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ORIGINAL ARTICLE

Transcriptomic modulation in response to high-intensity interval training in monocytes of older women with type 2 diabetes

Jovane Hamelin Morrissette[1] · Dominic Tremblay[2] · Alexis Marcotte-Chénard[1] · Farah Lizotte[2] · Marie A. Brunet[2,3] · Benoit Laurent[1,4] · Eléonor Riesco[1,5] · Pedro Geraldes[2,6]

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Abstract

Purpose Type 2 diabetes is associated with a higher risk of cardiovascular diseases, lowering the quality of life and increasing mortality rates of affected individuals. Circulating monocytes are tightly involved in the atherosclerosis process leading to cardiovascular diseases (CVD), and their inflammatory profile can be modified by exercise. The objective was to exploratory identify genes associated with CVD that could be regulated by high-intensity interval training (HIIT) in monocytes of type 2 diabetes patients.

Methods Next-generation RNA sequencing (RNA-seq) analyses were conducted on isolated circulating monocytes ($CD14^+$) of six women aged 60 and over with type 2 diabetes who completed a 12-week supervised HIIT intervention on a treadmill.

Results Following the intervention, a reduction of resting diastolic blood pressure was observed. Concomitant with this result, 56 genes were found to be downregulated following HIIT intervention in isolated monocytes. A large proportion of the regulated genes was involved in cellular adhesion, migration and differentiation into an "atherosclerosis-specific" macrophage phenotype.

Conclusion The downregulation of transcripts in monocytes globally suggests a favorable cardiovascular effect of the HIIT in older women with type 2 diabetes. In the context of precision medicine and personalized exercise prescription, shedding light on the fundamental mechanisms underlying HIIT effects on the gene profile of immune cells is essential to develop efficient nonpharmacological strategies to prevent CVD in high-risk population.

Keywords Cardiovascular diseases · HIIT · Inflammation · Monocytes · RNA-seq · Type 2 diabetes

Introduction

Type 2 diabetes (T2D) is a multifactorial pathology that lowers the health-related quality of life and increases mortality rates of affected individuals (Trikkalinou et al. 2017). T2D incidence steadily grows, and its complications are a major public health problem worldwide (Trikkalinou et al.

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Jovane Hamelin Morrissette and Dominic Tremblay have equally contributed to this work.

✉ Pedro Geraldes
pedro.geraldes@USherbrooke.ca

[1] Research Center on Aging, CIUSSS de l'Estrie-CHUS, Sherbrooke, QC J1H 4C4, Canada

[2] Faculty of Medicine and Health Sciences, Research Center of the Centre Hospitalier Universitaire de Sherbrooke (CHUS), 3001 12e Avenue N, Sherbrooke, QC J1H 5H3, Canada

[3] Medical Genetics Service, Department of Pediatrics, Université de Sherbrooke, Sherbrooke, QC, Canada

[4] Department of Biochemistry and Functional Genomics, Faculty of Medicine and Health Sciences, Université de Sherbrooke, Sherbrooke, QC J1H 5N4, Canada

[5] Faculty of Physical Activity Sciences, Université de Sherbrooke, Sherbrooke, QC J1K 2R1, Canada

[6] Division of Endocrinology, Department of Medicine, Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, QC J1H 5N4, Canada

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2017). For instance, insulin resistance and hyperglycemia are linked to vascular dysfunction and hypertension (Muniyappa and Sowers 2013), which directly contributes to the risk of developing cardiovascular diseases (CVD), in particular in women and the elderly population (Shalev et al. 2005). Although cholesterol-lowering molecules such as atorvastatin are the primary prevention treatment for CVD in T2D patients, it has been demonstrated that reducing chronic inflammation can also reduce the risk of atherothrombosis independently of lipid-lowering drugs (Ridker et al. 2017). Indeed, a controlled balance of the inflammatory state (anti- vs pro-inflammatory) is necessary to maintain cellular homeostasis, whereas chronic pro-inflammation condition is associated with physical inactivity, obesity, diabetes, and CVD (Roberto Carlos et al. 2020). The latter remains the leading cause of morbidity and premature mortality in industrialized countries (Townsend et al. 2016).

It is well recognized that chronic inflammation plays an important role in the pathophysiology of atherosclerosis (Libby et al. 2011). Inflammatory signals trigger vascular cell dysfunction and promote the recruitment of immune cells inside the arterial wall, which participate in the development of atherosclerotic plaque formation and complications (Arslan et al. 2017). As these monocytes/macrophages uptake cholesterol and become foam cells, they trigger the recruitment and activation of other immune cells such as T cells and sustain a chronic inflammation environment (Davis and Gallagher 2019). Monocytes are also involved in the initiation, progression, and manifestation of arterial hypertension (Rodriguez-Iturbe et al. 2017). In adipose tissue of obese patients, immune cell infiltration promotes the systemic release of inflammatory cytokines and chemokines. Elevated levels of these cytokines and chemokines have been shown to correlate with insulin resistance (Davis and Gallagher 2019). Therefore, any non-pharmacological therapeutic strategies that could modulate monocyte inflammatory state could represent an interesting avenue to prevent hypertension and CVD (Wenzel 2019), especially in older adults with chronic disease potentially dealing with polypharmacy.

Lifestyle modifications are the first line of treatment for T2D. In particular, physical exercise has been shown to effectively improve glycemic control (Winding et al. 2018), reduce blood pressure (Cornelissen and Smart 2013) and prevent CVD (Kubota et al. 2017). Interestingly, the protective actions of exercise against inflammatory diseases, particularly CVD, have been associated with an anti-inflammatory effect on monocytes (Dimitrov et al. 2017). Although different exercise training programs have been shown to be favorable, high-intensity interval training (HIIT) has the advantage to maximize cardiovascular fitness benefits (Ross et al. 2016), while also improving vascular health markers (Schjerve et al. 2008) and ambulatory blood pressure over 24 h (Molmen-Hansen et al. 2012). In addition to being a less time-consuming exercise modality, HIIT protocol could be more effective at controlling the glycemia of T2D patients than continuous exercise training (Terada et al. 2016). Moreover, in older adults, HIIT can shift immune cells toward an anti-inflammatory phenotype (Bartlett et al. 2018). In this context, it is not surprising that organizations such as Diabetes Canada recommend 150 min of aerobic exercises per week, including HIIT for the prevention and management of T2D (Sigal et al. 2018).

Although the HIIT intervention could be a valuable treatment modality for T2D patients, the mechanism of action on the monocyte inflammatory state is only partially understood. The leading hypothesis of this study was that the HIIT intervention may reduce blood pressure by regulating atherosclerosis-associated processes, in particular by altering the inflammatory profile of monocytes from T2D older women. In this context, the objective was to uncover the molecular effects of a 12-week HIIT intervention on the transcriptomic gene expression profiling of circulating monocytes in older women with T2D.

Methods

Study design

The data presented in this manuscript are the results of secondary analyses of a randomized study with two parallel groups conducted between January 2016 and September 2019 (Marcotte-Chénard et al. 2021; Marcotte-Chenard et al. 2021). The study was conducted according to the guidelines laid down in the Declaration of Helsinki and approved by the Research Ethics Committee of the CIUSSS de l'Estrie-CHUS.

Study protocol and participants

The recruitment of participants was conducted using the platform of the Research Center on Aging (CdRV), the geriatric diabetes clinic of the University of Sherbrooke Institute, the Estrie diabetes day center (Centre Hospitalier Universitaire de Sherbrooke) or through advertisements in local media and community organizations (Sercovie, Diabète Estrie). Inclusion criteria: women with diagnosed T2D aged between 60 and 85 years old, non-smoker, light drinker (≤ 7 alcoholic beverages per week), and physically inactive (< 75 min of structured exercise per week for the past year). Exclusion criteria: women taking hormone replacement therapy, insulin therapy, having unstable medication for the past 6 months, uncontrolled hypertension ($> 160/90$ mmHg), uncontrolled lipid profile (total cholesterol > 8 mmol/L, triglycerides > 10 mmol/L and LDL-C > 4 mmol/L), unstable weight (± 2.27 kg) in the past 6 months, physical incapacity

limiting the practice of physical activity, known diabetic complications (nephropathy; retinopathy; neuropathy), a surgery planned during the intervention or coronary artery disease without revascularization, or other vascular diseases. A total of six participants were selected based on the mean metabolic profile (A1c, fasting glucose, fasting insulin, HOMA2-IR).

Exercise intervention

For 12 weeks, participants performed three sessions per week on a treadmill (Life Fitness, Club Series, FlexDeck®, Illinois, USA), one hour after their usual breakfast. All exercise sessions were performed under the supervision of an exercise physiologist at the Research Centre on Aging training facility. The HIIT program consisted of a 3-min warm-up (2 min at 55% HR reserve (HRR) and 1 min at 75% HRR) followed by six 1-min intervals at 90% HRR with active 2-min recovery at 45% HRR and a 2-min cooldown at 40% HRR (25 min/session including 6 min at high intensity/session). A 4-week adaptation period was performed to achieve the prescribed intensity. To reach the target intensity, speed and slope were adjusted in accordance with the participant preference, but the slope was limited to 6%. The treadmill speed and incline were set 15 s before the beginning of each interval to make sure that the participant had 60 s at the targeted mechanical intensity. If the HRR was not reached during the 60 s, the speed or incline was adjusted for the next interval. Participants received verbal encouragement throughout the exercise session. Blood pressure was measured after a 5-min rest in a sitting position before the exercise session using a manual sphygmomanometer (ADC Diagnostix 703, American Diagnostic Corporation, New York, USA). Participants were instructed to report any issues during and after the training session and adverse events related to exercise were documented by an exercise physiologist.

Dietary habits and nutritional recommendations

Each participant had an individualized consultation with a nutritionist prior to the intervention. The objective of the meeting was to remind the participants about the nutritional guidelines of Diabetes Canada. The dietician focused on a low glycemic index diet considering that this approach does not require any drastic changes and has no marked side effects (Sievenpiper et al. 2018).

Monocyte isolation

Fasting blood samples (pre and post-exercise training intervention) from one EDTA tube per participant were used for $CD14^+$ cell isolation. Post-intervention blood samples were drawn at least 72 h (3 days) and at most 120 h (5 days) after the last bout of exercise. This timing was of utmost importance to ensure we were not measuring the acute effects of the last exercise bout, but rather the chronic effects of the 12-week intervention. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood using Histopaque-1077 reagent (Sigma, USA) and monocytes were positively selected using CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and columns (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocyte pellet was homogenized in 1 mL of Tri Reagent (BioShop Canada, Canada) and stored at − 80 °C.

RNA isolation

Total RNA was extracted using the phenol–chloroform method and RNA quantity was assessed with a NanoDrop 2000 instrument (Thermo Fisher Scientific, Wilmington, Delaware, USA) as previously described (Lizotte et al. 2016). Isolation of mRNA from total RNA was performed using the magnetic Dynabeads mRNA DIRECT Kit according to the manufacturer's protocol (Life Technologies, USA). NEBNext Ultra II Directional RNA Library Prep Kit for Illumina kit (New England Biolabs, USA) was used for the RNA-seq library construction according to the manufacturer's protocol and as previously done (Iberg-Badeaux et al. 2017). For PCR amplification, Phusion High-Fidelity PCR Master Mix (New England Biolabs; USA), Multiplexing PCR Primer 1.0 (Illumina, USA) and ScriptSeq PCR index primers (Illumina, USA) were used. DNA quantification Qubit fluorometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) was used for DNA quantification. For quality assessment, a microfluidic electrophoresis was done with Agilent 2100 Bioanalyser (Agilent Technologies, Santa Clara, California, USA) using Agilent High Sensitivity DNA Kit protocol (Agilent Technologies, USA). Library sequencing was performed on Illumina NextSeq 500 instrument to a depth of ~ 20–60 million pass-filter reads per library at the RNomics platform of Université de Sherbrooke. Data quality was assessed, and sequences were aligned on the human genome.

RNA-seq analysis

The quality of fastq files was assessed using FastQC (v 0.11.4) and trimmed using TrimGalore (v 0.6.4) with a Phred score threshold set at 20, a trim of 3 bp from the 5′ and 3′ end to avoid poor qualities or biases, and a maximum of *N* set at 5 per read. Thus, adaptors and low-quality reads were removed. Reads were then aligned to the human genome build hg38 using the annotation file from Gencode (v32) with the STAR software (v 2.7.3a) using default parameters and the following options: --outFilterMultimapNmax 10

--outSAMprimaryFlag AllBestScore --outFilterMismatchNmax 5. Aligned reads were then assigned to genes using the featureCounts algorithm from the Subread package (v 2.0.0). The parameters for featureCounts were set as follows: -C -M --fraction -Q 15 -g gene_id -t exon --fracOverlap 0.25 --largestOverlap --minOverlap 15. Differential expression analysis was conducted in an R environment (version 3.5.3) using the DeSeq2 package (version 1.8.2) with a FDR set at 5%. GSEA analyses were performed using the open-source GSEA software (v 2.0, Broad Institute, MA, USA). Figures were produced using in-house python scripts (v 3.7.3).

Gene expression analysis by qPCR

To confirm changes in expression uncovered by RNA-seq, individual gene expression was quantified by qPCR as previously described (Lizotte et al. 2016). The genes selected for qPCR were based on their association with cardiovascular diseases such as genes related to monocyte/cell adhesion, inflammation and immunology. RNA was reverse transcribed using Superscript IV reverse transcriptase kit (ThermoFisher Scientific, USA). Real-time PCR was performed to evaluate mRNA expression of genes of interest in isolated monocytes. PCR primers are listed in Table S1 (available at https://figshare.com/s/0a18f9457c67146b7fd5). GAPDH expression was used for normalization.

Statistical analysis

Statistical analyses were performed with SPSS (version 26, IBM SPSS Statistics, Chicago, IL, US) and RStudio (version 1.1.414, RStudio Inc., Boston, MA, US) unless stated otherwise. Basic characteristics and clinical variables are presented as median (interquartile range). Systolic and diastolic blood pressure before and after the intervention were analyzed using the Wilcoxon's test. Unpaired Mann–Whitney tests were used on qPCR data to compare before- and after-intervention samples.

Results

Participant characteristics

This project is an exploratory analysis of a randomized study recently published (Marcotte-Chénard et al. 2021). Characteristics of the 6 randomly recruited participants for transcriptomic analyses are presented in Table 1. All participant

Table 1 Participants baseline characteristics and variation post training

	Subsample ($N=6$)	Variation	p
Age	66.2 [63.0–70.3]	–	–
T2D duration (years)	7.5 [2.4–17.5]	–	–
Weight (kg)	75.7 [71.5–113.8]	−0.40 (4.90)	0.387
BMI (kg/m^2)	31.2 [28.2–44.5]	0.12 (1.80)	0.192
Lean body mass (kg)	42.3 [40.3–48.5]	–	–
Fat mass (kg)	29.1 [26.9–45.4]	–	–
A1C (%)	6.3 [5.9–7.7]	−0.10 (0.60)	0.624
Fasting glucose (mmol/L)	6.7 [5.7–7.7]	0.00 (1.80)	0.352
Fasting insulin (mIU/L)	71.5 [29.8–217.5]	23.0 (32.0)	0.018
Total cholesterol (mmol/L)	4.12 [3.9–4.22]	0.02 ± 0.31	0.970
HDL-cholesterol (mmol/L)	1.04 [0.99–1.60]	0.02 ± 0.12	0.931
LDL-cholesterol (mmol/L)	2.39 [1.90–2.51]	−0.03 ± 0.27	0.984
TG (mmol/L)	1.18 [0.68–1.61]	0.02 ± 0.12	0.599
Systolic BP (mmHg)	122[114–139]	−12.4 ± 9.8	0.324
Diastolic BP (mmHg)	77 [74–79]	−7.7 ± 6.8	0.490
Medication			
Nb of medication	6.5 [4.0–9.8]	–	–
Glucose lowering medication n (%)	6 (100)	–	–
Hypotensive medication n (%)	6 (100)	–	–
Lipid-lowering medication n (%)	5 (83)	–	–

Data are presented as median [interquartile range], N (%) for the medication and variation (and p value) between pre and post intervention

T2D type 2 diabetes, *BMI* body mass index, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *BP* blood pressure

characteristics were normally distributed, except for plasma total cholesterol concentration.

Change in resting systolic and diastolic blood pressure following the HIIT intervention

Although the sample size is small ($n=6$), there was a statistically significant reduction in resting diastolic blood pressure of the participants initially chosen to be included in the transcriptomic analysis (76.5 [74.00–79.25] mmHg to 73.00 [69.00–76.25] mmHg; $p=0.024$). A reduction of systolic blood pressure was observed in the full sample ($n=14$; $p=0.011$ data not shown), although a mild change was observed in this subsample (121.5 [113.75–138.5] mmHg to 120.5 [115.5–128.25] mmHg; $p=0.686$).

RNA-Seq analysis revealed 56 negatively regulated, CVD-associated genes with the intervention

Six samples were analyzed by RNA-seq. Samples of four participants were paired with pre- and post-intervention. Unfortunately, because of the limited amount of isolated RNA, pre-intervention from one participant and post-intervention from another participant were not examined.

The RNA-seq analysis revealed that the expression of 56 genes was negatively regulated by the HIIT intervention and surprisingly none were upregulated (Fig. 1). Change in expression, gene function and the potential link with CVD are presented in Table S2 (available at https://figshare.com/s/0a18f9457c67146b7fd5). A considerable number of these genes are well characterized in the non-monocytic cell types, often of hematopoietic origins (i.e., T cells, NK, dendritic cells or eosinophils). Noteworthy, 4 identified genes (CHRM3 antisense RNA 2, *CHRM3-AS2*; Long Intergenic Non-Protein Coding RNA 861, *LINC00861*; PRKCQ antisense RNA 1, *PRKCQ-AS1*; Myocardial infarction associated transcript, *MIAT*) produce non-coding long RNAs, three of which have unknown function.

Differentially expressed genes were regrouped according to similarities in their expression patterns and function (Fig. 2). Several genes are associated with T cell activation and differentiation pathways and a large cluster of them are related to TCR signaling (*CD247/CD3ζ, CD3G, CD3D, TRAC, LCK, ITK, ZAP70, GRAP2, NFAT2, CD28,*

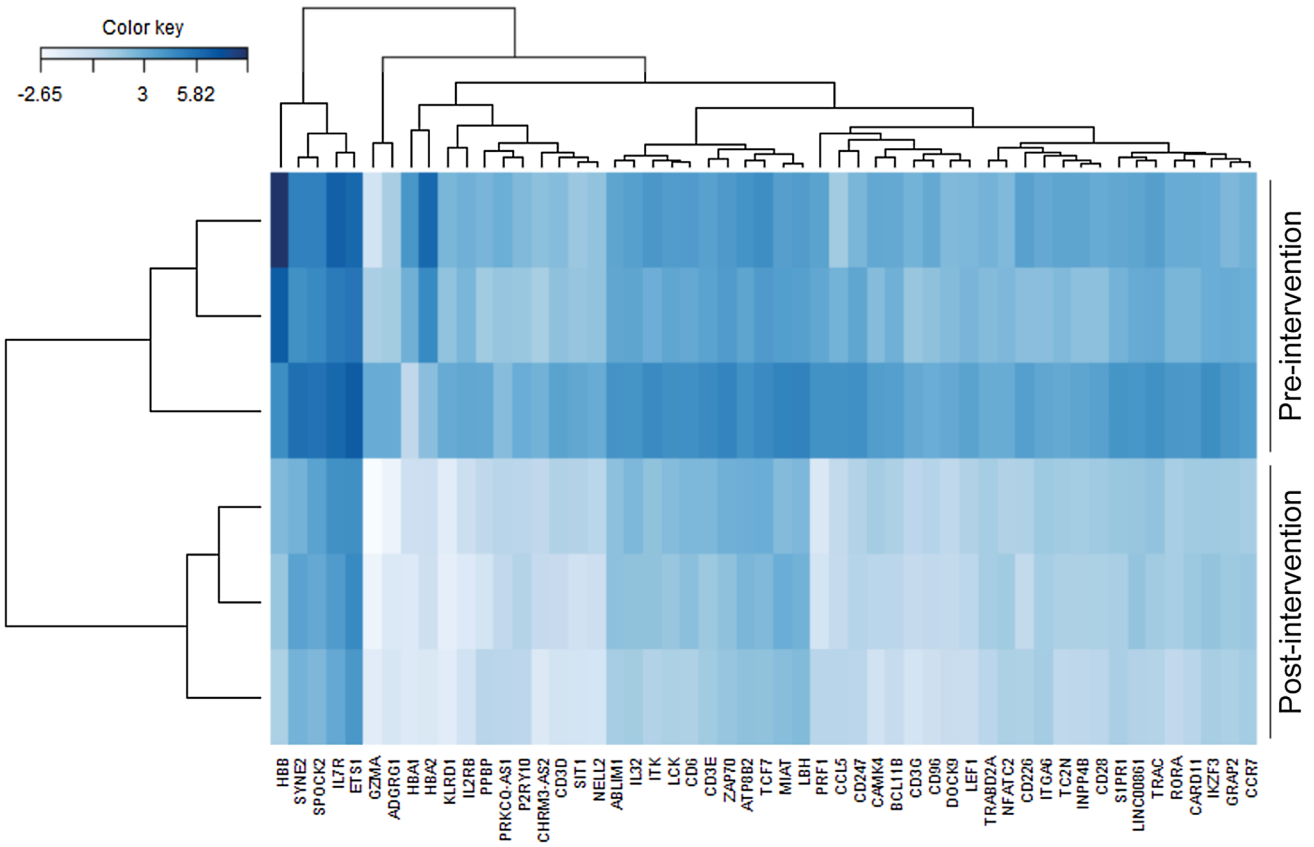

Fig. 1 Heatmap of differential gene expression before and after HIIT intervention. Numbers in legend correspond to the binary log counts per million (logCPM), representing the ratio of gene-specific aligned transcripts over the total number of sequenced transcripts in a sample, multiplied by a million. Raw data for logCPM are presented in Table S1

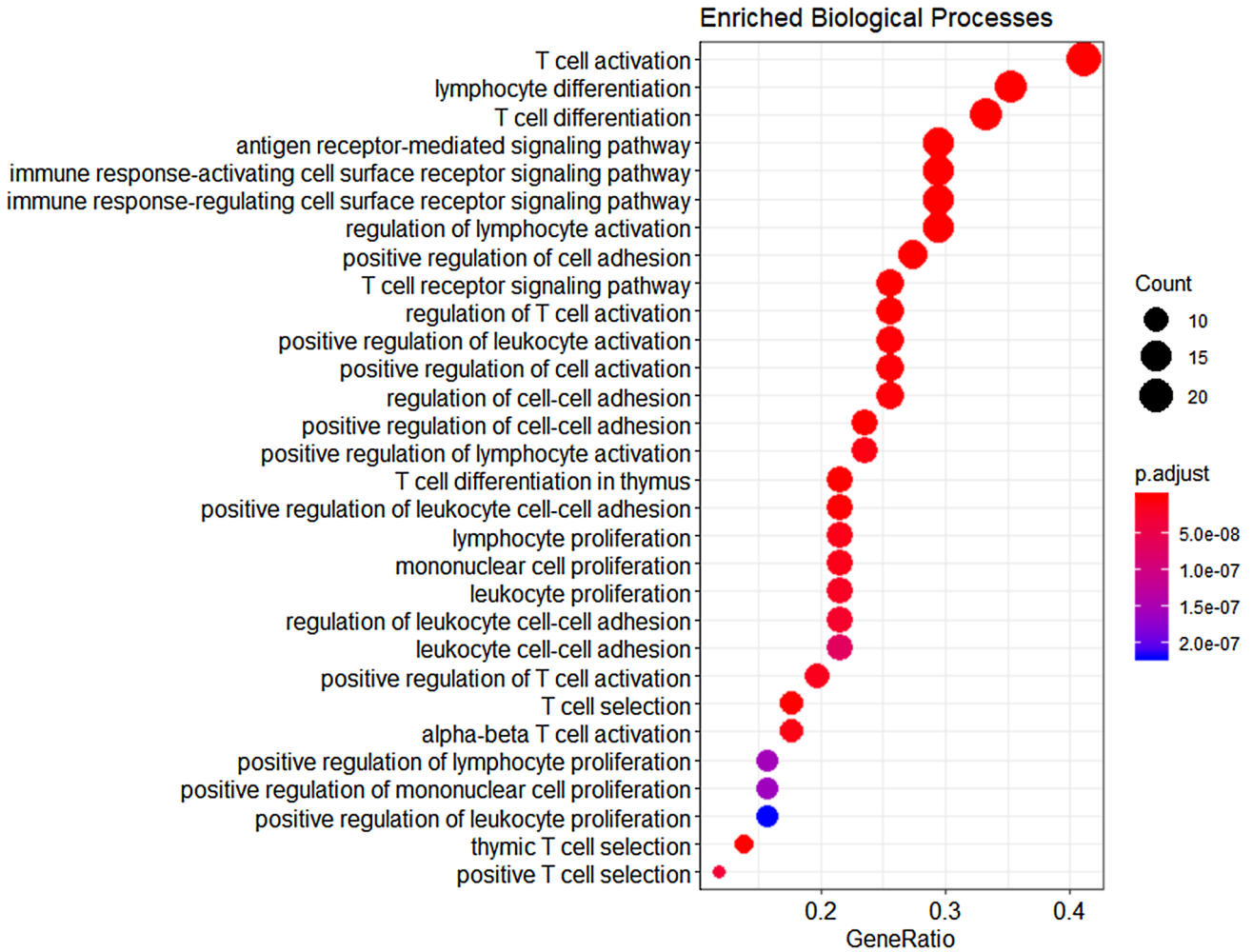

Fig. 2 Graphical representation of differentially expressed genes. The vertical axis lists the names of the categories of pathways/functions (*MSigDB* database), and the horizontal axis shows the proportion of annotated genes in each category versus the total number of annotated genes. Dots diameter represents the number of individual genes associated with each of the listed function. *p* values were adjusted with Benjamini–Hochberg post-hoc test (FDR 5%)

SIT1, CARD11/CARMA1). To help the comparison of our data with existing literature, KEGG database annotations are presented separately in Table 2. Similarl to the annotations presented in Fig. 2, most of the regulated genes have functions that are associated with immune cell activation as well as pathologies and conditions with a major immune component such as measles, malaria and infection to human T-lymphotropic virus.

qPCR gene expression analysis on 10 chosen samples confirmed the RNA-Seq data

To validate RNA-Seq data, quantitative PCR was performed using samples pre- and post-intervention of five participants ($n=10$) of the six participants' subgroup. Mean fold changes of eight selected genes were evaluated and their expression was decreased by HIIT intervention (Fig. 3). Three of them were significantly reduced (*HBB*, −90.8%, $p=0.019$; *HBA2*, −92.2%, $p=0.007$; *CD247*, −28.9%, $p=0.047$; Fig. 3A–C) and two others showed a trend toward significance (*ZAP*, −47.6%, $p=0.075$; *CD226*, −27,1%, $p=0.075$; Fig. 3D, E). In addition, there was an average reduction of 51.1%, 36.5% and 51% for *CCL5*, *IL-2RB* and *PPBP*, respectively, but the changes were not statistically significant, due to individual variability and low sample size (Fig. 3F–H).

Discussion

The objective of this study was to evaluate for the first time the effect of a 12-week HIIT intervention on the circulating monocyte transcriptome in older women with T2D. We hypothesized that the intervention would lower the expression of genes associated with atherosclerosis development

Table 2 KEGG of differentially expressed genes

KEGG annotation	Number of genes	$p^{\dagger}$	Ajusted $p^{\ddagger}$
T cell receptor signaling pathway	12	1.6×10^{-12}	1.6×10^{-10}
Primary immunodeficiency	5	2.6×10^{-5}	1.3×10^{-3}
Natural killer cell mediated cytotoxicity	7	3.5×10^{-5}	1.2×10^{-3}
Hematopoietic cell lineage	5	1.0×10^{-3}	2.6×10^{-2}
NF-kappa B signaling pathway	5	1.0×10^{-3}	2.6×10^{-2}
HTLV-I infection	7	1.8×10^{-3}	3.6×10^{-2}
Chagas disease	5	2.0×10^{-3}	3.3×10^{-2}
Measles	5	4.9×10^{-3}	6.8×10^{-2}
African trypanosomiasis	3	1.3×10^{-2}	1.5×10^{-1}
Malaria	3	2.7×10^{-2}	2.6×10^{-1}
Cytokine-cytokine receptor interaction	5	3.7×10^{-2}	3.2×10^{-1}
Cell adhesion molecules (CAMs)	4	3.7×10^{-2}	3.0×10^{-1}
Colorectal cancer	3	4.1×10^{-2}	3.0×10^{-1}
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	3	4.8×10^{-2}	3.2×10^{-1}
Pathways in cancer	6	5.2×10^{-2}	3.2×10^{-1}
Chemokine signaling pathway	4	7.2×10^{-2}	4.0×10^{-1}
Prostate cancer	3	7.7×10^{-2}	4.0×10^{-1}

Analyses performed with DAVID Bioinformatics Resources (Huang et al. 2007)

KEGG Kyoto Encyclopedia of Genes and Genomes

†Fischer's modified exact test (EASE score) was used to compare the proportion of annotated genes in each pathway/function versus the total number of annotated genes. $^{\ddagger}p$ values were adjusted with Benjamini–Hochberg post-hoc test (FDR 5%)

(i.e., inflammatory response, cellular adhesion, oxidative stress, macrophages polarization and lipid metabolism) in circulating monocytes of the studied population while reducing resting blood pressure. Transcriptome analysis was performed on circulating monocytes ($CD14^{+}$) of subgroup of 6 women who completed the study. We uncovered that 56 genes were negatively regulated and several of them are associated with atherosclerosis progression. Concomitant with the downregulation of those genes, a reduction of resting diastolic blood pressure was observed, associating the molecular signature with clinical outcomes. Although several hypotheses including the anti-inflammatory effect of β2 adrenergic stimulation (Galvez et al. 2019) and exercise-induced hemodynamic changes (Van Craenenbroeck et al. 2014) have been proposed to explain the cardiovascular benefits of exercise, the mechanism by which exercise influences monocyte function remains unclear.

Interestingly, all the genes identified in our study were negatively regulated by the intervention. This phenomenon has been previously reported in the literature. A study on the acute effect of one HIIT workout session found 33 over 35 genes to be downregulated in circulating monocytes (Wang et al. 2015). Other explanations for these unidirectional changes may be due to the adaptative effects of aerobic exercise and resistance training on PBMC activity as well as the influence of physical activity levels, dietary and sleep habits (Sakharov et al. 2012). Interestingly, other groups have shown that reduction of gene expression such as *CDKN2A/2B/2BAS* in leucocytes is associated with pro-inflammatory monocytes in T1D and T2D (Vinu et al. 2019; Martinez-Hervas et al. 2019). In addition, because the quantity of isolated circulating monocyte was variable among participants, some extracted RNA samples were not analyzed by RNA-seq. Therefore, differentially expressed genes may have not been identified due to the sample size and heterogenous response caused by physical exercise. However, despite the complexity of the innate immune system, our results appear to suggest that the HIIT intervention could shift a large proportion of the circulating monocyte population toward a less activated phenotype. We cannot exclude the possibility that diabetes and exercise intervention may upregulate genes that were not captured in our study.

Genes associated with monocyte migration and pro-inflammatory activation

The expression of genes associated with CD14+ cells adhesion and endothelial transmigration was negatively regulated by the HIIT intervention. Our data demonstrated that the chemokine (C–C motif) ligand 5 (*CCL5/RANTES*) gene, a leucocyte recruiting chemokine that acts similarly as CCL2/MCP-1 by recruiting immune cells from the peripheral blood to sites of inflammation, seems downregulated with the exercise training. It has been shown that CCL5 can promote the formation of atheroma in mice (Blin et al. 2019). Interestingly, the expression of CCL5 in the adipose tissue of adults living with obesity can be decreased following a mixed program of aerobic and resistance training for a 3-month period (Baturcam et al. 2014). To our knowledge, the present study is the first to suggest a reduction of the expression of *CCL5* in human blood monocytes following a physical exercise intervention.

Our exploratory results suggest that genes differentially regulated by HIIT are involved in monocyte proliferation, differentiation, and activation. Lymphoid enhancer-binding factor 1 (LEF1), a transcription factor classically activated by the Wnt-/β-catenin pathway, could influence the differentiation of monocytes. Borrell-Pagès and colleagues have shown that monocytes/macrophage activation of the LEF1 transcription factor is involved in LDL uptake and cell migration, two critical processes of atherosclerosis progression (Borrell-Pages et al. 2014).

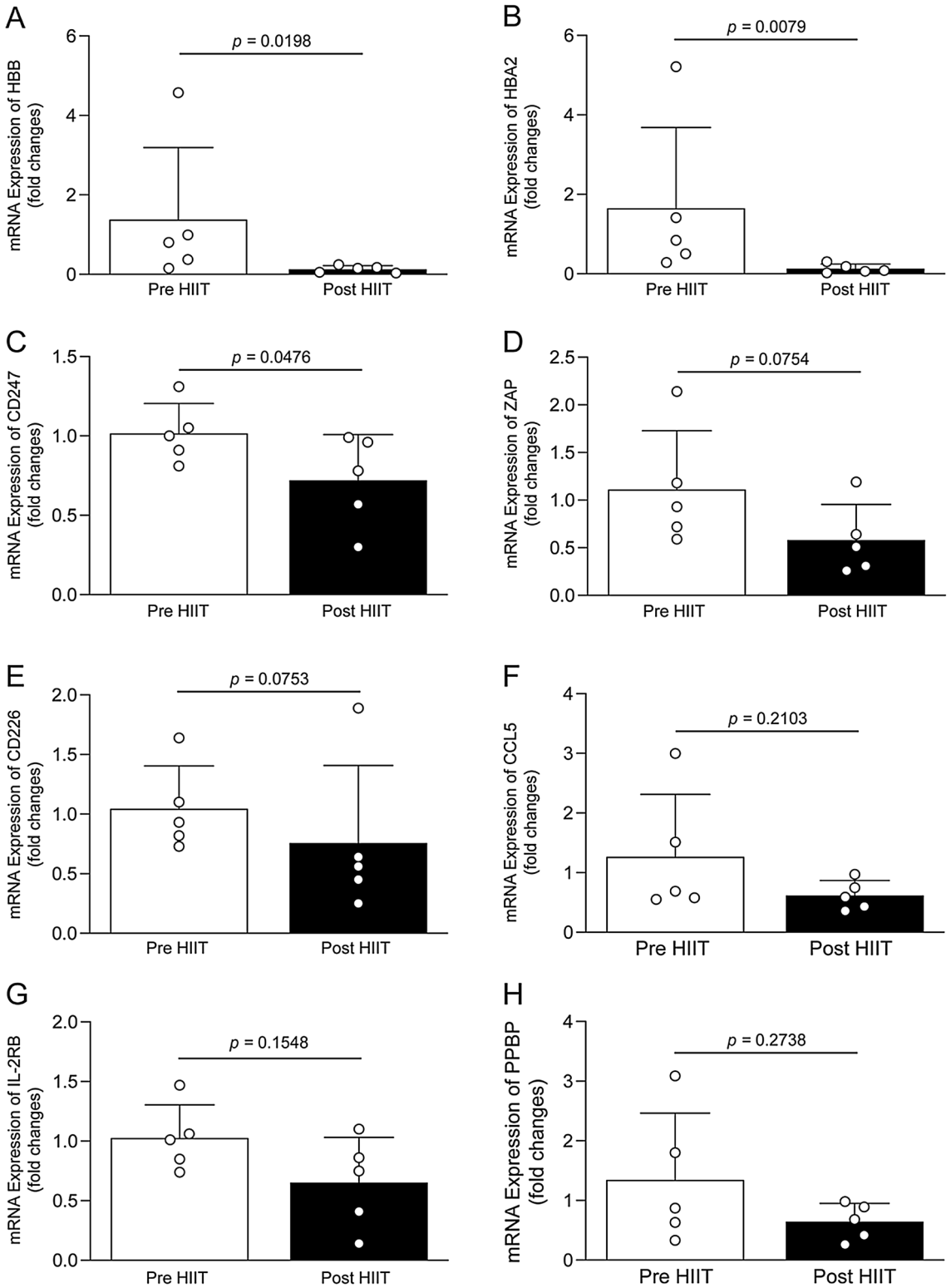

Fig. 3 Quantitative PCR analysis of selected genes. **A** Hemoglobin subunit beta (HBB), **B** hemoglobin subunit alpha 2 (HBA2), **C** cluster of differentiation 247 (CD247), **D** zeta-chain of T cell receptor-associated protein kinase 70 kDa (ZAP70), **E** cluster of differentiation 226 (CD226), **F** C–C motif chemokine ligand 5 (CCL5), **G** interleukin 2 receptor subunit beta (IL-2RB), **H** pro-platelet basic protein (PPBP) mRNA expression in monocytes of post-menopausal women prior and following a 12-week high-intensity interval training (HIIT). Results are shown as mean ± SD. *p* values represent unpaired Mann–Whitney tests

Genes with function associated with other immune cells

Although the monocyte isolation was confirmed by FASC, our RNAseq data have unexpectedly revealed that exercise training seems to modulate gene expression not typically associated with monocyte function. CD247/CD3ζ, CD3G and CD3D are transmembrane proteins part of the T-cell antigen receptor (TCR) that play in TCR signaling (Rudemiller et al. 2014). T cell receptor alpha constant (*TRAC*) gene expression may also be downregulated by the HIIT intervention in our study. In T cells, *TRAC* encodes for the alpha chain of the TCR, which is responsible for recognizing antigens bound to the major histocompatibility complex of other cells. Moreover, we have identified several genes that code for proteins in TCR signal transduction and regulation (*LCK, ITK, ZAP70, GRAP2, NFAT2, CD28, SIT1, CARD11*). Although these genes are associated with immunity, a previous study reported that ZAP70 is modulated in lymphocytes of endurance-trained athletes (Alack et al. 2020), suggesting that TCR signal transduction could be regulated by exercise. Whereas it has been accepted that the TCR complex expression is exclusive to T cells, the TCRαβ and molecules necessary for its signaling are expressed in circulating monocytes and monocyte-derived macrophages (Chavez-Galan et al. 2015). Leukocyte C-terminal Src kinase (*LCK*) mRNA has only recently been found to be expressed in the monocyte/macrophage lineage (Al-Mossawi et al. 2019). Although monocyte TCR signaling pathway is still unknown, a previous study reported that TCRαβ+ positive macrophages accumulate in the atherosclerotic lesions contributing to disease progression (Fuchs et al. 2015). Nevertheless, we cannot exclude the possibility that a very small number of T cells may have been included in our analysis, which may explain the presence of T cell-related genes.

Long coding RNAs

With the recent advancements in transcriptomics, the deregulation of long non-coding RNAs (lncRNAs) is associated with various human diseases, including cancers, neurological disorders and CVD (Sun et al. 2018). Numerous lncRNAs, including the myocardial infarction-associated transcript (MIAT), have been linked to the progression of atherosclerosis (Zhong et al. 2018). Of the four long non-coding RNAs that could be regulated by the HIIT intervention, only MIAT has a known function. However, genetic susceptibility to CVD can result in multiple common single nucleotide polymorphisms (SNPs), most of them in non-coding regions of the genome (Fiatal and Adany 2017). Therefore, the assumption that the three other lncRNAs identified (*CHRM3-AS2*, *LINC00861* and *PRKCQ-AS1*) could be potential targets to better understand the impact of exercise training on monocytes function in CVD needs further investigations.

Altogether, considering that monocyte recruitment, differentiation and pro-inflammatory activation play an important role in the pathogenesis of atherosclerosis (Kita et al. 2001), the genes identified with RNAseq, such as *CCL5*, *LEF1* and *ZAP70* may suggest a positive impact of exercise on CVD risk. Nonetheless, future studies regarding the function of these individual genes in the context of atherosclerosis will be required to fully appreciate the effects of HIIT on circulating monocyte activation.

Conclusion

In summary, a 12-week HIIT intervention reduced the expression of 56 genes in circulating monocytes of older women with T2D, many of them are associated with inflammation and/or the development of CVD. The negative modulation of these genes globally may suggest a favorable cardiovascular impact of HIIT in this population. However, we cannot exclude the possibility that other physical exercise intervention may also produce similar effects in older women with T2D. Nonetheless, shedding light on the mechanisms underlying HIIT effects on cells involved in CVD development is of critical importance to develop efficient non-pharmacologic prevention strategies to further guide research investigations.

Author contributions DT, AM-C and FL performed experiments. JHM, DT, AM-C, FL, MAB, BL, ER and PG analyzed the data. JHM, ER and PG wrote the manuscript. MAB, BL and ER reviewed the manuscript and provided comments.

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Declarations

Conflict of interest The Author(s) declare(s) that there is no conflict of interest.

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