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The beneficial effects of 15 units of high-intensity circuit training in women is modified by age, baseline insulin resistance and physical capacity

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ABSTRACT

Aim: To investigate the effect of a single and 15 units of high-intensity circuit training (HICT) programme on glucose metabolism, myokines' response and selected genes' expression in women.

Methods: Thirty-three, non-active women (mean age: 38 ± 12) were split into a HICT (n = 20) or a control group (CON, n = 13). The training protocol included three circuits of nine exercises with own body weight as a workload performed 3 times a week for five weeks. The CON group performed HICT twice. Blood samples were taken before, 1 h and 24 h after the first and last unit to determine IGF-1, myostatin, irisin, decorin, HSP27, interleukin-15 concentrations using the ELISA immunoenzymatic method. To evaluate *HSPB1, TNF-α* and *DCN* mRNA, real-time PCR was used. Pre- and post-intervention, the oral glucose test and body composition assessment were completed.

Results: The following parameters tended to decrease after the 5-week HICT program: insulin and HOMA-IR Training diminished insulin/IGF-1 ratio (51% CI: −63% to −34%) and induced the drop of myostatin concentration but significantly only among middle-aged women and at baseline insulin resistance.

Conclusion: Obtained data revealed that HICT improved an insulin sensitivity and diminished myostatin concentration among older, insulin-resistant women with lower baseline physical capacity.

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1. Introduction

Physical activity deficit has become an important public health issue in recent years. In the long-term, a sedentary lifestyle is associated with an increased risk of mortality, cardiovascular disease, and type 2 diabetes mellitus (T2DM), which is commonly registered among adults as well as young patients [1]. At the same time, physical activity promotes better functioning of the human body through adaptive changes. Regular exercise supports improved insulin sensitivity, thus, helping to prevent diabetes [2].

The most common reason for not engaging in physical activity is lack of time. Since 2014, the American College of Sports Medicine (ACSM) has been consistently recommending high-intensity interval training (HIIT) as the most effective and time-efficient form of exercise [3,4]. Not only is HIIT thought to induce similar changes as moderate-intensity continuous training, but it can be practiced by people with an impaired glucose tolerance and T2DM [5]. Furthermore, a modification of HIIT, high-intensity circuit training (HICT) using one's own body weight as a workload, is considered as a safe way of practicing resistance training [6].

Data published by Gmiat et al. showed that in a group of women, a single session of HICT had a positive impact on cognitive function and immunological response, modulated by circulating myokines. Still, the effect depended on participants' age [7]. These findings carry a particular importance, as few studies so far have investigated the combined effect of resistance training and endocrine function of muscle mass (myokines released during contractions) [8].

It is worth noting that skeletal muscles are responsible for >75% of insulin-mediated glucose metabolism [9]. Moreover, reduced muscle mass contributes to skeletal muscles' insulin resistance [10]. Considering this observation and the anabolic action of some myokines (IL-15), during maintenance or development of muscle mass through resistance training [11], it appears to be crucial for counteracting adverse metabolic changes. Nonetheless, a recently published review emphasised that among 51 published papers relating to resistance training, only 12 involved female participants [12].

The potential treatment and prevention of metabolic diseases associated with regular physical activity is ascribed to the myokines released during exercise. Another protein, decorin, secreted during exercise, has been shown to have an inhibitive impact on breast cancer progression [13] and a blinding effect on myostatin that consequently conduces to muscle hypertrophy [14].

Myostatin as a member of the TGF-β superfamily is a negative regulator of muscle development [15], and is also mentioned to be involved in energy expenditure and glucose homeostasis [16]. Higher expression of myostatin mRNA in skeletal muscles has been observed in obese, insulin-resistant subjects [17]. Moreover, a study on mice has indicated that the animals lacking the myostatin gene ($Mstn^{-/-}$) have exhibited an improved glucose uptake and insulin sensitivity [18]. Data published by Dong et al. revealed that inhibition of myostatin improves insulin sensitivity via irisin-mediated crosstalk between muscles and adipose tissue [9]. Involvement of irisin in glucose homeostasis [19] may be modified by signalling between insulin and insulin-like growth factor 1 (IGF-1) [20].

Interestingly, insulin resistance (IR) may be affected by heat shock protein beta 1 (gene expression as *HSPB1*), also known as heat shock protein 27 (HSP27). Yuan et al. have demonstrated that AMPK-mediated *HSPB1* expression enhanced insulin sensitivity in skeletal muscles [21]. Hence, the increase of HSP27 24 h after a single bout of HICT recorded by Gmiat et al. should encourage further investigations to verify this form of exercise as an effective, health-promoting strategy [7].

We have decided therefore to investigate the effect of a single session as well as a 5-week HICT programme applied in adult women on glucose homeostasis and myokines' secretion, which can modulate and enhance the response to training. Given that blood cells are known to be the best way of describing the whole-body response to exercise [22], together with proteins: HSP27, IGF-1, IL-15, irisin, myostatin and decorin, a genetic assessment of *HSPB1*, *DCN* and *TNF-α* mRNA was also evaluated.

2. Materials and methods

2.1. Subjects

Thirty-six healthy, inactive women (within last year) without diabetes characterised by a fair level of relative maximal oxygen uptake (average VO_{2max} 29 ± 6.8 mL·kg^{-1}·min^{-1}) [23] participated in the experiment. At baseline women were examined by a doctor in order to eliminate those with medical contraindications. Written, informed consent was obtained from all participants. Based on the values of relative maximal oxygen uptake, women were divided into two groups trained HICT (n = 20, age: 40 ± 11 years) or control CON (n = 13, age: 45 ± 13 years). Women were additionally divided according to the young (YG n = 11, age ≤30 years) and middle-aged (MG n = 9, age >30 years) group, in order to assess age-depended changes [7]. Only women whose training attendance exceeded 85% were included in to statistical analysis and three women were therefore excluded in final analysis.

One week prior to the start of the experiment and directly (within four days) after 5 weeks of completed training the following tests were performed: body composition, aerobic capacity assessment, blood collection and functional movement screen (FMS).

Women were asked to not change their daily habits. The study was approved by the Bioethical Committee of the Regional Medical Society in Gdansk KB-14/17 in accordance with Declaration of Helsinki.

2.2. Preliminary testing

2.2.1. Body composition assessment

Skeletal muscle mass (SMM), body fat mass (BFM), and percent body fat (PBF) were evaluated by using a multifrequency impedance Analyser In Body 720 (Biospace, Korea). Impedance of segments of the body parts (trunk, arms and legs) was measured at diverse 6 frequencies (1, 5, 50, 250,

500, and 1000 kHz) using an eight-polar tactile-electrode. Percent of body fat mass repeated measurement precision was expressed as the coefficient of variation, which was on average, 0.6% [24]. Additionally, the amount of the visceral fat area (VFA) expressed in cm^2 was determined [25,26].

2.2.2. Cardiorespiratory fitness measurement

To assign a VO_{2max} value participants performed a graded cycle test on an electromagnetically-braked, cycle ergometer (884E Sprint Bike Monark, Sweden). The test began with 5-minute warm up with the intensity $1 \, W \cdot kg^{-1}$ and pedalling cadence 60 rpm. Directly after the warm up, participants began VO_{2max} testing by cycling with the same pedalling cadence but with progressively increased workload by $25 \, W \cdot min^{-1}$ until the subject reached the point of volitional exhaustion. During testing breath by breath pulmonary gas exchange was measured (MetaMax 3B, Cortex, Germany) [27]. The highest values of relative oxygen uptake were considered to calculate maximal aerobic power. Participants were identified according to cardiorespiratory fitness level. The cut-off point was set at the level of $28 \, mL \cdot kg^{-1} \cdot min^{-1}$, women above were described as high fitness level (VO_{2max}) and those below as low fitness level (VO_{2max}).

2.2.3. Functional Movement Screen (FMS)

FMS was used to assess participants' movement patterns, the mobility and stability of certain joints and the coordination of certain kinetic chains. It includes seven tasks: deep squat, hurdle step, inline lunge, shoulder mobility, active straight leg raises, trunk stability push-up and rotary stability. Each task is evaluated by the quantity and the quality of the movement by the scale from 0 to 3. The sum score of all tasks is calculated [28].

2.3. Training procedure

HICT training protocol was the same as in our previous study [7] based on ACSM recommendations and the original protocol [6]. Before the main experiment we recorded a movie with instructions (whole units of training) with music and time visualisation, that was easy to play on mobile devices. One week before beginning the experiment, participants had been familiarised with the protocol in order to perform each exercise correctly at an appropriate intensity (80%–90% of maximal heart rate HR_{max}). The first and last unit of HICT were performed under instructor's supervision, in case of the need to correct and control the quality of the training. Directly after the first and the last HICT sessions participants were asked to assess the rating of perceived exertion (RPE), based on the Borg scale [29]. During the training programme participants were allowed to train individually at home, and they were also asked to train always at the same time of a day. Each week the instructor called (or directly asked) to confirm the attendance of HICT sessions and assess the RPE values. After 2 weeks of training the additional series were added. Subjects from training group completed 15 HICT sessions 3 times per week (on Monday, Wednesday and Friday) within 5 weeks. One HICT session consisted of 3 circuits with 2-minute break between. Each HICT training consisted 9 exercises with one's

body as a workload performed as follow: jumping jacks, push-ups, sit-ups, side plank, squats, plank, running in place, lunges and push-ups with rotation. Subjects from the CON group completed HICT twice at the baseline and after 5 weeks. Supplementary Material 1 shows the schedule of the study.

2.4. Blood collection

Blood samples were taken at two time points during the experiment: before and after the whole intervention and also before and after single bout of HICT. A professional nurse collected the blood from the antecubital vein into the vacutainer tubes: with $EDTAK_3$ for plasma analysis, Vacutainer SST™ II Advance for serum analysis and into the vacutainer tubes with sodium fluoride to estimate glucose concentration. One week before and two days after 5 weeks of training blood samples were collected to perform oral glucose tolerance test (OGTT). Before, 1 h and 24 h after the first and last HICT sessions blood samples were collected. Samples were centrifuged at 2000 g for 10 min at 4 °C then stored at −80 °C by the time of immunoenzymatic analysis. Serum concentrations of irisin, IL-15, myostatin, IGF-1, HSP27 and plasma level of decorin were measured. Additionally, gene expression was performed.

Serum IL-15, myostatin, and IGF-1 were evaluated using ELISA kits (R&D Systems, USA, catalogue no. D1500, DGDF80, and DG100 respectively) in accordance with manufacturer's instructions. The maximal intra-assay coefficient of variability (CV) for IL-15 was 5.3%, for myostatin was 5% and for IGF-1 4.3%. The inter-assay coefficient and detection sensitivity were as follows: 9.1% and $2 \, pg \cdot mL^{-1}$ for IL-15; 6% and $5.32 \, pg \cdot mL^{-1}$ for myostatin and 8.3% and $0.056 \, ng \cdot mL^{-1}$ for IGF-1. The serum concentration of irisin was evaluated using competitive enzyme immunoassay sandwiches from Phoenix Pharmaceuticals Inc (catalog no EK 067-16). Intra-assay CV was 4%–6% and inter-assay CV was 8%–10%. The procedure was the same as described previously [7].

Quantification of plasma decorin was determined via Human Decorin DuoSet ELISA (R&D Systems, USA, catalogue no. DY143) and DuoSet ELISA Ancillary Reagent Kit (catalogue no. DY008) according to the manufacturer´s protocol.

Serum HSP27 was evaluated using a Cloud-Clone Corp. ELISA kit (USA). The minimum detection limits were $0.31 \, ng \cdot mL^{-1}$ and the intra-assay and inter-assay CV were <10% and <12% respectively.

Glucose level was assessed using the Cobas 6000 analyser. To evaluate insulin values the immunoassay kit from DiaMetra (catalogue no DKO076) was used. The within intra-assay CV was ≤5% and the inter-assay CV was ≤10%.

Homoeostasis model assessment HOMA-IR (fasting serum insulin $\mu U \cdot mL^{-1} \times$ fasting plasma glucose $mmol \cdot L^{-1}/22.5$), was calculated [30].

2.5. Genetic research

Genetic methodology was conducted according to a protocol described previously by Zychowska et al. [31].

2.5.1. RNA isolation

During analysis 2 mL of blood was collected into the vacutainer tube with $EDTAK_3$ and used for mRNA extraction. Erythrocytes were lysed and discarded using RBCL buffer (A&A Biotechnology, Poland) and the obtained leukocytes were lysed using Fenozol (A&A Biotechnology, Poland). Further isolation of RNA was performed according to the methodology described by Chomczynski and Sacchi [32].

2.5.2. Reverse transcription and quantitative real-time polymerase chain reaction

Quality and quantity obtained RNA was analised using spectrophotometery (photometer, Eppendorf, BioPhotometer Plus, Germany), and 1000 ng of total RNA were used to reverse transcription (Transcriptor First Strand cDNA Synthesis Kit, Roche, Poland).

To quantitative real-time PCR (qRT-PCR) 10-fold diluted cDNA was applied. To amplify tested genes, a reaction mix containing the following was used: 5 μL polymerase (LightCycler polymerase; Roche, Poland), 0.4 μL reverse and forward primers and 2.2 μL H_2O for each reaction. The thermal profile was used according to the manufacturer's instructions. qRT-PCR was applied using AriaMx real-time PCR System (Agilent, Department Poland) and amplification of tested genes was performed in three replications for each sample. *DCN* (decorin) and *TNF-α* (tumor necrosis factor α) expression were checked using two different primer pairs.

For amplification of tested genes following the primers were used:

For *TUBB*: R:TCTGTCGGCTCCGCTCTGAGAT and F:ACTCCCGTTGTCCCAAGGCTCT

For *DCN*: F: AAGTTCCTGATGACCGCGACTT and

R: TTGCAGGTCTAGCAGAGTTGTG (F: GATGAGGCTTCTGGGATA

and R: CAATGCGTGAAGGTTCTT)

For *TNF-α*: F:GCCATTGGCCAGGAGGGC and

R:CGCCACCACGCTCTTCTG (F:TTCTCCTTCCTGATCGTGCA R:TACAGGCTTGTCACTCGG)

For *HSPB1*: F: AAGGATGGCGTGGTGGAGATCA and

R: GAGGAAACTTGGGTGGGGTCCA

Target genes expression were calculated to the expression of the reference gene *TUBB* according to the delta Ct method [33].

2.6. Statistical calculation

All measures were compiled in a spreadsheet for the analysis of parallel-group trials and the effects were interpreted using magnitude-based inferences [34]. All data were log-transformed to reduce bias arising from non-uniformity of the error. To improve precision of estimates, mean changes were adjusted to the overall mean of baseline in the CON and HICT groups. Baseline values were expressed in measurement units. Means of the observed and adjusted changes, standard deviations of the observed changes, and adjusted effects (differences in changes of the means and their confidence intervals) were back-transformed to percentages. Magnitudes of the effects were evaluated with the log-transformed data by standardizing with the standard deviation of the overall

baseline values. Threshold values for assessing magnitudes of standardised effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large respectively Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial or trivial as follows: * – possible, ** – likely, *** – very likely and **** – most likely [35].

3. Results

Overall, data obtained from thirty-three participants were evaluated. Table 1 presents the anthropometrical and physiological data. Although any significant changes were recorded in body composition, it is worth noting that the applied HICT programme reduced body fat content expressed in kg as well as in percentage of body mass. Women who completed 15 training sessions gained better results in FMS and were characterised by the elevated relative maximal oxygen consumption (VO_{2max}) compared to the baseline values (Table 1). Ratings of perceived exertion differed between groups. Among the HICT group values of RPE decreased from 18 ± 3 after the first to 16 ± 2 after the last training session, whereas in the CON group values remained unchanged (16 ± 2 and 16 ± 8, respectively).

The training programme tended to improve glucose homeostasis in the HICT group. The 15th units of HICT caused some decrease in resting glucose, insulin concentrations and diminished the HOMA-IR (insulin −20% CI: −42% to 10%; glucose −1% Cl: −11 to 10% and HOMA-IR −11% CI: −27% to 9%, respectively) as depicted in Fig. 1 which in fact only shows a slight difference among the two groups. Still, in the CON group those indicators remained unchanged or presented an opposite tendency.

The applied training programme induced a significant 34% ±45% rise of IGF-1, whereas in the CON group the decrease (−29% ±58%) of this protein was noted and the adjusted effect for this change was 87%, CI: 34% to 162%. This effect was firstly noted after single HICT, especially prominent 24 h post training. Furthermore, the ratio insulin to IGF-1 declined significantly (−21% ±54%) only in the HICT group (Fig. 1d). These alterations were independent of participants age, VO_{2max} and IR. The exercise-induced changes of myostatin in response to a single session of HICT were small, nonsignificant and registered only 24 h after effort. Still, the whole HICT program caused a decrease in resting serum myostatin (Fig. 2a). Interestingly, the drop in resting values of myostatin, recorded after 5 training weeks, was much more pronounced and significant among the MG than YG group (Fig. 2b). Based on diversification of HOMA-IR in the HICT group, the higher decline of myostatin was observed in women, who were classified at baseline as IR and the range of changes was higher compared to non-insulin-resistant group (nonIR) (Fig. 2c). Moreover, when considering individual values of relative oxygen uptake of women from the HICT group, a higher and significant decrease of myostatin was registered in participants with VO_{2max} below 28 mL·kg^{-1}·min^{-1} (Fig. 2d).

The intervention did not modify the resting concentration of decorin in either group (9.91 ± 1.9 ng·mL^{-1} and 9.44 ± 1.7 ng·mL^{-1} in HICT and CON group, respectively). Values recorded at rest as well after first and last training session

Table 1 – Characteristic of participants divided into groups.

	Group	Baseline	Adjusted change	Adjusted effect		
				Mean	CI	Inference
Anthropometric parameters						
BFM (kg)	CON	25.9 ± 10.5	4 ± 3%	−8%	−10 to −5%	Trivial
	HICT	20.5 ± 9.6	−4 ± 5%			
SMM (kg)	CON	26.4 ± 3.3	−1 ± 3%	2%	0 to 3%	Trivial
	HICT	26.6 ± 3.5	1 ± 3%			
PBF (%)	CON	33.9 ± 8.4	4 ± 3%	−7%	−9 to −5%	Small[*]
	HICT	28.6 ± 8.5	−3 ± 4%			
VFA (cm^3)	CON	110.9 ± 48.9	−1 ± 4%	0%	−3 to 3%	Trivial
	HICT	80.8 ± 35.4	−2 ± 7%			
Physiological performance						
VO$_2$max	CON	30.2 ± 8.7	5 ± 5%	15%	10 to 20%	Moderate[****]
(mL·kg^{-1}·min^{-1})	HICT	28.6 ± 5.1	20 ± 9%			
AP	CON	2.03 ± 0.6	−1 ± 10%	9%	2 to 16%	Small[**]
(W·kg^{-1})	HICT	2.25 ± 0.5	8 ± 12%			
FMS	CON	13.5 ± 2.1	6 ± 9%	6%	1 to 11%	Small[**]
	HICT	14.9 ± 1.5	12 ± 5%			

HICT – high intensity circuit training group, CON – control group, BFM – body fat mass, SMM – skeletal muscle mass, PBF – percentage body fat, VFA – visceral fat area, VO$_{2max}$ – maximal oxygen uptake, AP – aerobic power, FMS – functional movement screen. Asterisks indicate effects clear at the 5% level (90% CL) and likelihood that the true effect is substantial or trivial. as follows: * possible, ** likely, **** most likely. Magnitude thresholds (for difference in means divided by SD of HICT group): <0.20, trivial; 0.20–0.59, small; 0.60–1.19, moderate; >1.20, large.
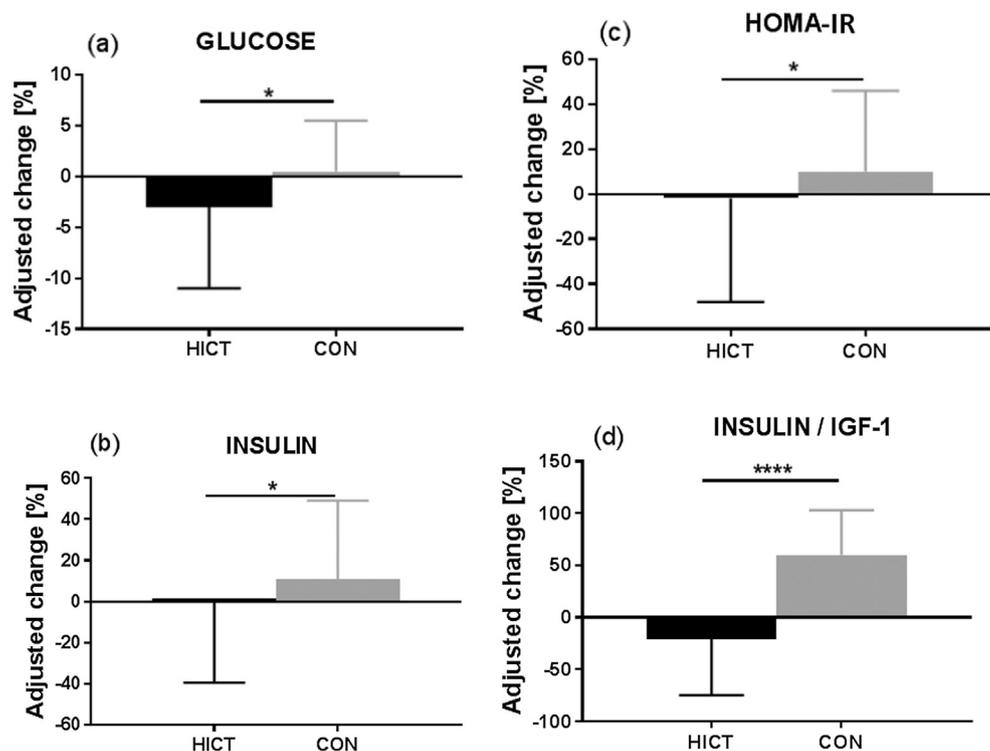

Fig. 1 – Adjusted changes in indicators of glucose homeostasis in response to 5 week high- intensity circuit training in high-intensity interval in group (HICT) and control group (CON): (a) Glucose level. (b) Insulin level. (c) the Homeostasis Model Assessment (HOMA-IR). (d) Insulin/IGF-1 ratio. Asterisks indicate effects clear at the 5% level and likelihood that the true effect is substantial: * – possible, ** – likely, *** – very likely, **** – most likely.

remained unchanged. On the other hand, the single HICT unit significantly altered IL-15 concentration, and this change was different among groups. The first HICT session involved a decrease of IL-15 in the HICT as well as in the CON group (Fig. 3a), whereas after the last HICT training session, concentration of IL-15 increased only in the HICT group with the tendency being opposite in the CON (24%, CI: 9% to 40%) (Fig. 3b). Notably, obtained changes were sustained 24 h post exercise.
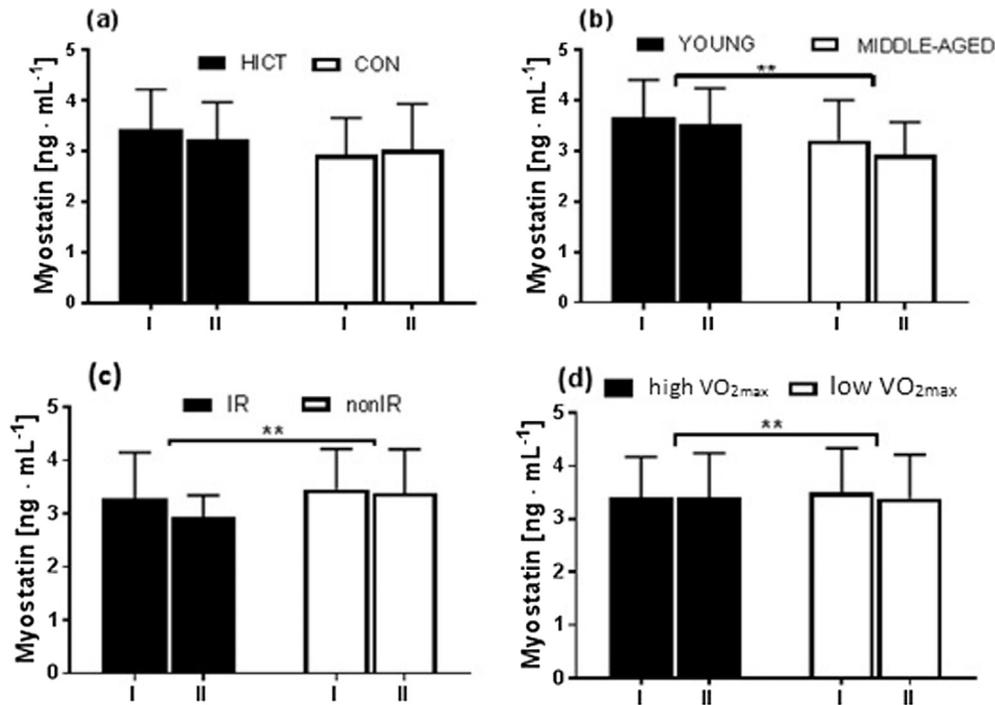
Fig. 2 – Changes in resting myostatin concentration recorded before (I) and after 5 weeks (II): (a) among trained (HICT) and control (CON) groups. (b) among young and middle-aged group. (c) among women with insulin resistance (IR) and without insulin resistance (nonIR). (d) among women with high and low relative oxygen uptake (VO$_{2max}$).
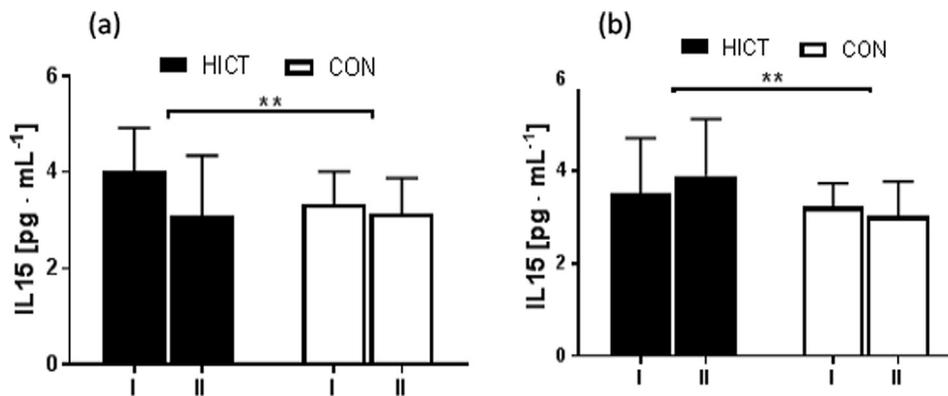

Fig. 3 – Comparison of shifts in circulating IL-15 between high-intensity interval training (HICT) and control group (CON). Values registered at rest (I) and 1 h after (II) single training: (a) First HICT session. (b) Last HICT session.

The first and last session of HICT caused a decline of irisin, especially visible 24 h after effort in the trained group. This range was also depended on age, IR and the level of relative oxygen uptake of women. The adjusted effect between YG and MG was −27%, CI: −52% to 10%. Interestingly, IR women from the HICT group had significantly lower baseline values of irisin (3.2 ± 2.58 ng·mL^{-1}) compared to those without IR (9.01 ± 8.44 ng·mL^{-1}). Moreover, shifts of irisin were dependent on oxygen uptake and the adjusted effect was 54%, CI: −7% to 54%. In women with higher fitness level a drop of irisin was noted (-13% ±40%), whereas in women with a VO$_{2max}$ below 28 mL·kg^{-1}·min^{-1} the adverse shift was noted (34% ±100%). This change was significant. After 5 weeks of HICT independently from the age and insulin resistance, resting

irisin concentration significantly correlated with HOMA-IR (r = 0.48, p = 0.03). At the same time point measurements of resting decorin in exercising women correlated with glucose (r = 0.46, p = 0.04). Similar to irisin, at the beginning of the intervention, this correlation was inverse. In the CON group the above described relationships were not presented.

Resting serum HSP27 values remained unchanged after 5 weeks, in both groups. Interestingly, the last HICT session contributed a significant increase in HSP27 in the HICT group (15% ±13%), whereas in the CON group the tendency was opposite (−28% ±42%). The adjusted effect was moderate and most likely (60%, CI: 29% to 99%). Still, those changes were most pronounced 24 h post training. To assess the whole-body response to the exercise, genes analyses in blood

cells were performed. Any significant differences were observed in *HSPB1* expression in response to intervention (Fig. 4a). Still, in HICT group the *HSPB1* mRNA was higher than in the CON group ($2^{0.59} \pm 0.50$ and $2^{0.24} \pm 0.19$, respectively). This effect was also visible at the transcriptional level of *HSPB1* mRNA, but these changes in leukocytes were insignificant. Low expression of pro-inflammatory cytokine *TNF-α* was detected in response to the first and last HICT session (Fig. 4c, 4d). Despite no significant changes and a low number of copies of *TNF-α* mRNA after the last HICT session, a slight average reduction of *TNF-α* mRNA (from $2^{0.06}$ to $2^{0.02}$ in HICT and from $2^{0.05}$ to $2^{0.02}$ in CON) was observed. However, large individual differences in *TNF-α* expression between individuals were noted. Additionally, *DCN* mRNA was assessed. Only below cut-off *DCN* mRNA was detected in leukocytes in all participants at every stage of the experiment (mean value for groups was 0.001 and in many participants no Qt was received).

4. Discussion

Our study shows that 15 sessions of HICT significantly improved glucose homeostasis, with the effect being accompanied by shifts in myokines. Although muscle mass did not change significantly, all indicators of characterised muscle sensitivity on insulin were ameliorated. The drop of glucose, insulin, and HOMA-IR was accompanied by a decrease of serum myostatin concentration. Notably, this alteration depended on age and IR at the baseline. Sharma et al. have pointed out that myostatin is associated with obesity and T2DM [36]. Data from an animal study indicated that inhibition of myostatin improves insulin sensitivity [9]. Moreover, in humans (advanced-aged women) there was reported elevation of serum myostatin, compared with younger subjects [37]. Thus, this observation may explain the potential role of

increased myostatin concentration, occurring along with ageing, in muscle-wasting processes. Neither Peng et al. has not proven myostatin age-related dependence in elderly women [38]. According to this myokine secretion during exercise, Hittel et al. have claimed that in insulin-resistant men, 6 months of low intensity endurance training contributed to a decrease in the expression of and plasma myostatin concentration, with the effect correlating with an improvement of insulin sensitivity [39]. In our experiment, 15 units of the applied training programme, using subjects' body weight as a workload, contributed to a decrease of myostatin. This effect was mostly visible among the women characterised by higher age, IR and lower VO2max. This response may suggest the anti-inflammatory effect of the applied procedure. Moreover, the lower expression of *TNF-α* can support this reasoning.

The action of myostatin can be modified by IGF-1 and irisin concentration [9,40]. Recent studies have shown that IGF-1 stimulated beneficial glucose metabolism and its level increased post-exercise [41–43]. Gregory et al. have shown that 8 weeks of resistance training (3 RM to 12 RM, 90–180 s rest between sets) caused the level of IGF-1 to rise in young women, whereas no similar effect occurred in participants who attended endurance training instead [41]. In our study, based on using body weight as a workload, 15 units of HICT caused a significant elevation of IGF-1. This rise was accompanied by an improvement of glucose and HOMA-IR, with such a beneficial effect absent in the CON group. Until now, the assessment of IGF-1 in glucose homeostasis has been focused on patients with type 1 diabetes [42,43]. A reduction of physical activity induced IR [44] and contributed to T2DM development [10]. Thus, the pursuit of effective preventative methods is justified. Furthermore, we noted a statistically significant decline of the insulin/IGF-1 ratio after completion of the training programme. To our knowledge, the decrease of this ratio reported in this study is to the first observed in
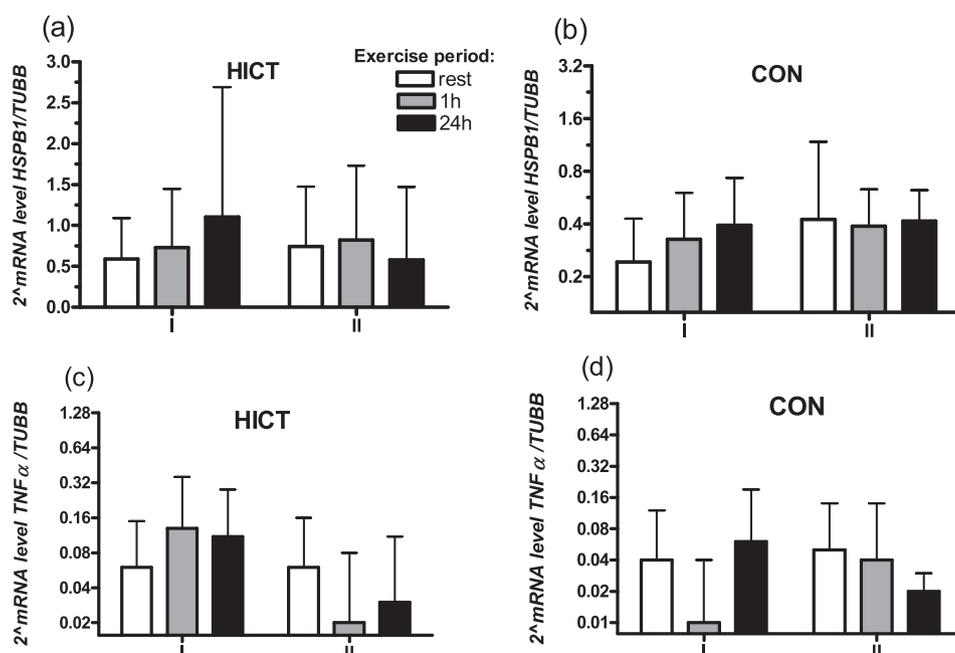

Fig. 4 – Relative expression of genes *HSPB1* and *TNF-α* in response to first (I) and last (II) of HICT session: (a) *HSPB1* expression in HICT group. (b) *HSPB1* expression in CON group. (c) *TNF-α* expression in HICT group. (d) *TNF-α* expression in CON group.

human subjects. Most available data about insulin/IGF-1 signalling are based on animal studies [45]. Thus, further investigations are needed to verify our results.

Together with IGF-1, another myokine, irisin, is the link between myostatin and glucose metabolism [9]. Recent studies have demonstrated that irisin was not only involved in maintaining glucose homeostasis [19], but it could also regulate exercise-induced adaptation [46]. Results obtained in our experiment appear ambiguous. Still, both the first and the last training session caused a drop of irisin in both groups and changes recorded after the last session of training were significant only in the HICT group. The small decrease of irisin was noted among training women with a higher level expressed in $VO_{2max,}$ which suggests applied training is insufficient for inducing adaptive changes in women who had presumably better cardiovascular fitness and higher muscle capacity. It might suggest that active women have lower circulating irisin concentration due to muscle adaptation process. On the other hand, obtained results revealed that decreased irisin was characteristic at the baseline for subjects with IR and the HICT program did not affect its circulating concentration. Nonetheless, irisin values correlated significantly and positively with HOMA-IR results. Previously published data indicated that differences in HOMA-IR are age-and sex-dependent in subjects, who do not suffer from diabetes. However, the HOMA-IR threshold is not clearly established, and the consideration is needed in relationship to the population. Following Gayoso-Diz et al. averaged smooth HOMA-IR on 75 percentile for Caucasian women aged 30–39 years is 2.36 [47] and this value was considered as a cut-off level in our study. To validate the accuracy of our results, we have also checked individual shifts in analysed blood proteins. It transpired that in women with IR, who attended 15 HICT units, there was a significant correlation between insulin and irisin level.

Modulations of glucose metabolism are known to be connected with circulating irisin [19]. It is worth establishing that irisin modulations induced by training might be crucial in the prevention of many metabolic disease due to the fact that irisin is a well-known metabolic biomarker and even a detector of breast cancer [48]. According to the latest data, patients with T2DM exhibited lower levels of irisin [49]. This condition may be modified by lifestyle habits such as physical activity [19]. Thus, a drop of HOMA-IR and irisin in women might have had an influence on an improvement of tissue' insulin sensitivity via better muscle endocrine function promoting glucose uptake after the applied training. So far, the tissue mechanism of irisin uptake remains unknown. Loffer et al have recorded an increase of irisin, detectable only immediately after the exercise. It dissipated considerably within 30 min time following the exercise, suggesting a growing uptake of this myokine. According to the results presented by Loffer et al., we cannot rule out a possibility that the training period was too short to achieve significant resting rise of irisin [50].

Previous studies have demonstrated that irisin, myostatin and IL-15 are all exercise-modulated myokines that participate in the regulation of glucose metabolism [51,52]. While Nadeau and Aguer have reported that IL-15 treatment protected against the development of IR in rodents, the link between circulating IL-15 levels and IR in humans remains

unclear [51]. Some data showed that circulating IL-15 levels increased after resistance exercise [53,54]. In our study, the first session of HICT induced a drop, while the last session induced a rise of IL-15 among women in the HICT group. This inconsistent response to exercise of IL-15 may have resulted from not completely understood secretion and regulation of IL-15, which can be released by several kinds of tissues and regulated by its receptor isoforms in diverse responsible [11]. Still, elevated circulating IL-15 is considered to be a response to muscle contraction [51].

Moreover, our study verifies if HICT sessions may have induced changes of HSP concentration and expression. Exercise stimulates an increase of HSP expression and this response may contribute to beneficial metabolic effects in insulin-resistant tissues [21]. In the current experiment, increased HSP27 concentration was recorded in the HICT group after the whole HICT programme along with the higher expression in leukocytes. In contrast, serum HSP27 was observed to be significantly lower in subjects with T2DM compared to normal glucose tolerance [55]. It has also been postulated that changes in the expression of *HSPB1* in leukocytes can be associated with the intensity and duration of exercise [56]. However, high stability of *HSPB1* mRNA was noted after moderate exercise [57].

To the best of our knowledge our study is the first, to investigate the effect of high intensity circuit training protocol on decorin secretion and blood cells *DCN* expression in women. Available data showed that a single resistance training session caused an elevation of circulating decorin and an increase of the muscle expression of *DCN* in response to 12 weeks of combined resistance and endurance training in T2DM men [14]. On the other hand, previously published data revealed that decorin might be a good marker of pathophysiology of breast cancer [13] or obesity and accompanying its IR [58]. The higher expression of *DCN* was documented only in chronic lymphocytic leukaemia patients [59]. This discrepancy might be caused by different time points of blood collection and diverse physical workload of exercise. In our study blood samples were collected 1 h and 24 h after training and women performed exercise without extra weights, whereas among T2DM men, who performed strength training with an 8 RM load blood samples were taken during 3 set of exercise, directly after training and every half hour to 2 h after exercise. Thus, further investigations are needed, to assess the appropriative mode, frequency and duration of exercise to find out the best stimuli to elevate/or diminish decorin concentration among women. Moreover, the optimal time point of blood collection is required to determine to assess the decorin changes because data are inconsistent.

A few limitations of this study must to be mentioned. Firstly, women from the HICT group were allowed to train at home, with the indirectly supervision. Secondly, the number of participants might have impacted on the statistical power and disturbed detection of significant differences between the groups, hence further investigations are needed.

To summarize, 15 units of HICT led to an improved glucose homeostasis via a reduction of resting glucose concentration, insulin level and HOMA-IR. Induced changes were modified by myostatin, IGF-1 and irisin shifts. Myostatin dropped mainly in older women and especially among those with IR

and low cardiorespiratory fitness at the baseline. Moreover, the insulin/IGF-1 ratio decreased after the applied training program, suggesting that it may be a good predictor for future investigations of any improvements of insulin sensitivity mechanism after training. Consequently, HICT programme with own body weight as a workload can be considered a time-efficient and health-promoting form of physical activity.

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Declaration of Competing Interest

No conflict of interests regarding the publication of this manuscript is declared by the authors.

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