Etude n°III : Effet de l'exercice physique sur le contenu en miRs des microparticules circulantes chez les sujets sains

Aerobic exercise training modulates microparticles microRNAs in healthy subjects

Saloua DIMASSI, Esma KARKENI, Soumaya BOUMIZA, Pascal LAURANT, Karim CHAHED, Zouhair TABKA, Jean-Francois LANDRIER, Catherine RIVA.

(Soumis dans Vascular Pharmacology impact factor 2015: 3.63)

But de l'étude

Le but de cette étude était d'analyser les effets d'un programme d'entraînement physique sur une population de jeunes femmes saines (sans aucun facteur de risque cardiovasculaire) d'une part, par l'exploration de leur fonction endothéliale et du statut inflammatoire, et d'autre part, par l'étude de leurs MPs circulantes et leur contenu en miRs.

Méthodologie

Un groupe de six jeunes femmes saines normo-pondérées et ne présentant aucun facteur de risque cardiovasculaire, a été enrôlé pour cette étude. La fonction endothéliale de ces sujets a été explorée par l'étude de la réponse endothélium-dépendante au niveau de la microcirculation cutanée. De plus, l'inflammation a été évaluée en analysant le taux de CRPus. L'étude des MPs a été effectuée par mesure des concentrations plasmatiques

par cytométrie en flux et l'analyse des miRs a été effectuée par RT-PCR. Le choix des miRs étudiés dans cette étude a été effectué sur la base de leur implication dans différents processus vasculaires (inflammation, fonction vasculaire). Ces sujets ont participé à un programme d'entraînement physique de 8 semaines de type aérobie intermittent à forte intensité. Les différentes mesures ont été effectuées avant et après le programme d'entraînement physique afin d'analyser les effets de l'exercice.

Principaux résultats

Au bout de 8 semaines d'exercice physique, nous avons remarqué une amélioration de la fonction endothéliale pour notre population d'étude ainsi qu'en diminution de la CRPus suggérant une amélioration de statut inflammatoire. Le taux de MPs plasmatiques n'a toutefois pas été diminué par le programme d'entraînement. Par contre, nous avons pu observer une augmentation de l'expression des miR-21, miR-146a, miR-124a, miR-150 et miR-223 qui, pour certains ont été rapportés comme jouant des rôles anti-inflammatoires.

Conclusion

Cette étude nous a permis dans un premier temps de constater que l'exercice physique permettait une augmentation de la réponse endothélium-dépendante ainsi qu'une diminution de l'inflammation même chez des sujets sains. Par ailleurs, même si l'exercice physique n'a pas joué sur la concentration des MPs circulantes, nous avons pu observer une augmentation de l'expression de certains miRs de leur contenu en miR vers un profil anti-inflammatoire. De par l'importance des miRs dans la régulation de

nombreux gènes impliqués dans l'homéostasie vasculaire, et du rôle des MPs circulantes leur assurant un transport intercellulaire, la modulation du contenu en miR des MPs observé suite au programme d'entrainement serait une adaptation physiologique à l'exercice physique qui pourrait bien intervenir dans les différents effets bénéfiques de cet exercice au niveau vasculaire.

Article

Aerobic exercise training modulates microparticles miRNAs in healthy subjects

Saloua DIMASSI[1,2], Esma KARKENI[3,4,5], Soumaya BOUMIZA[2], Pascal LAURANT[1], Karim CHAHED[2], Zouhair TABKA[2], Jean-Francois LANDRIER[3,4,5], Catherine RIVA[1].

1. Avignon University, LaPEC EA4278, F-84000, Avignon, France
2. Faculty of Medicine, Sousse University, UR12ES06, Physiologie de l'Exercice et Physiopathologie: de l'Intégré au Moléculaire « Biologie, Médecine et Santé », Sousse, Tunisia
3. INRA, UMR 1260, F-13385, Marseille, France.
4. INSERM, UMR 1062, Nutrition, Obésité et Risque Thrombotique F-13385, Marseille, France.
5. Aix-Marseille Université, Faculté de Médecine, F-13385, Marseille, France.

Running title: Endothelial function, MP-miRNAs and exercise

Corresponding author: Catherine RIVA

Address: Avignon University, LaPEC EA4278, 74 rue Louis Pasteur, 84000 Avignon, France

Telephone number: +33 (0) 490162933 / Fax number +33 (0) 490162901

E-mail: catherine.riva@univ-avignon.fr

Key words: miRNA, Microparticles, Physical exercise, Endothelial function, inflammation

Words count: 2071 words

Total number of figures and tables: 2 tables and 1 figure

Prior Presentation:

Part of this study was presented in abstract form at the ISHR Bordeaux, France 2015

Author Contributions:

S.D. researched data/wrote the manuscript, E.K. researched data, S. B. researched data, K.C. reviewed the manuscript, Z.T. reviewed the manuscript, P.L. reviewed the manuscript, JF.L. designed experiments and reviewed the manuscript, C.R. designed experiments, researched data/wrote/edited the manuscript and is the guarantor of this work and, as such, had full access to all study data and takes responsibility for the data integrity and the data analysis accuracy.

Disclosure:

The authors have no potential conflicts of interest

Acknowledgement:

The authors want to thank Prof MC Alessi from INSERM, UMR 1062, INRA, UMR 1260, Dr H Debbabi, from the Faculty of Medicine, Sousse University, UR12ES06, and Dr A Miled from the Faculty of Medicine, Sousse University, Department of Clinical Biochemistry for the access to their core facility for biological measurements.

Abbreviation list:

ACh: Acetylcholine

BMI: Body Mass Index

CBF: Cutaneous Blood Flow

CVC: Cutaneous Vascular Conductance

eMPs: Endothelial-derived MicroParticles

HDLc: High density Lipoprotein Cholesterol

HsCRP: High Sensitive C-Reactive Protein

LDF: Laser Doppler Flowmetry

LDLc: Low Density Lipoprotein Cholesterol

LSH: Local Skin Heating

MPs: Microparticles

PFP: Platelet-Free Plasma

PRP: Platelet-Rich Plasma

RT: Room Temperature

RT-PCR: Real Rime Polymerase Chain Reaction

TBARS: ThioBarbituric Acid Reactive Substances

THR: Target Heart Rate

Abstract:

Exercise training is known to stimulate vascular function and remodeling in a shear stress and inflammation dependent manner. Microparticles (MPs) released from vascular cells in response to shear stress, play a role in cell-cell crosstalk through carrying bioactive molecules such as miRNAs. Thus, the aim of our study was to explore whether exercise training impacts vascular wall cells and contributes to vascular function improvement through the modulation of miRNAs-containing MPs release. Therefore, we investigated the presence in MPs of 9 miRNAs potentially associated to vascular function and inflammation: endothelial-related miRNAs, such as miR-126, miR-21 and miR-320a, monocyte/macrophage–related miRNAs, such as miR-155, miR-146a, miR-223, miR-124a, and miR-150, platelets–related miR-223 and miRNA-302a found in aorta. A group of sedentary women (n=6, BMI<25Kg/m²) recruited at F. Hached Hospital (Sousse, Tunisia) was enrolled in an 8-weeks training program. Vascular function was assessed by Laser Doppler Flowmetry, circulating MPs quantification, by flow cytometry, and miRNAs by real-time PCR, before and after exercise training. While exercise training improved significantly the endothelial-dependent vasorelaxation and decreased systemic inflammation, circulating MPs level and oxidant stress remained unchanged. The miRNA profile revealed that 1/ miR-155 and miR-302a were not detected in MPs neither before nor after training program; 2/ miR-21, miR-150, miR-320a, miR-146a, miR-124a, miR-126 and miR-223 were expressed in circulating MPs of sedentary women; and 3/ after training program, a significant increase of miR-21, miR-146a, miR-124a, miR-150 and miR-223 content was observed while miR-126 and miR-223 remained unchanged. Our results highlight the role of MPs as vehicle for miRNAs and the potential implication of miRNAs as effectors in the vascular wall in order to generate an optimal vascular function and to control inflammation.

1- Introduction

The study of miRNAs (small noncoding RNA molecules ~22 nt in length) is rapidly growing because they are involved in many human diseases in particular cardiovascular diseases. Recent studies demonstrated that miRNAs can be detected in circulating blood and may be useful as biomarkers for disease [1,2] . The mechanism of how circulating miRNAs are release into circulation remains unclear. However, increasing evidence suggests that miRNAs are actively secreted in microvesicles [2–4]. Extracellular membrane vesicles are important for cell-cell communication through numerous biological processes. In particular, microparticles (MPs) are typically defined by their size (0.1-1 μm in diameter) [5], exposure of phosphatidylserine (PS) and the expression of surface antigens originating from their donor cells [5,6]. MPs vesiculation occurs as a cellular response to various physiological conditions including, apoptosis, senescence, cellular activation [7], shear stress and biochemical triggers (such as cytokines). Furthermore, several studies have reported that MPs could contribute to endothelial vascular homeostasis [8,9]. On the other side, very little is known about dynamic changes in microRNAs in response to common physiological perturbations such as physical exercise. Understanding how exercise can alter gene regulation at the level of microRNAs will likely prove important in optimizing therapeutic uses of exercise to benefit human health, in particular, for cardiovascular diseases in which both exercise and microRNAs play a key role in pathogenesis [10–12]. Among them, miRNAs potentially associated to vascular function and inflammation were investigated such as endothelial-related miRNAs, miR-126, miR-21 and miR-320a, monocyte/macrophage–related miRNAs, miR-155, miR-146a, miR-223, miR-124a, and miR-150, platelets–related miR-223 and miRNA-302a found in aorta. We proposed in this study to explore the effects of 8 week-training exercise program on vascular modulation, through

endothelial function exploration and inflammatory parameter measurements, and miRNAs-containing MPs profile in a sedentary population.

2- Materials and methods

2-1- Subject population

The study, approved by Farhat Hached Hospital Ethical Committee for research on humans in Tunisia, included 6 sedentary (self-reported, regular exercise aerobic trainers ≤ 2 days per week) Caucasian healthy women who signed an informed consent before inclusion. All participants underwent history and medical evaluations and had to meet the following criteria before enrollment in the study: 1) no participation in regular physical activity; 2) no current chronic health problems; 3) no past or present history of smoking; 4) no cardiovascular, metabolic or respiratory disease; 5) no overweight or obesity and 5) no consumption of any antioxidant supplementation within the past 6 months.

2-2- Anthropometric, biochemical and blood pressure measurements

Body mass index (BMI) and mean arterial pressure (MAP= 1/3 (systolic blood pressure) + 2/3 (diastolic blood pressure)) was recorded. All subjects underwent biological parameters evaluation such as fasting glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDLc) levels, apolipoproteins (ApoA and ApoB) and high sensitive C-reactive proteins (hsCRP). Low-density lipoprotein cholesterol (LDLc) levels were calculated according to Friedewald's formula. Oxidative damages

were estimated by measuring the lipid peroxidation level i.e. plasma levels of Thiobarbituric Acid Reactive substances (TBARS).

All measurements, blood collection and functional exploration were assessed at rest and 48h after the last session of exercise training, to avoid any short term effects of exercise training.

2-3- Endothelial function assessment

Endothelial function was explored by assessing the forearm microvascular cutaneous vasoreactivity using Laser Doppler Flowmetry coupled with iontophoresis (Periflux System 5000, Perimed, Jarfalla Sweden). Endothelium-dependent vasodilation was evaluated by stimulation with 2% acetylcholine chloride (ACh) (Sigma Aldrich, Switzerland) and endothelium-independent vasodilation, after local skin heating (LSH) [13]. Briefly, cutaneous blood flow (CBF) was recorded at rest for 2min and during the functional exploration. Three doses of ACh were delivered using an anodal current (0,1mA for 10s) at 2-min intervals. Finally, the local skin temperature, initially maintained at 32°C, was increased to 44°C for 5min (LSH) [14]. Data were expressed as cutaneous vascular conductance (CVC), which represents the ratio between the CBF and MAP values, to take into account variations in blood pressure between subjects [15]. The endothelium-dependent response was calculated as the difference between the peak CVC upon ACh stimulation, (i.e. the CVC after the third dose of ACh) and the baseline CVC (Δ ACh CVC). The endothelium-independent response, was calculated as the difference between the peak CVC following LSH-induced vasodilation and the baseline CVC (Δ LSH CVC) [16].

2-4- MPs preparation and flow cytometry analysis

2-4-1- MPs preparation

Citrated blood samples were collected and processed within 2h. Platelet-rich plasma (PRP) was collected after centrifugation at 1,500g for 15min at room temperature (RT). Platelet-free plasma (PFP) was obtained by further centrifugation at 13,000g for 2min at RT and the MP pellets after ultracentrifugation of the PFP at 20,000g for 90min at 4°C. MPs and PFP aliquots were stored at -80°C until analysis [17].

2-4-2- MPs quantification by Flow cytometry

MPs samples were analyzed using Accuri C6 flow cytometer and software (Accuri Cytometers, Ann Arbor, MI) to define the MPs gate and to quantify the absolute numbers of MPs per µl of plasma. Regions corresponding to MPs were identified in forward and side-angle light scatter intensity dot plot representation set at logarithmic gain, based on their diameter using standard microbeads (0.1 and 1.1µm).

2-4-3- MPs-containing miRNAs assessment

Total RNAs including miRNAs were isolated from the MPs contained in 150µL of PFP using 700µL of Qiazol Lysis Reagent of the miRNeasy Micro Kit according to the manufacturer's instructions (Qiagen, Courtabœuf, France). Then, 3.5µL of miRNeasy Plasma Spike-In Control (Ce_miR-39_1; 1.6 x 10^8 copies/µL working solution) (Qiagen, Courtabœuf, France) was added to the samples. cDNAs were synthesized from 75ng of total RNA in 20µL using 5X miScript Hiflex Buffer, 10x dNTP mix and miScript Reverse Transcriptase according to the manufacturer's instructions Qiagen (Courtabœuf, France). Real-time quantitative RT-PCR analysis was performed using the Mx3005P Real-Time PCR System (Stratagene, La Jolla, CA, USA) as previously described [18]. Reactions were performed in a 12.5µL volume containing 6.25µL of 2X QuantiTect SYBR Green

PCR Master Mix (Qiagen, Courtabœuf, France), 1.25µL of 10X miScript Universal Primer (Qiagen, Courtabœuf, France), 1.25µL of 10X miScript Primer Assay (Hs_miR-21_2 miScript Primer Assay, Hs_miR-124a_1 miScript Primer Assay, Hs_miR-126*_1 miScript Primer Assay, Hs_miR-146a_1 miScript Primer Assay, Hs_miR-150_1 miScript Primer Assay, Hs_miR-155_2 miScript Primer Assay, Hs_miR-223_1 miScript Primer Assay, Hs_miR-302a_2 miScript Primer Assay, Hs_miR-320a_1 miScript Primer Assay, Ce_miR-39_1 miScript Primer Assay) (Qiagen, Courtabœuf, France) and 2.5µL of RNase-free water. After an initial incubation for 15min at 95°C, amplification reaction was performed in 40 cycles comprising 3 steps (94°C, 15s; 55°C, 30s and 70°C, 30s). For each condition, the expression was quantified in duplicate and the Ce_miR-39_1 miScript Primer Assay (Qiagen, Courtabœuf, France) was used as endogenous control in the comparative cycle threshold (C_T) method [19].

2-5- Exercise-training program

Subjects performed a high intensity interval aerobic exercise training program 3 times a week for 8 weeks. Heart rate monitoring was processed during sessions (Polar-NV-Finland) and exercise intensity was adjusted on an individual basis to ensure women exercised at their target heart rate (THR) calculated with Karvonen's formula [20] and based on 70 to 80% of heart rate reserve which correspond to a high intensity training zone [21]. Maximal heart rate was obtained with a multi-stage 20m shuttle run Luc Leger test before the program training [22]. Each training session consisted of a 15min warm-up before performing 3 rounds of 10min intervals at THR with ergometer or treadmill and 5min active recovery, giving a total exercise time of 45min. Each session ended with 20min of cool down relaxation.

2-6- Statistical analysis

Data analysis was performed using SPSS 17.0 software package (SPSS Inc, Chicago, IL, USA). Results are expressed as means ± standard error (SEM). Data were checked for normality using the Shapiro-Wilk test and tested by paired t-test, for parametric data, and by Wilcoxon signed rank test, for nonparametric data. Statistical significance was set at a p-value of $p<0.05$.

3- Results

A cohort of 6 young adult women of similar age, body mass index and fasting glucose was studied. An intervention of high interval training program was chosen, based on several studies which had proved its efficiency to stimulate changes in skeletal muscle metabolism, cardiovascular regulation and work performance [23]. In fact, after an 8 week-training program, significant reduction of weight was obtained but no modification of lipidemic, glycemic parameters and oxidative stress were observed (Table 1).

Table 1 - Clinical parameters and biochemical measurements of subjects before and after training program

Characteristics	Before	After	p
Age (years)	24.33 ± 1.47		
BMI (kg/m²)	22.14 ± 0.95	21.86 ± 0.95 *	**0.04**
MAP (mmHg)	81.11 ± 1.64	81.66 ± 1.87	0.31
Fasting glucose (mmol/l)	5.04 ± 0.16	5.10 ± 0.13	0.49
Total cholesterol (mmol/l)	4.12 ± 0.29	4.16 ± 0.26	0.89
Triglycerides (mmol/l)	0.68 ± 0.11	0.75 ± 0.13	0.83
HDLc (mmol/l)	1.29 ± 0.13	1.16 ± 0.05	0.33
LDLc (mmol/l)	2.52 ± 0.18	2.41 ± 0.36	0.91
ApoA (g/l)	1.49 ± 0.07	1.45 ± 0.06	0.46
ApoB (g/l)	0.72 ± 0.05	0.71 ± 0.05	0.68
TBARS (µmol/l)	4.03 ± 2.27	3.47 ± 3.09	0.24

Abbreviations: BMI. Body Mass Index; WHR. Waist-to-Hip Ratio; HDLc. High Density Lipoprotein cholesterol; LDLc. low Density Lipoprotein cholesterol; ApoA. ApolipoproteinA; ApoB. ApolipoproteinB; TBARS: ThioBarbituric Acid Reactive Substances. Data are expressed as mean ± SEM; * :$p<0.05$.

However, a significant decrease of inflammatory marker, i.e. hsCRP was noticed, revealing a diminished systemic inflammation (Table 2). Moreover, the exploration of endothelial function by laser Doppler displayed no modification of vascular conductance

at baseline (Basal CVC) after the 8 week-training program, but the flow-mediated endothelial-dependent relaxation (Δ ACh CVC) was significantly increased from 0.18 to 0.32 PU/mmg Hg ($p<0.05$) after the 8 week-training program while the endothelium-independent vasodilatation remained unchanged (Table 2).

Table 2 – Inflammatory parameter, endothelial function and MPs measurements for subjects before and after training program

Characteristics	Before	After	p
Inflammatory marker			
hsCRP (mg/l)	0.55 ± 0.16	0.35 ± 0.10*	**0.04**
Endothelial function parameters			
Basal CVC (PU/mmHg)	0.05 ± 0.01	0.06 ± 0.01	0.34
Δ ACh CVC (PU/mmHg)	0.18 ± 0.05	0.32 ± 0.10*	**0.04**
Δ LSH CVC (PU/mmHg)	0.60 ± 0.19	0.80 ± 0.11	0.17
MPs measurement			
MPs (MPs/µl plasma)	4444.40 ± 439.55	5663.00 ±807.12	0.22

Abbreviations: hsCRP. high sensitive C-Reactive Protein; PU: Perfusion Unit; ACh: Acetylcholine; LSH : Local Skin Heat; CVC: cutaneous vascular conductance; Δ ACh CVC : peak ACh CVC minus baseline; Δ LSH CVC: peak LSH CVC minus baseline;; MPs: microparticles. Data are expressed as mean ± SEM.*:$p<0.05$.

The analysis of circulating MPs before and after 8 week-training program did not display any significant change of MPs plasmatic levels (Table 2). Furthermore, miRNAs in MPs were detected using quantitative reverse transcription PCR analysis. The analysis of a subset of 9 miRNAs revealed that, two miRNAs (miR-155 and miR-302a) were

undetected in all samples, while miR-150, miR-320a, miR-146a, miR-124a, miR-21, miR-126 and miR-223 were present in circulating MPs. Moreover, after 8 week-training program, miR-150, miR-320a, miR-146a, miR-124a and miR-21 expression in MPs was significantly enhanced while miR-126 and miR-223 expression remained unchanged (Figure 1).

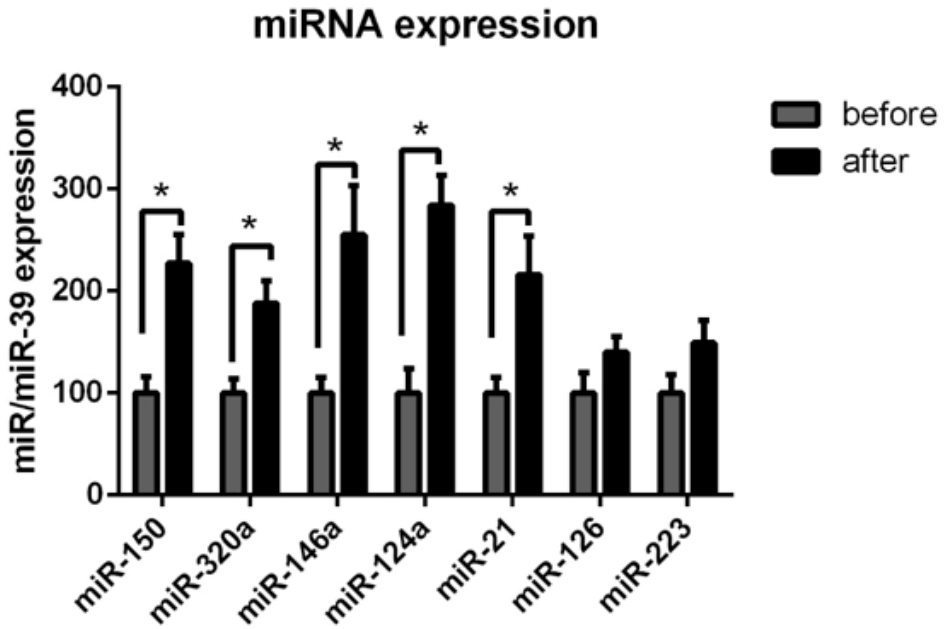

Figure 1: MPs-containing miR expression before and after training exercise; Values are mean ± SEM; *:$p<0.05$.

4- Discussion

The present study focused on the effects of a 8 week-training program on vascular reactivity, MPs quantification and MP-miRNAs content in a group of healthy young sedentary women. Our principal finding demonstrated that aerobic-type exercise training 1/ improved endothelial vasorelaxation and reduced systemic inflammation; but 2/ didn't

modify circulating MPs levels. In addition, this is the first study, we believe, to demonstrate substantial changes in miRNAs expression in MPs in healthy individuals following 8 week-training program.

In this study we have shown that circulating MPs were retrieved at low concentrations in plasma samples of healthy sedentary women. These circulating MPs from healthy subjects were at basal levels and lower than the circulating level encountered in pathological situations such as hypertension [24], diabetes [25], obesity [26] or coronary arteries diseases [27]. The paracrine secretion of MPs has been proved to be key effectors in inflammation, angiogenesis and vascular disorders [9,28], and is generally thought to be the principal transport vehicles for miRNAs in circulation [29]. In fact, because of their membrane vesicles constitution, MPs can protect from RNases in the plasma or serum, are also resistant to repetitive freezing and thawing cycle [30], and might facilitate communication within different cells. Therefore, we investigated the presence and the abundance in MPs of 9 miRNAs potentially associated to vascular function and/or dysfunction and inflammation: endothelial-related miRNAs, such as miR-126 and miR-21, miR-320a, monocyte/macrophage–related miRNAs, such as miR-155, miR-146a, miR-223, and miR-124a, and miR-150, platelet –related miR-223 [31] and miRNA-302a found in aorta [32].

Firstly, the miRNAs are secreted from cells into the circulation or are taken up from circulation into cells, suggesting that minimal miRNA degradation occurs due to RNases present in body fluids [1]. This may be attributed to the protection of miRNAs from RNases by intracellular small vesicles such as exosomes, microvesicles, and apoptotic bodies [33,34]. This hypothesis can be confirmed by our data, since we were able to detect significant level of several miRNAs in circulating MPs from healthy subjects. Furthermore, it has been suggested that exercise transiently or adaptively

changes the level of c-miRNAs in humans [35–37], leading to post-transcriptional regulation of proteins associated with energy metabolism and angiogenesis in adipocytes, hepatocytes, and endothelial cells. Among the miRNAs analysed, miR-155 and miR-302a have been reported to be highly implicated in cardiovascular disease, such as miR-302 a modulator of cholesterol homeostasis and atherosclerosis [32] and miR-155 implicated in atherogenesis in vivo [38] and inflammation during cardiovascular diseases, the metabolic syndrome, ageing [39] and obesity [40]. In our study, these two miRNAs couldn't be detected in circulating MPs of healthy women. It is conceivable to think that, these miRNAs would be under-expressed in non-pathological condition and therefore not present in MPs of healthy subjects. On the other hand, miR-21, miR-150, miR-320a, miR-146a, miR-124a, miR-126 and miR-223 were detected in circulating MPs of healthy women. All these miRNAs have been implicated as negative regulators of inflammatory processes at the transcriptional level [41]. Among them miR-146a and miR-223 have been reported to be a negative regulator of the immune response through inhibition of the expression of the mRNAs encoding TRAF6 and IRAK1, two proteins involved in the transcription of TLR signaling leading to NF-kB activation [42], and anti-inflammatory effects were also observed for miR-124a by the inhibition of adhesion and infiltration of inflammatory cells into the endothelial space [43].

Exercise training is considered as an efficient way to increase resistance to cardiovascular diseases through mediating adaptive responses. In the present study, we reported that a 8 week-training program reduced systemic inflammation, through reduction of circulating CRP levels, in healthy young women which is in total accordance with previous results [44,45]. Aside, we also observed an increased blood flow, and likely increased shear stress as reported by Green et al. [46] resulting in an improved endothelial function through an enhanced endothelial-dependent vasodilation. Wang et al. [47] have

already observed enhanced skin blood flow and cutaneous vascular conductance of healthy sedentary subjects in response to a moderate-intensity exercise (8 weeks). Moreover, regular aerobic exercise could restore the loss in endothelial-dependent vasodilation in healthy middle-aged and older men [48]. Only few studies were interested on the effect of exercise on shear stress with consequently on circulating MPs and on miRNAs. We reported no significant changes in MPs circulating levels after 8 week-training program in the healthy young women. While Babbit et al. [49] observed a reduction in endothelial-derived MPs (EMPs) circulating levels after a 6-month aerobic exercise training for sedentary middle-to-older-aged subjects, they did not quantified total MPs circulating level. Moreover, other studies reported an elevation of EMPs circulating levels associated with reduced daily physical activity and inactivation [50,51] but no correlations were found between EMPs levels and flow mediated dilation measurement [50].

Furthermore, after 8-week training, the exploration of MPs-containing miRNAs showed that pro-inflammatory miR-302a and miR-155 were still not detected. But, a significant increase of miR-21, miR-146a, miR-124a, miR-150 and miR-223 abundance was observed after 8 week-training program while miR-126 and miR-223 remained unchanged. It is well known that aerobic exercise is a potent physiological stimulant by inducing laminar shear stress [52], Interestingly, studies reported that high unidirectional shear stress could enhance expression of a distinct group of miRNAs in endothelial cells, and among them miR-21 [53]. It is conceivable to speculate that enhanced expression of miRNAs in MPs could be a result of the 8 week-training program. Moreover, Weber et al. observed that HUVEC overexpressing miR-21, after shear stress stimulation, had decreased apoptosis and increased eNOS phosphorylation and nitric oxide production through a PI3K/Akt/eNOS pathway, which would suggest an implication of these

miRNAs in the beneficial effects of exercise on endothelial function [53]. However, studies investigating miRNA modulation by exercise, reported contradictory results [54]. In fact, Baggish et al. [35] observed enhanced plasmatic miR-21, mi-146a or miR-126 expression, after 90 days exercise training for a group of trained men [55], whereas, Nielson et al. [56] identified seven miRNAs, among them miR-21, with decreased levels in plasma of young healthy men, in response to a 12 week chronic training. These discrepancy could be the result of population characteristics as it was suggested that plasmatic miRNA levels in response to exercise differ between trained and non-trained subjects [57]. Moreover, studies reported that exercise type, duration and intensity could largely influence circulating miRNAs expression and so could also partially explain these controversial results [37,58].

In total, exercise training can be considered as an efficient way prevent cardiovascular diseases through mediating adaptive reactions. Since chronic inflammation is clearly associated to cardiovascular disease, diabetes mellitus, adipogenesis and obesity [59,60], regulatory miRNAs could be involved in the control of vascular inflammation and vascular function. On the other hand, paracrine release of MPs, by transporting these miRNAs through the circulation, may highly contribute to these mechanisms. Besides, further research is warranted for the characterization of detailed mechanisms and the physiological and pathological changes in miRNAs implicated into this vascular adaptive response to physical exercise. Nevertheless, the field of miRNAs research is attractive and is expected to present more novel findings for the field of therapeutics.

5- References

[1] P.S. Mitchell, R.K. Parkin, E.M. Kroh, B.R. Fritz, S.K. Wyman, E.L. Pogosova-Agadjanyan, et al., Circulating microRNAs as stable blood-based markers for cancer detection, Proc. Natl. Acad. Sci. 105 (2008) 10513–10518. doi:10.1073/pnas.0804549105.

[2] J. Skog, T. Würdinger, S. van Rijn, D.H. Meijer, L. Gainche, W.T. Curry, et al., Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. 10 (2008) 1470–1476. doi:10.1038/ncb1800.

[3] M.P. Hunter, N. Ismail, X. Zhang, B.D. Aguda, E.J. Lee, L. Yu, et al., Detection of microRNA Expression in Human Peripheral Blood Microvesicles, PLoS ONE. 3 (2008) e3694. doi:10.1371/journal.pone.0003694.

[4] A. Zernecke, K. Bidzhekov, H. Noels, E. Shagdarsuren, L. Gan, B. Denecke, et al., Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection, Sci. Signal. 2 (2009) ra81. doi:10.1126/scisignal.2000610.

[5] J. Gong, R. Jaiswal, J.-M. Mathys, V. Combes, G.E.R. Grau, M. Bebawy, Microparticles and their emerging role in cancer multidrug resistance, Cancer Treat. Rev. 38 (2012) 226–234. doi:10.1016/j.ctrv.2011.06.005.

[6] O. Morel, F. Toti, B. Hugel, B. Bakouboula, L. Camoin-Jau, F. Dignat-George, et al., Procoagulant Microparticles Disrupting the Vascular Homeostasis Equation?, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 2594–2604. doi:10.1161/01.ATV.0000246775.14471.26.

[7] A. Janowska-Wieczorek, L.A. Marquez-Curtis, M. Wysoczynski, M.Z. Ratajczak, Enhancing effect of platelet-derived microvesicles on the invasive potential of breast cancer cells, Transfusion (Paris). 46 (2006) 1199–1209. doi:10.1111/j.1537-2995.2006.00871.x.

[8] G.N. Chironi, C.M. Boulanger, A. Simon, F. Dignat-George, J.-M. Freyssinet, A. Tedgui, Endothelial microparticles in diseases, Cell Tissue Res. 335 (2008) 143–151. doi:10.1007/s00441-008-0710-9.

[9] M.C. Martinez, S. Tual-Chalot, D. Leonetti, R. Andriantsitohaina, Microparticles: targets and tools in cardiovascular disease, Trends Pharmacol. Sci. 32 (2011) 659–665. doi:10.1016/j.tips.2011.06.005.

[10] M. Franco, R.S. Cooper, U. Bilal, V. Fuster, Challenges and Opportunities for Cardiovascular Disease Prevention, Am. J. Med. 124 (2011) 95–102. doi:10.1016/j.amjmed.2010.08.015.

[11] N. Garbacki, E. Di Valentin, V.A. Huynh-Thu, P. Geurts, A. Irrthum, C. Crahay, et al., MicroRNAs Profiling in Murine Models of Acute and Chronic Asthma: A Relationship with mRNAs Targets, PLoS ONE. 6 (2011) e16509. doi:10.1371/journal.pone.0016509.

[12] M. Hoekstra, C.A.C. van der Lans, B. Halvorsen, L. Gullestad, J. Kuiper, P. Aukrust, et al., The peripheral blood mononuclear cell microRNA signature of coronary artery disease, Biochem. Biophys. Res. Commun. 394 (2010) 792–797. doi:10.1016/j.bbrc.2010.03.075.

[13] T.T. van Sloten, S. Czernichow, A.J. Houben, A.D. Protogerou, R.M. Henry, D.M. Muris, et al., Association Between Arterial Stiffness and Skin Microvascular Function: The SUVIMAX2 Study and The Maastricht Study, Am. J. Hypertens. (2014). doi:10.1093/ajh/hpu246.

[14] J.-J. Mourad, G. des Guetz, H. Debbabi, B.I. Levy, Blood pressure rise following angiogenesis inhibition by bevacizumab. A crucial role for microcirculation, Ann. Oncol. (2007) mdm550. doi:10.1093/annonc/mdm550.

[15] D.S. O'Leary, Regional vascular resistance vs. conductance: which index for baroreflex responses?, Am. J. Physiol. - Heart Circ. Physiol. 260 (1991) H632–H637.
[16] R. de Moraes, D. Van Bavel, B.S. de Moraes, E. Tibiriçá, Effects of dietary creatine supplementation on systemic microvascular density and reactivity in healthy young adults, Nutr. J. 13 (2014). doi:10.1186/1475-2891-13-115.
[17] S. Robert, P. Poncelet, R. Lacroix, L. Arnaud, L. Giraudo, A. Hauchard, et al., Standardization of platelet-derived microparticle counting using calibrated beads and a Cytomics FC500 routine flow cytometer: a first step towards multicenter studies?, J. Thromb. Haemost. 7 (2009) 190–197. doi:10.1111/j.1538-7836.2008.03200.x.
[18] J.-F. Landrier, C. Malezet-Desmoulins, E. Reboul, A. Marie Lorec, M. Josèphe Amiot, P. Borel, Comparison of different vehicles to study the effect of tocopherols on gene expression in intestinal cells, Free Radic. Res. 42 (2008) 523–530. doi:10.1080/10715760802098859.
[19] K.J. Livak, T.D. Schmittgen, Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method, Methods. 25 (2001) 402–408. doi:10.1006/meth.2001.1262.
[20] M. Karvonen, The effects of training on heart rate. A longitudinal study, Ann Ned Exp Biol Fenn. 35 (1957) 307–315.
[21] L. Vanhees, N. Geladas, D. Hansen, E. Kouidi, J. Niebauer, Z. Reiner, et al., Importance of characteristics and modalities of physical activity and exercise in the management of cardiovascular health in individuals with cardiovascular risk factors: recommendations from the EACPR (Part II), Eur. J. Prev. Cardiol. 19 (2012) 1005–1033. doi:10.1177/1741826711430926.
[22] L.A. Léger, J. Lambert, A maximal multistage 20-m shuttle run test to predict VO2 max, Eur. J. Appl. Physiol. 49 (1982) 1–12. doi:10.1007/BF00428958.
[23] M.J. Gibala, S.L. McGee, Metabolic Adaptations to Short-term High-Intensity Interval Training: A Little Pain for a Lot of Gain?, Exerc. Sport Sci. Rev. 36 (2008) 58–63. doi:10.1097/JES.0b013e318168ec1f.
[24] S. Nomura, N. Inami, A. Shouzu, F. Urase, Y. Maeda, Correlation and association between plasma platelet-, monocyte- and endothelial cell-derived microparticles in hypertensive patients with type 2 diabetes mellitus, Platelets. 20 (2009) 406–414. doi:10.1080/09537100903114545.
[25] B. Feng, Y. Chen, Y. Luo, M. Chen, X. Li, Y. Ni, Circulating level of microparticles and their correlation with arterial elasticity and endothelium-dependent dilation in patients with type 2 diabetes mellitus, Atherosclerosis. 208 (2010) 264–269. doi:10.1016/j.atherosclerosis.2009.06.037.
[26] K. Esposito, M. Ciotola, B. Schisano, R. Gualdiero, L. Sardelli, L. Misso, et al., Endothelial Microparticles Correlate with Endothelial Dysfunction in Obese Women, J. Clin. Endocrinol. Metab. 91 (2006) 3676–3679. doi:10.1210/jc.2006-0851.
[27] N. Werner, S. Wassmann, P. Ahlers, S. Kosiol, G. Nickenig, Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 26 (2006) 112–116. doi:10.1161/01.ATV.0000191634.13057.15.
[28] F. Sabatier, R. Lacroix, L. Camoin-Jau, F. Anfosso, J. Sampol, F. Dignat-George, Cellules endothéliales circulantes, microparticules et progéniteurs : vers la définition de la « vasculocompétence », Rev. Médecine Interne. 32 (2011) 54–63. doi:10.1016/j.revmed.2010.03.341.

[29] P. Diehl, A. Fricke, L. Sander, J. Stamm, N. Bassler, N. Htun, et al., Microparticles: major transport vehicles for distinct microRNAs in circulation, Cardiovasc. Res. 93 (2012) 633–644. doi:10.1093/cvr/cvs007.
[30] T.S. Chen, R.C. Lai, M.M. Lee, A.B.H. Choo, C.N. Lee, S.K. Lim, Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs, Nucleic Acids Res. 38 (2010) 215–224. doi:10.1093/nar/gkp857.
[31] K. Kin, S. Miyagawa, S. Fukushima, Y. Shirakawa, K. Torikai, K. Shimamura, et al., Tissue- and Plasma-Specific MicroRNA Signatures for Atherosclerotic Abdominal Aortic Aneurysm, J. Am. Heart Assoc. 1 (2012) e000745. doi:10.1161/JAHA.112.000745.
[32] S. Meiler, Y. Baumer, E. Toulmin, K. Seng, W.A. Boisvert, MicroRNA 302a Is a Novel Modulator of Cholesterol Homeostasis and Atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 323–331. doi:10.1161/ATVBAHA.114.304878.
[33] K.C. Vickers, A.T. Remaley, Lipid-based carriers of microRNAs and intercellular communication:, Curr. Opin. Lipidol. 23 (2012) 91–97. doi:10.1097/MOL.0b013e328350a425.
[34] G. Raposo, W. Stoorvogel, Extracellular vesicles: Exosomes, microvesicles, and friends, J. Cell Biol. 200 (2013) 373–383. doi:10.1083/jcb.201211138.
[35] A.L. Baggish, A. Hale, R.B. Weiner, G.D. Lewis, D. Systrom, F. Wang, et al., Dynamic regulation of circulating microRNA during acute exhaustive exercise and sustained aerobic exercise training, J. Physiol. 589 (2011) 3983–3994. doi:10.1113/jphysiol.2011.213363.
[36] D. de Gonzalo-Calvo, A. Dávalos, A. Montero, Á. García-González, I. Tyshkovska, A.G. Medina, et al., Circulating inflammatory miRNA signature in response to different doses of aerobic exercise, J. Appl. Physiol. (2015) jap.00077.2015. doi:10.1152/japplphysiol.00077.2015.
[37] T. Xu, Q. Liu, J. Yao, Y. Dai, H. Wang, J. Xiao, Circulating microRNAs in response to exercise, Scand. J. Med. Sci. Sports. 25 (2015) e149–e154. doi:10.1111/sms.12421.
[38] X. Ma, C. Ma, X. Zheng, MicroRNA-155 in the Pathogenesis of Atherosclerosis: A Conflicting Role?, Heart Lung Circ. 22 (2013) 811–818. doi:10.1016/j.hlc.2013.05.651.
[39] B. Schroen, S. Heymans, Small but smart—microRNAs in the centre of inflammatory processes during cardiovascular diseases, the metabolic syndrome, and ageing, Cardiovasc. Res. 93 (2012) 605–613. doi:10.1093/cvr/cvr268.
[40] E. Karkeni, J. Astier, F. Tourniaire, M. El Abed, B. Romier, E. Gouranton, Obesity-associated inflammation induces microRNA-155 expression in adipocytes and adipose tissue: outcome on adipocyte function. J Clin Endocrinol Metab. 2016 Feb 1:jc20153410.
[41] L.A. O'Neill, F.J. Sheedy, C.E. McCoy, MicroRNAs: the fine-tuners of Toll-like receptor signalling, Nat. Rev. Immunol. 11 (2011) 163–175. doi:10.1038/nri2957.
[42] K.D. Taganov, M.P. Boldin, K.-J. Chang, D. Baltimore, NF-κB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 12481–12486. doi:10.1073/pnas.0605298103.
[43] M. Hulsmans, D.D. Keyzer, P. Holvoet, MicroRNAs regulating oxidative stress and inflammation in relation to obesity and atherosclerosis, FASEB J. 25 (2011) 2515–2527. doi:10.1096/fj.11-181149.
[44] E.S. Ford, Does exercise reduce inflammation? Physical activity and C-reactive protein among U.S. adults: Epidemiology, 13 (2002). 5:561-8.

[45] F. Mattusch, B. Dufaux, O. Heine, I. Mertens, R. Rost, Reduction of the Plasma Concentration of C-Reactive Protein Following Nine Months of Endurance Training, Int. J. Sports Med. 21 (2000) 21–24. doi:10.1055/s-2000-8852.
[46] D.J. Green, H.H. Carter, M.G. Fitzsimons, N.T. Cable, D.H.J. Thijssen, L.H. Naylor. Obligatory rol of hyperaemia and stress stress in icrovascular adaptation to repeated heating in humans. J. Physiol. (2010) 588, 9: 1571-1577 doi: 10.1113/jphysiol.2010.186965.
[47] J.S. Wang, Effects of exercise training and detraining on cutaneous microvascular function in man: the regulatory role of endothelium-dependent dilation in skin vasculature, Eur. J. Appl. Physiol. 93 (2004) 429–434. doi:10.1007/s00421-004-1176-4.
[48] C.A. DeSouza, L.F. Shapiro, C.M. Clevenger, F.A. Dinenno, K.D. Monahan, H. Tanaka, et al., Regular Aerobic Exercise Prevents and Restores Age-Related Declines in Endothelium-Dependent Vasodilation in Healthy Men, Circulation. 102 (2000) 1351–1357. doi:10.1161/01.CIR.102.12.1351.
[49] D.M. Babbitt, K.M. Diaz, D.L. Feairheller, K.M. Sturgeon, A.M. Perkins, P. Veerabhadrappa, et al., Endothelial Activation Microparticles and Inflammation Status Improve with Exercise Training in African Americans, Int. J. Hypertens. 2013 (2013) 1–8. doi:10.1155/2013/538017.
[50] L.J. Boyle, D.P. Credeur, N.T. Jenkins, J. Padilla, H.J. Leidy, J.P. Thyfault, et al., Impact of reduced daily physical activity on conduit artery flow-mediated dilation and circulating endothelial microparticles, J. Appl. Physiol. 115 (2013) 1519–1525. doi:10.1152/japplphysiol.00837.2013.
[51] N.M. Navasiolava, F. Dignat-George, F. Sabatier, I.M. Larina, C. Demiot, J.-O. Fortrat, et al., Enforced physical inactivity increases endothelial microparticle levels in healthy volunteers, Am. J. Physiol. - Heart Circ. Physiol. 299 (2010) H248–H256. doi:10.1152/ajpheart.00152.2010.
[52] R. Hambrecht, A. Wolf, S. Gielen, A. Linke, J. Hofer, S. Erbs, et al., Effect of exercise on coronary endothelial function in patients with coronary artery disease, N. Engl. J. Med. 342 (2000) 454–460. doi:10.1056/NEJM200002173420702.
[53] M. Weber, M.B. Baker, J.P. Moore, C.D. Searles, MiR-21 is induced in endothelial cells by shear stress and modulates apoptosis and eNOS activity, Biochem. Biophys. Res. Commun. 393 (2010) 643–648. doi:10.1016/j.bbrc.2010.02.045.
[54] S. Radom-Aizik, F. Zaldivar, S.-Y. Leu, G.R. Adams, S. Oliver, D.M. Cooper, Effects of Exercise on microRNA Expression in Young Males Peripheral Blood Mononuclear Cells, Clin. Transl. Sci. 5 (2012) 32–38. doi:10.1111/j.1752-8062.2011.00384.x.
[55] M. Uhlemann, S. Möbius-Winkler, S. Fikenzer, J. Adam, M. Redlich, S. Möhlenkamp, et al., Circulating microRNA-126 increases after different forms of endurance exercise in healthy adults, Eur. J. Prev. Cardiol. 21 (2014) 484–491. doi:10.1177/2047487312467902.
[56] S. Nielsen, T. Åkerström, A. Rinnov, C. Yfanti, C. Scheele, B.K. Pedersen, et al., The miRNA Plasma Signature in Response to Acute Aerobic Exercise and Endurance Training, PLoS ONE. 9 (2014) e87308. doi:10.1371/journal.pone.0087308.
[57] A. Bye, H. Røsjø, S.T. Aspenes, G. Condorelli, T. Omland, U. Wisløff, Circulating MicroRNAs and Aerobic Fitness – The HUNT-Study, PLoS ONE. 8 (2013) e57496. doi:10.1371/journal.pone.0057496.

[58] S. Banzet, M. Chennaoui, O. Girard, S. Racinais, C. Drogou, H. Chalabi, et al., Changes in circulating microRNAs levels with exercise modality, J. Appl. Physiol. 115 (2013) 1237–1244. doi:10.1152/japplphysiol.00075.2013.
[59] G. Singer, N. Granger, Inflammatory Responses Underlying the Microvascular Dysfunction Associated with Obesity and Insulin Resistance, Microcirculation. 14 (2007) 375–387. doi:10.1080/10739680701283158.
[60] R. Ross, Atherosclerosis — An Inflammatory Disease, N. Engl. J. Med. 340 (1999) 115–126. doi:10.1056/NEJM199901143400207.