2.1 | 3.2 | 0.480 | 0.8 | −3.1 | 1.5 |
| IL-1$\beta_{24h}$ (pg/ml) | 1.2 | 1.2 | 1.1 | 1.6 | 0.940 | 0.0 | −1.2 | 1.3 |
| Healthy CG | | | | | | | | |
| TGF-$\beta_{rest}$ (pg/ml) | 118.7 | 19.8 | 130.4 | 16.9 | 0.150 | −11.7 | −28.6 | 5.1 |
| TGF-$\beta_{max}$ (pg/ml) | 137.8 | 29.3 | 120.6 | 21.7 | **0.020** | 17.2 | 2.1 | 32.3 |

| Variable | Hyp | | Nor | | p | 95% CI for differences | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | | Mean differences | Lower bound | Upper bound |
| TGF-$\beta_{24h}$ (pg/ml) | 141.3 | 40.5 | 127.7 | 27.9 | 0.310 | 13.5 | −14.9 | 41.9 |
| TNF-$\alpha_{rest}$ (pg/ml) | 42.1 | 14.4 | 33.8 | 9.7 | 0.160 | 8.3 | −4.0 | 20.6 |
| TNF-$\alpha_{max}$ (pg/ml) | 51.7 | 30.9 | 62.1 | 44.9 | 0.580 | −10.2 | −50.2 | 29.8 |
| TNF-$\alpha_{24h}$ (pg/ml) | 61.8 | 43.9 | 36.3 | 9.9 | 0.070 | 46.9 | −2.9 | 53.8 |
| IL-6 $_{rest}$ (pg/ml) | 1.9 | 1.1 | 0.9 | 0.6 | 0.330 | 0.3 | −0.1 | 1.6 |
| IL-6$_{max}$ (pg/ml) | 1.9 | 3.1 | 1.0 | 0.7 | 0.320 | 0.9 | −0.9 | 2.8 |
| IL-6$_{24h}$ (pg/ml) | 1.6 | 1.2 | 0.7 | 0.6 | **0.010** | 0.9 | 0.2 | 1.6 |
| IL-1$\beta_{rest}$ (pg/ml) | 2.8 | 4.5 | 3.0 | 3.4 | 0.890 | 0.2 | −3.7 | 3.3 |
| IL-1$\beta_{max}$ (pg/ml) | 2.9 | 5.8 | 2.3 | 3.9 | 0.790 | 0.5 | −3.9 | 5.1 |
| IL-1$\beta_{24h}$ (pg/ml) | 2.6 | 5.4 | 2.2 | 4.2 | 0.850 | 0.4 | −4.1 | 4.9 |

24 h, 24 h after the end of the test; CG, control group; Hyp, hypoxia; IL-6, interleukin 6; IL-1β, interleukin 1 beta; max, immediately after Ex; Nor, normoxia; rest, before Ex; T1D, type 1 diabetes; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha.

($p < 0.05$) (Table 4). ANOVA revealed a significant effect for the test conditions (F = 5.1 $p < 0.01$) and interaction effects for group and Ex (F = 3.8, $p < 0.05$) on TNF-α serum concentration. In the T1D and CG, a tendency to TNF-α levels to increase in the 24 h post-Ex was observed. ANOVA and *post hoc* analysis did not reveal any significant effects of the factors or significant differences in serum levels of IL-6 and IL-1β in T1D group (Table 4). Significant interactions between Ex, oxygen conditions and type of INS treatment was observed in serum IL-6 (F = 4.7, $p < 0.01$) and 1L-1β levels (F = 3.4, $p < 0.04$ in T1D. Lower post-Ex IL-1β levels were observed in patients using MDII in Nor ($p < 0.01$) and Hyp ($p < 0.01$) compared with patients using CSII.

In T1D and CG groups, continuous Ex combined with Hyp significantly reduced SatO$_2$ compared with Nor (97.0 ± 0.7 *versus* 91.0 ± 2.8, $p < 0.01$ and 95.6 ± 1.7 *versus* 91.6 ± 2.6, $p < 0.01$, respectively).

In Hyp, there were positive correlations between post-Ex BDNF and total IGF-1 levels in T1D group and CG (r = 0.73, $p = 0.02$ and r = 0.68, $p = 0.011$). Serum pre-Ex BDNF levels were associated with BG concentrations in T1D group in Nor (r = 0.68, $p = 0.04$). In the T1D group, immediately post-Ex BDNF correlated positively with BGΔ in Hyp (r = 0.72, $p = 0.03$). There were no correlations observed in CG.

## Discussion

This study aimed to assess the effects of Hyp and Ex on myokines and proinflammatory cytokines involved in regulating neuromuscular function in patients with T1D. The assessment was based on the analysis of glycaemia changes and serum concentrations of selected myokines (BDNF, IGF-1, irisin), and proinflammatory cytokines (IL-6, IL-1β, TNF-α, TGF-β) known to be involved in regulating muscle and/or nerve cell growth.[21,22,44–46] A major finding of the study is that continuous Ex in different oxygen conditions modified secretion of the factors regulating neuromuscular function. Continuous Ex in Nor resulted in significant increases in BDNF and IGF-1 levels, whereas no significant changes occurred in free IGF-1 levels. When Ex was combined with Hyp, a significant elevation of serum concentration of free IGF-1 and irisin levels immediately after Ex as well as a greater BGΔ compared with Ex alone were observed. Hyp caused a greater Ex-induced serum IGF-1 level increase, whereas no significant changes occurred in IGFBP-3 levels. IGF-1/IGFB-3 ratios were

significantly elevated by Hyp. Thus, IGF-1 bioavailability was increased as a result of Hyp compared with Nor in individuals with T1D. It was also interesting to note that resting BDNF and IGF-1 levels were significantly lower and irisin levels significantly higher in patients with T1D than in age-matched healthy controls.

Myokines are synthesised and released by contracting myocytes.[44] Myokines exhibit an autocrine and para/endocrine function regulating the metabolism of the muscles and of organs and tissues such as brain, liver and adipose tissue.[18,23,31,47,48] In the present study, the profiles of most of the selected myokines significantly differed between the groups. It has been documented previously that skeletal muscle ability to secrete some myokines (BDNF, IGF-1 and irisin) may decline with age,[39,49,50] and age-related changes in skeletal muscles affect individuals from approximately the fourth decade of life.[51] In the present study, there was an insignificant ($p = 0.07$) age difference between the two groups and pre-Ex serum levels of the myokines varied. Nevertheless, with a mean age of 28.7 (7.3) years, comparable skeletal muscle mass (Table 1) and miscellaneous serum concentrations of BDNF and IGF-1 (decreased) and irisin (increased) in T1D compared with healthy subjects, it seems that the differences in myokines secretion could not be attributed to age difference, but potentially to hyperglycaemia. A study by Ates *et al.* found a positive correlation between irisin and HbA1c and observed that chronic inflammation and autoimmunity seem to increase serum irisin levels. [52] On the contrary, Tentolouris *et al.* observed significantly lower levels of circulating irisin in patients with T1D compared with healthy adults.[53] The authors observed a positive correlation between irisin levels and waist circumference in subjects with diabetes.

In the present study, individuals with T1D had a higher waist-to-hip ratio (WHR) than healthy controls and comparable body fat mass (~18% of body mass). The higher irisin levels among T1D compared with healthy individuals in the current study might be associated with intergroup differences in WHR; however, other factors should also be considered.[16,18,54]

It has been noted that irisin levels increase in response to Ex, and fitness level and BMI were identified as significant predictive variables for post-Ex irisin concentration in healthy subjects.[55] In our study, in T1D group irisin increased in response to Ex in Hyp and remained elevated in 24h post-Ex. The benefits of Hyp should be treated with caution as higher irisin levels in 24h post-Ex in Nor Ex were also observed. Hyp stimulated irisin secretion in response to Ex more effectively in healthy subjects. These findings suggest that Hyp is an important factor in increasing serum levels of irisin. Similarly, significantly higher serum irisin levels in response to the 8-week Ex training in a Hyp environment compared with Ex training alone in obese rats have been reported.[37] Interestingly, serum irisin level decreased post-Ex in CG but not in the T1D group, where its levels remained elevated. These different responses of circulating irisin to Ex protocols in both groups suggest either impaired secretion of irisin or irisin resistance in T1D group. Ex has been considered to be the main factor stimulating the synthesis of myokine; nevertheless, inflammation and autoimmunity, the factors characterising T1D, may also play a role.[38,52,55]

Previous studies have shown that irisin promotes glucose uptake in skeletal muscle, thus improving glucose metabolism.[19,48] In the present study, Ex- and Hyp-induced increases in serum irisin were observed in parallel with significant BG decrease in T1D patients and considered as a positive observation. It has been suggested recently that irisin plays a role in regulating glucose homeostasis through inducing translocation of glucose transporter type 4 (GLUT 4) to skeletal muscle cell membrane and by stimulating GLUT 4 expression in human adipocytes.[39,40] However, whether it also leads to the translocation of GLUT 4 to the membrane and, consequently, increased glucose uptake by adipose tissue, remains unknown.[56]

In patients with diabetes, the loss of INS secretion with the consequent dysregulated secretion of counter-regulatory hormones (glucagon, cortisol, catecholamines, growth hormone) predispose to an increased risk of Ex-induced glucose fluctuations,[12] which depend on the initial BG levels as well as type of INS therapy.[10,11,57] Thus, precise Ex recommendations should be tailored to meet the specific needs of each individual.[41] As recommended, in patients with T1D, BG concentrations should always be checked prior to Ex and the target range for pre-Ex BG level is between 90 and 250 mg/dl. Appropriate CHO intake and adjustment of INS doses to starting

BG levels and the intensity of Ex are essential.[9,10,41] The initial BG levels of T1D patients tended to be higher before Hyp Ex compared with Nor in patients with diabetes. No significant interactions between pre-Ex glycaemia, oxygen conditions, Ex and BG levels seemed to confirm that continuous Ex in Hyp may be more effective in reducing hyperglycaemia compared with continuous Ex alone. The patients were treated with different types of INS therapy (pump *versus* injections and rapid-acting INS only *versus* rapid- and long-acting INS); however, no significant effect of the type of treatment on pre- and post-Ex BG levels was determined. Therefore, the interpretation of BG response to Ex with and without Hyp has proven to be challenging.

The study patients were found with significantly lower serum INS concentrations (hypoinsulinaemia) compared with healthy subjects. This observation could have been a result of pre-Ex reduction of INS administration. Nevertheless, inadequate adherence to INS therapy cannot be ruled out.[9]

Studies have confirmed that, in patients with T1D hypoinsulinaemia, chronic hyperglycaemia and elevation in IL-6 reduce the synthesis of neurotrophic factors such as BDNF and IGF-1,[5,7,31,58–60] leading to muscle atrophy and consequently reduced muscle strength and cognitive decline.[1,6,60] Indeed, in the present study, the baseline serum BDNF and total IGF-1 concentrations were significantly lower in the T1D group compared with healthy controls. In the T1D group, IGF-1 increased significantly in response to both Ex protocols. The increased total IGF-1 levels and lower IGFBP-3 concentrations in response to Ex in Hyp suggested a positive effect of the Ex in Hyp on IGF-1/IGFBP-3 ratio and the bioavailability of IGF-1 in T1D. IGFBPs extend the half-life of IGF-1 in the circulatory system, decreasing its degradation by proteolysis.[61] Thus, it is thought that the higher molar ratio of IGF-1/IGFBP-3 due to IGFBP-3 proteolysis may contribute to benefits of Hyp by increasing free IGF-1 bioavailability. Apart from its neurotrophic and neuroregenerative effect, alterations in the IGF-1 system may be attributed to better control of glucose homoeostasis,[60,62,63] as well as improving endothelial function and muscle strength.[22,61,64] These IGF-1-induced effects will be of great importance for T1D patients, who are at higher risk of muscle atrophy as well as nervous and cardiovascular system complications.

In the present study, serum BDNF levels increased significantly in response to continuous Ex in Nor and tended to be elevated in Hyp. Due to the fact that no functional test of neuromuscular performance was used in this study, we can only speculate that the observed elevated BDNF levels might be associated with improved cognitive function. The effect of different forms of aerobic Ex on enhanced serum BDNF levels,[65] INS sensitivity and glucose homeostasis in T1D and T2D has been well documented.[7,66] In addition, BDNF has been shown to protect pancreatic β cells. This may be attributed to its effect on expression of the genes encoding proteins implicated in stress resistance and cell survival.[27,67] It is important to point out that some inconsistencies in literature data on BDNF benefits can be explained by differences in the applied methodologies of BDNF assessment (serum *versus* plasma).[68] Since peripheral BDNF is stored mainly in platelets, serum BDNF may be a more reliable method of assessing Ex-induced BDNF production.[68]

The present study revealed a significant positive correlation between Ex with Hyp-induced IGF-1 and BDNF serum levels in patients with T1D. The increase of total IGF-1 and free IGF-1 was associated with greater glycaemia decline compared with that in response to Ex in Nor. IGF-1 has been suggested to be a key factor involved in the modulation of BDNF and INS signalling pathways, contributing to motoneurons remodelling and muscle growth.[5,7] Both Ex protocols stimulated secretion of IGF-1 and BDNF to a similar extent. Nevertheless, Hyp had a significant effect on serum free IGF-1 levels, which were significantly higher compared with Nor conditions.

It has been shown that hyperglycaemia and Hyp increase secretion of cytokines by inflammatory cells.[60,69] However, in the present study, diabetes did not have a significant effect on TNFα, TGF-β, IL-1β and IL-6 serum concentrations, which were similar in both groups. Since the evaluation of proinflammatory cytokines is a valuable tool for evaluation of the risk of developing diabetes-related complications,[34,65,70,71] the study findings suggest that T1D patients may not be at risk of developing such complications; however, more tests would need to be carried out to confirm that.

Interestingly a tendency to IL-6 and IL-1β increase and slightly higher serum IGF-1 concentrations were observed in patients treated with INS pumps compared with those using MDII. Still, a question arises whether the method of glycaemia management could maintain adequate serum myokines concentrations in T1D patients. This will require further investigation.

The proinflammatory cytokines analysed are also classed as myokines and secreted by working skeletal muscles.[72] TGF-β and TNF-α tended to increase immediately after and 24h post-Ex, respectively. These cytokines are involved in the acute inflammatory response to myocyte microdamage occurring during Ex, and in muscle repair and regeneration.[73,74] Despite the beneficial role of TNF-α in post-Ex muscle repair, its persistent elevated level may have a detrimental effect on glucose and lipid metabolism.[75] Nevertheless, it can be expected that regular Ex will have an anti-inflammatory effect and reduce TNF-α levels and moreover increase secretion of anti-inflammatory cytokines.[76] Whether or not this Ex regimen will result in long-term clinical benefits needs further investigation.

## Conclusion
The study results suggest that a single moderate intensity continuous Ex significantly increases IGF-1 and BDNF levels and has a beneficial effect on serum irisin levels. Ex in hypoxic conditions may be more effective in increasing availability of IGF-1. The alterations in the post-Ex irisin levels and IGF-1 system may be contributing to more effective glycaemia control in patients with T1D.

### Limitations
There are several limitations of this study. The sample size was small and the patients with T1D were slightly older. The low serum INS levels in patients with diabetes compared with healthy subjects might have had a possible impact on Ex-induced biochemical changes. Finally, it is not a longitudinal study, which made it impossible to observe the clinical impacts of the intervention on myokine profiles.

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## Supplemental material
Supplemental material for this article is available online.

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