

**lek. Michał Szyszka**

**Badanie ATHENA:  
patogeneza nadciśnienia tętniczego  
- mikroRNA jako brakujące ogniwo  
w rozwoju nadciśnienia tętniczego u dzieci**

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu  
w dyscyplinie nauki medyczne**

**Promotor: dr hab. n. med. i n. o zdr. Piotr Skrzypczyk**

**Katedra i Klinika Pediatrii i Nefrologii  
Warszawski Uniwersytet Medyczny**



**Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych  
Warszawskiego Uniwersytetu Medycznego**

**Warszawa, 2026 r.**

**Słowa kluczowe:** mikroRNA; nadciśnienie tętnicze pierwotne; dzieci; czynniki epigenetyczne; uszkodzenie narządowe; biomarker.

**Key words:** microRNA; primary hypertension; children; epigenetic factors; hypertension-mediated organ damage; biomarker.

# Podziękowania

Pragnę złożyć serdeczne podziękowania wszystkim Osobom, które przyczyniły się do powstania niniejszej rozprawy doktorskiej, a których nie sposób tu wymienić każdej z osobna, choć ich wkład i życzliwość miały istotne znaczenie dla realizacji tej pracy.

Szczególne wyrazy wdzięczności kieruję do mojego Promotora, Pana dr hab. Piotra Skrzypczyka, za nieocenione wsparcie merytoryczne, życzliwość, cierpliwość oraz inspirację naukową, która towarzyszy mi od początku mojej drogi akademickiej i której istotny etap rozpoczął się jeszcze w czasie studiów pod Jego opieką. Dziękuję za poświęcony czas, stałą dostępność, także w późnych godzinach, za gotowość do wsparcia, za cenne wskazówki, za inspirujące dyskusje naukowe oraz za rozmowy wykraczające poza ramy nauki, a także za nieustanną motywację do rozwoju, stawiania pytań i poszukiwania odpowiedzi.

Dziękuję również Pani Profesor Małgorzacie Mizerskiej-Wasiak, obecnemu Kierownikowi Katedry i Kliniki Pediatrii i Nefrologii, za stworzenie sprzyjających warunków do prowadzenia pracy naukowej oraz za wsparcie organizacyjne i merytoryczne.

Wyrazy wdzięczności składam także Pani Profesor Małgorzacie Pańczyk-Tomaszewskiej, poprzedniemu Kierownikowi Katedry i Kliniki Pediatrii i Nefrologii, za umożliwienie rozpoczęcia pracy naukowej oraz stworzenie fundamentów do jej realizacji.

Szczególne podziękowania kieruję do mojej Rodziny — w szczególności do mojej Mamy, Taty i Brata — za nieustające wsparcie, wyrozumiałość i cierpliwość. Bez Waszej obecności, stworzenia bezpiecznego i pełnego życzliwości środowiska, opartego na szacunku dla nauki, a także wiary we mnie, nie byłbym w miejscu, w którym jestem, a realizacja tej pracy nie byłaby możliwa. Szczególne podziękowania należą się mojej Babci Krystynie, która zaszczepiła we mnie ciekawość świata i pokazała piękno stawiania pytań.

Szczególne miejsce w tych podziękowaniach zajmuje osoba mi najbliższa, której obecność, wsparcie i spokój — których często mi brakowało — pozwalały mi przejść przez najbardziej wymagające momenty pracy nad rozprawą. Dziękuję za wyrozumiałość i cierpliwość, za towarzyszenie mi w czasie wielu godzin, dni i miesięcy intensywnej pracy naukowej, za akceptację mojej częstej nieobecności oraz za codzienne wsparcie w chwilach największego zaangażowania w realizację tej pracy. Dziękuję także za zrozumienie dla codziennych niedoskonałości, które nieodłącznie towarzyszyły jej powstawaniu.

## Wykaz publikacji stanowiących pracę doktorską

1. Michał Szyszka, Piotr Skrzypczyk. MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension - A Preliminary Study. *J Clin Med* 2024; 13(22): 6929. doi:10.3390/jcm13226929.  
IF: 2,9      MNiSW: 140 pkt
2. Michał Szyszka, Ceren Eyileten, Jolanta Małyszko, Karolina Skubisz, Radosław Pietrzak, Hubert Krysztofiak, Piotr Skrzypczyk. ATHENA Study – The Role of Circulating Plasma MicroRNAs in Paediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes. *Kidney Blood Pres Res* 2026; 51(1): 468-487. doi: 10.1159/000552984.  
IF: 2,1      MNiSW: 70 pkt
3. Michał Szyszka, Radosław Pietrzak, Klaudia Obsznajczyk, Karolina Skubisz, Ceren Eyileten, Piotr Skrzypczyk. Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension. *Int J Mol Sci* 2026; 27(6): 2806. doi:10.3390/ijms27062806.  
IF: 4,9      MNiSW: 140 pkt
4. Michał Szyszka, Piotr Skrzypczyk. MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review. *Biomolecules*. 2026; 16(5):636. <https://doi.org/10.3390/biom16050636>  
IF: 4,8      MNiSW: 100 pkt

**Łącznie punktacja MNiSW: 450 pkt**

**Łącznie Impact Factor: 14,7**



Nr referencyjny  
BIBG/Punktacja/322/2026/MW

Warszawa, 10.06.2026

Sz. Pan  
Lek. Michał Szyszka  
Katedra i Klinika Pediatrii i Nefrologii WUM

ANALIZA BIBLIOMETRYCZNA PUBLIKACJI  
PANA MICHAŁA SZYSZKI,  
WCHODZĄCYCH W SKŁAD CYKLU PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

| LP       | OPIS                                                                                                                                                                                                                                                                                                                                              | IF     | MNiSW | NAJWYŻSZY KWARTYL* |
|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|-------|--------------------|
| 1        | Szyszka M, Skrzypczyk P. MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension—A Preliminary Study. Journal of Clinical Medicine. 2024;13(22):1-14.<br><i>Rodzaj pracy: praca oryginalna</i>                                                              | 2,900  | 140   | Q1                 |
| 2        | Szyszka M, Eyileten C, Małyszko J, Skubisz K, Pietrzak R, Krysztofiak H, Skrzypczyk P. ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes. Kidney & Blood Pressure Research. 2026;1-31.<br><i>Rodzaj pracy: praca oryginalna</i> | 2,100  | 70    | Q2                 |
| 3        | Szyszka M, Pietrzak R, Obsznajczyk K, Skubisz K, Eyileten C, Skrzypczyk P. Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension. International Journal of Molecular Sciences. 2026;27(6):1-18.<br><i>Rodzaj pracy: praca oryginalna</i>                                         | 4,900  | 140   | Q1                 |
| 4        | Szyszka M, Skrzypczyk P. MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review. Biomolecules. 2026;16(5):1-21.<br><i>Rodzaj pracy: praca poglądowa</i>                                                                                                                            | 4,800  | 100   | Q1                 |
| Łącznie: |                                                                                                                                                                                                                                                                                                                                                   | 14,700 | 450   | -                  |

| KSIĄŻKI |      |
|---------|------|
| LP      | OPIS |
| 1.      | -    |

| ROZDZIAŁY |      |
|-----------|------|
| LP        | OPIS |
| 1.        | -    |

\* Kwartył z roku publikacji, według Impact Factor.



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UNIwersytet  
MEDYCZNY

BIBLIOTEKA UCZELNIANA

Nr referencyjny  
BIBG/Punktacja/322/2026/MW

Warszawa, 10.06.2026

Sz. Pan  
Lek. Michał Szyszka  
Katedra i Klinika Pediatrii i Nefrologii WUM

ANALIZA BIBLIOMETRYCZNA CAŁOKSZTAŁTU DOROBKU PUBLIKACYJNEGO  
PANA MICHAŁA SZYSZKI,  
W POSTĘPOWANIU O NADANIE STOPNIA NAUKOWEGO DOKTORA

| LP                                                                                                                                                      | OPIS                                                                                                                                                                                                                                                                                                                                  | IF    | MNiSW | NAJWYŻSZY<br>KWARTYL* |
|---------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-------|-----------------------|
| I. Artykuły opublikowane w czasopismach naukowych lub w recenzowanych materiałach z konferencji międzynarodowych ujętych w aktualnym wykazie MNiSW [1]. |                                                                                                                                                                                                                                                                                                                                       |       |       |                       |
| 1                                                                                                                                                       | Skrzypczyk P, Ofiara A, Szyszka M, Dziedzic-Jankowska K, Sołtyski J, Pańczyk-Tomaszewska M. Vitamin D in children with primary hypertension. Arterial Hypertension. 2018;22(3):127-134.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                                                      | -     | 8     | -                     |
| 2                                                                                                                                                       | Skrzypczyk P, Mizerska-Wasiak M, Ofiara A, Szyszka M, Kułagowska J, Bieja A, Brzewski M, Kucińska B, Werner B, Pańczyk-Tomaszewska M. Zatorowość płucna u dziewczynki z zespołem nerczycowym i czynnikiem V Leiden – opis przypadku. Polski Merkuriusz Lekarski. 2018;45(267):114-118.<br><i>Rodzaj pracy: opis przypadku</i>         | -     | 7     | -                     |
| 3                                                                                                                                                       | Skrzypczyk P, Szyszka M, Ofiara A, Leszczyńska B, Adamczuk D, Daniel M, Pańczyk-Tomaszewska M. Ambulatory blood pressure monitoring and subclinical inflammation in children with chronic kidney disease. Arterial Hypertension. 2019;23(1):14-21.<br><i>Rodzaj pracy: praca oryginalna</i>                                           | -     | 70    | -                     |
| 4                                                                                                                                                       | Skrzypczyk P, Ozimek A, Ofiara A, Szyszka M, Sołtyski J, Stelmaszczyk-Emmel A, Górka E, Pańczyk-Tomaszewska M. Markers of endothelial injury and subclinical inflammation in children and adolescents with primary hypertension. Central European Journal of Immunology. 2019;44(3):253-261.<br><i>Rodzaj pracy: praca oryginalna</i> | 1,415 | 70    | Q4                    |

|    |                                                                                                                                                                                                                                                                                                                                               |       |     |    |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-----|----|
| 5  | Skrzypczyk P, Ozimek A, Szyszka M, Ofiara A, Sołtyski J, Stelmaszczyk-Emmel A, Górka E, Pańczyk-Tomaszewska M. Zależność pomiędzy stężeniem sklerostyny, ciśnieniem tętniczym i sztywnością tętnic u dzieci i młodzieży z nadciśnieniem tętniczym pierwotnym. Przegląd Lekarski. 2019;76(5):220-226.<br><i>Rodzaj pracy: praca oryginalna</i> | -     | 20  | -  |
| 6  | Skrzypczyk P, Zacharzewska A, Szyszka M, Ofiara A, Pańczyk-Tomaszewska M. Arterial stiffness in children with primary hypertension is related to subclinical inflammation. Central European Journal of Immunology. 2021;46(3):336-343.<br><i>Rodzaj pracy: praca oryginalna</i>                                                               | 1,634 | 70  | Q4 |
| 7  | Skrzypczyk P, Stelmaszczyk-Emmel A, Szyszka M, Ofiara A, Pańczyk-Tomaszewska M. Circulating calcification inhibitors are associated with arterial damage in pediatric patients with primary hypertension. Pediatric Nephrology. 2021;36(8):2371-2382.<br><i>Rodzaj pracy: praca oryginalna</i>                                                | 3,652 | 140 | Q2 |
| 8  | Skrzypczyk P, Wabik A, Szyszka M, Jóźwiak S, Bombiński P, Jakimów-Kostrzewa A, Brzewski M, Pańczyk-Tomaszewska M. Early Vascular Aging in Children With Tuberous Sclerosis Complex. Frontiers in Pediatrics. 2021;9:1-11.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                            | 3,569 | 70  | Q2 |
| 9  | Szyszka M, Skrzypczyk P, Pańczyk-Tomaszewska M. Kwas moczowy u dzieci i młodzieży z nadciśnieniem tętniczym pierwotnym. Polski Merkuriusz Lekarski. 2021;49(290):119-124.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                                                                            | -     | 20  | -  |
| 10 | Szyszka M, Skrzypczyk P, Stelmaszczyk-Emmel A, Pańczyk-Tomaszewska M. Serum Periostin as a Potential Biomarker in Pediatric Patients with Primary Hypertension. Journal of Clinical Medicine. 2021;10(10):1-14.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                                      | 4,964 | 140 | Q2 |
| 11 | Skrzypczyk P, Ofiara A, Szyszka M, Stelmaszczyk-Emmel A, Górka E, Pańczyk-Tomaszewska M. Serum Sclerostin Is Associated with Peripheral and Central Systolic Blood Pressure in Pediatric Patients with Primary Hypertension. Journal of Clinical Medicine. 2021;10(16):1-13.<br><i>Rodzaj pracy: praca oryginalna</i>                         | 4,964 | 140 | Q2 |
| 12 | Skrzypczyk P, Bujanowicz A, Ofiara A, Szyszka M, Pańczyk-Tomaszewska M. 24-hour central blood pressure and immune system activation in adolescents with primary hypertension – a preliminary study. Central European Journal of Immunology. 2022;47(2):160-167.<br><i>Rodzaj pracy: praca oryginalna</i>                                      | 1,300 | 70  | Q4 |
| 13 | Bujanowicz A, Szyszka M, Skrzypczyk P. An adolescent with isolated systolic hypertension – case report and diagnostic and therapeutic approach. Pediaatria Polska. 2022;97(3):269-274.<br><i>Rodzaj pracy: praca poglądowa, opis przypadku</i>                                                                                                | -     | 100 | -  |
| 14 | Szyszka M, Skrzypczyk P, Ofiara A, Wabik A, Pietrzak R, Werner B, Pańczyk-Tomaszewska M. Circadian Blood Pressure Profile in Pediatric Patients with Primary Hypertension. Journal of Clinical Medicine. 2022;11(18):1-14.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                           | 3,900 | 140 | Q2 |

|    |                                                                                                                                                                                                                                                                                                                                                             |       |     |    |
|----|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|-----|----|
| 15 | Deja A, Skrzypczyk P, Nowak M, Wrońska M, Szyszka M, Ofiara A, Lesiak-Kosmatka J, Stelmaszczyk-Emmel A, Pańczyk-Tomaszewska M. Evaluation of Active Renin Concentration in A Cohort of Adolescents with Primary Hypertension. International Journal of Environmental Research and Public Health. 2022;19(10):1-13.<br><i>Rodzaj pracy: praca oryginalna</i> | -     | 140 | -  |
| 16 | Szmigielska A, Skrzypczyk P, Szyszka M, Bukowska M, Wojtas M, Jakimów-Kostrzewa A. IVCA in a Boy with Multilocular Renal Cyst as a Risk Factor for Deep Vein Thrombosis. Journal of Mother and Child. 2024;28(1):146-150.<br><i>Rodzaj pracy: opis przypadku</i>                                                                                            | -     | 20  | -  |
| 17 | Szyszka M, Skrzypczyk P. MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension—A Preliminary Study. Journal of Clinical Medicine. 2024;13(22):1-14.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                        | 2,900 | 140 | Q1 |
| 18 | Dziedzic-Jankowska K, Bujanowicz A, Szyszka M, Stelmaszczyk-Emmel A, Skrzypczyk P. Subclinical inflammation in paediatric patients with primary hypertension and white coat hypertension. Pediaatria i Medycyna Rodzinna. 2024;20(2):215-224.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                      | 0,100 | 20  | Q4 |
| 19 | Dziedzic-Jankowska K, Szyszka M, Bujanowicz A, Stelmaszczyk-Emmel A, Skrzypczyk P. Blood Pressure Variability and Low-Grade Inflammation in Pediatric Patients with Primary Hypertension. Journal of Clinical Medicine. 2025;14(16):1-17.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                          | 2,900 | 140 | Q1 |
| 20 | Dziedzic-Jankowska K, Pietrzak R, Szyszka M, Bujanowicz A, Stelmaszczyk-Emmel A, Werner B, Skrzypczyk P. Monocyte-to-Neutrophil Ratio as an Immunological Marker of Left Ventricular Hypertrophy in Children with Primary Hypertension. Journal of Clinical Medicine. 2025;14(11):1-17.<br><i>Rodzaj pracy: praca oryginalna</i>                            | 2,900 | 140 | Q1 |
| 21 | Sota M, Armuła M, Szyszka M [korespondencyjny], Skrzypczyk P. Urine Sodium Excretion in Children with Primary Hypertension: A Single-Center Retrospective Study. Journal of Clinical Medicine. 2025;14(24):1-20.<br><i>Rodzaj pracy: praca oryginalna</i>                                                                                                   | 2,900 | 140 | Q1 |
| 22 | Szyszka M, Eyileten C, Małyszko J, Skubisz K, Pietrzak R, Krysztosiak H, Skrzypczyk P. ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes. Kidney & Blood Pressure Research. 2026:1-31.<br><i>Rodzaj pracy: praca oryginalna</i>           | 2,100 | 70  | Q2 |
| 23 | Szyszka M, Pietrzak R, Obsznajczyk K, Skubisz K, Eyileten C, Skrzypczyk P. Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension. International Journal of Molecular Sciences. 2026;27(6):1-18.<br><i>Rodzaj pracy: praca oryginalna</i>                                                   | 4,900 | 140 | Q1 |

|                                                                                                                                                                                      |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------|------|----|
| 24                                                                                                                                                                                   | Szyszk M, Skrzypczyk P. MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review. Biomolecules. 2026;16(5):1-21.<br><i>Rodzaj pracy: praca poglądowa</i>                                                                                                                                         | 4,800  | 100  | Q1 |
| 25                                                                                                                                                                                   | Dąbek M, Szyszk M [korespondencyjny], Skrzypczyk P. Sodium and Water Homeostasis in Children: Pathogenesis, Diagnosis, and Treatment. Journal of Clinical Medicine. 2026;15(2):1-18.<br><i>Rodzaj pracy: praca poglądowa</i>                                                                                                                                  | 2,900  | 140  | Q1 |
| 26                                                                                                                                                                                   | Pięta J, Szyszk M [korespondencyjny], Lipiński P, Skrzypczyk P. Urolithiasis in Children—Clinical Picture, Pathogenesis, and Diagnostic Approach. Biomolecules. 2026;16(1):1-28.<br><i>Rodzaj pracy: praca poglądowa</i>                                                                                                                                      | 4,800  | 100  | Q1 |
| 27                                                                                                                                                                                   | Woźniak-Szewczyk W, Skrzypczyk P, Szyszk M [korespondencyjny], Niszczo C, Kucińska B, Stelmaszczyk-Emmel A, Panczyk M, Zacharzewska A, Tkacz K, Kuchar E, Okarska-Napierała M. Vascular and blood pressure alterations in children two years after multisystem inflammatory syndrome. Scientific Reports. 2026:1-39.<br><i>Rodzaj pracy: praca oryginalna</i> | 3,900  | 140  | Q1 |
| Łącznie:                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                               | 60,498 | 2495 | -  |
| II. Artykuły opublikowane przed 1.01.2019 r. w czasopismach ujętych w wykazie czasopism MNiSW z dnia 25.01.2017 r., o ile czasopismo uzyskało co najmniej 10 pkt.                    |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| Łącznie:                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                               | -      | -    | -  |
| III. Pozostałe artykuły.                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| 28                                                                                                                                                                                   | Szyszk M, Skrzypczyk P. Kamica układu moczowego u dzieci. Klinika Pediatria. 2021;29(4):471-479.<br><i>Rodzaj pracy: praca poglądowa</i>                                                                                                                                                                                                                      | -      | -    | -  |
| Łącznie:                                                                                                                                                                             |                                                                                                                                                                                                                                                                                                                                                               | -      | -    | -  |
| Łącznie (cz. I-III):                                                                                                                                                                 |                                                                                                                                                                                                                                                                                                                                                               | 60,498 | 2495 | -  |
| IV. Monografie naukowe/rozdziały w monografiach wydane przez wydawnictwa ujęte w wykazie MEIN [2] lub jednostki organizacyjne podmiotów, których wydawnictwa są ujęte w tym wykazie. |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| -                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| V. Pozostałe monografie lub rozdziały w monografiach.                                                                                                                                |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| -                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| VI. Patenty.                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |
| -                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                               |        |      |    |

\* Kwartył z roku publikacji, według Impact Factor.

[1]. Wykaz sporządzony zgodnie z przepisami wydanymi na podstawie art. 267 ust. 2 pkt 2 lit. b Ustawy z dnia 20 lipca 2018 r. - Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2022 r., poz. 574 z późn. zm.). Wykaz stanowi załącznik do komunikatu MNiSW z 5 stycznia 2024 r. w sprawie wykazu czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych.

[2]. Wykaz sporządzony zgodnie z przepisami wydanymi na podstawie art. 267 ust. 2 pkt 2 lit. a Ustawy z dnia 20 lipca 2018 r. - Prawo o szkolnictwie wyższym i nauce (Dz. U. z 2022 r., poz. 574 z późn. zm.). Wykaz ogłoszony komunikatem MEiN z dnia 22 lipca 2021 r. w sprawie wykazu wydawnictw publikujących recenzowane monografie naukowe.

## Spis treści

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## 1. Wykaz zastosowanych skrótów

**95CI** – ang. 95 percentile confidence interval, 95-procentowy przedział ufności

**ABPM** – ang. ambulatory blood pressure monitoring, całodobowa rejestracja ciśnienia tętniczego

**AUC** – ang. area under curve, pole pod krzywą

**cfPWV** – ang. carotid–femoral pulse wave velocity, prędkość fali tętna mierzona pomiędzy tętnicą szyjną a udową

**cIMT** – ang. carotid intima–media thickness, grubość kompleksu błona wewnętrzna–błona środkowa tętnicy szyjnej wspólnej

**DBP** – ang. diastolic blood pressure, ciśnienie tętnicze rozkurczowe

**HMOD** – ang. hypertension–mediated organ damage, uszkodzenie narządowe spowodowane nadciśnieniem tętniczym

**IVRT** – ang. isovolumic relaxation time, czas rozkurczu izowolumetrycznego

**LVH** – ang. left ventricular hypertrophy, przerost mięśnia lewej komory serca

**LVMI** – ang. left ventricular mass index, indeks masy lewej komory serca

**LV GLS** - ang. left ventricular global longitudinal strain, globalne odkształcenie podłużne lewej komory serca

**MAP** – ang. mean arterial pressure, ciśnienie tętnicze średnie

**NT** – nadciśnienie tętnicze

**NTP** – nadciśnienie tętnicze pierwotne

**OR** – ang. odds ratio, iloraz szans

**PWV** – ang. pulse wave velocity, prędkość fali tętna

**RAA** – ang. renin-angiotensin-aldosterone, renina-angiotensyna-aldosteron

**ROC** – ang. receiver operating characteristic, krzywa charakterystyki operacyjnej

**SBP** – ang. systolic blood pressure, ciśnienie tętnicze skurczowe

**SD** – ang. standard deviation, odchylenie standardowe

**STE** – ang. speckle tracking echocardiography, echokardiografia śledząca markery akustyczne

## **2. Streszczenie w języku polskim**

### **Badanie ATHENA: Patogeneza nadciśnienia tętniczego - mikroRNA jako brakujące ogniwo w rozwoju nadciśnienia tętniczego u dzieci.**

#### **Wprowadzenie:**

Nadciśnienie tętnicze (NT) występuje u 3-5% dzieci, a ok. połowy przypadków stanowi nadciśnienie tętnicze pierwotne (NTP). Większość przypadków NT przebiega bezobjawowo. U dzieci i młodzieży z NT poważne powikłania należą do rzadkości. Częstsze jest występowanie subklinicznych powikłań narządowych, takich jak przerost lewej komory serca. Patogeneza nadciśnienia tętniczego pierwotnego (NTP) jest wieloczynnikowa (czynniki genetyczne i środowiskowe, substancje produkowane przez śródbłonek (np. tlenek azotu), aktywacja współczulnego układu nerwowego i układu renina-angiotensyna-aldosteron).

MikroRNA to małe, jednoniciowe cząsteczki kwasu rybonukleinowego, które powstają w jądrze komórki w procesie transkrypcji na matrycy DNA. Cząsteczki te łączą się ze specyficznymi mRNA, prowadząc do ich rozpadu lub zahamowania translacji – zjawisko to znane jest jako potranskrypcyjna regulacja ekspresji genów. Zaburzenia w powyższym procesie mogą powodować upośledzenie funkcji poszczególnych komórek organizmu, co prowadzi do rozwoju wielu chorób. Dobrze przebadanymi cząsteczkami mikroRNA u dorosłych chorych z NT są: mikroRNA-16, mikroRNA-21, mikroRNA-27a/b, mikroRNA-133a i mikroRNA-145. Regularna aktywność fizyczna jest jedną z nefarmakologicznych metod zapobiegania i leczenia NT. Krótkotrwały, jak i regularny, aerobowy wysiłek fizyczny obniża ciśnienie tętnicze u dorosłych. Mniej jest danych dotyczących tego zjawiska u pacjentów pediatrycznych. Brak jest danych na temat związku pomiędzy mikroRNA a NTP i uszkodzeniem narządowym u dzieci, a także brak informacji na temat związku mikroRNA z ciśnieniem tętniczym po pojedynczej sesji ćwiczeń aerobowych u pacjentów z nadciśnieniem tętniczym.

**Cele pracy:**

1. Ocena ekspresji mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a i mikroRNA-145 u dzieci i młodzieży z nadciśnieniem tętniczym pierwotnym i u zdrowych rówieśników.
2. Ocena zależności pomiędzy poziomem ekspresji powyższych mikroRNA a ciśnieniem tętniczym u nieleczonych farmakologicznie dzieci z nadciśnieniem tętniczym pierwotnym.
3. Ocena zależności pomiędzy poziomem ekspresji mikroRNA a zmianami narządowymi: masą lewej komory w badaniu echokardiograficznym, grubością kompleksu błona wewnętrzna–błona środkowa tętnic szyjnych wspólnych (cIMT) oraz sztywnością tętnic u dzieci z nadciśnieniem tętniczym pierwotnym.
4. Ocena krążących mikroRNA jako potencjalnych biomarkerów zaburzeń funkcji skurczowej serca wyrażonych jako odkształcenie podłużne lewej komory (LV GLS) u dzieci z nadciśnieniem tętniczym pierwotnym.
5. Ocena wpływu pojedynczej sesji standaryzowanego wysiłku aerobowego na ciśnienie tętnicze i ekspresję mikroRNA u dzieci i młodzieży z NTP i u zdrowych rówieśników.

**Materiał i metody:**

Badania miały charakter obserwacyjny, częściowo przekrojowy oraz częściowo typu badania pre-post – po pojedynczej sesji standaryzowanego wysiłku aerobowego. Do analiz włączono dzieci i młodzież z nowo rozpoznany, nieleczonym farmakologicznie nadciśnieniem tętniczym pierwotnym oraz zdrowych rówieśników stanowiących grupę kontrolną. Badaniem objęto 56 dzieci z nadciśnieniem tętniczym pierwotnym ( $15,4 \pm 1,9$  lat, 42 chłopców, 14 dziewczynek) oraz 59 zdrowych rówieśników ( $15,3 \pm 1,6$  lat, 41 chłopców, 18 dziewczynek). Nadciśnienie tętnicze rozpoznawano zgodnie z aktualnymi wytycznymi European Society of Hypertension oraz potwierdzano w ABPM.

U wszystkich uczestników oceniano podstawowe parametry antropometryczne, w tym wzrost, masę ciała, wskaźnik masy ciała (BMI) oraz ich wartości Z-score. Wykonywano pomiary gabinetowego ciśnienia tętniczego metodą oscylometryczną oraz ABPM z jednoczesną analizą parametrów hemodynamicznych i ciśnienia centralnego. Analizowano między innymi średnie wartości skurczowego, rozkurczowego i średniego ciśnienia tętniczego obwodowego (SBP, DBP, MAP) oraz ciśnienia centralnego (AoSBP, AoDBP, AoMAP).

W ramach oceny uszkodzenia narządowego wykonywano badania echokardiograficzne z oceną masy lewej komory (LVM) oraz wskaźnika masy lewej komory (LVMI). Dodatkowo

oceniano globalne odkształcenie podłużne lewej komory (LV GLS) metodą śledzenia markerów akustycznych w badaniu echokardiograficznym. Zmierzono również grubość kompleksu błona wewnętrzna–błona środkowa tętnic szyjnych wspólnych (cIMT), szyjno-udową prędkość fali tętna (cfPWV) oraz wskaźnik albumina-kreatynina w porannej porcji moczu (ACR).

W głównym badaniu analizowano wpływ pojedynczej, standaryzowanej sesji wysiłku aerobowego na ekspresję mikroRNA oraz parametry ciśnienia tętniczego. W tym celu wszyscy uczestnicy zostali poddani próbie spiroergometrycznej (CPET) w celu indywidualnego wyznaczenia intensywności wysiłku. Następnie, po 2–6 tygodniach, wykonywali 60-minutowy nadzorowany wysiłek na ergometrze rowerowym o intensywności odpowiadającej 90–110% obciążenia uzyskanego dla progu beztlenowego (AT), zgodnie z zaleceniami American College of Sports Medicine dotyczącymi wysiłku o umiarkowanej intensywności. Próbkę krwi pobierano rano, na czczo, przed oraz po wysiłku fizycznym.

Do analizy molekularnej pobierano osocze krwi żyłnej za pomocą zestawów próżniowych Vacutainer. Izolację mikroRNA przeprowadzano z użyciem komercyjnych zestawów diagnostycznych mirVana PARIS, a następnie wykonywano odwrotną transkrypcję oraz ilościową reakcję PCR w czasie rzeczywistym (qPCR) z zastosowaniem testów i sond TaqMan. Analizowano ekspresję wybranych mikroRNA: mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145. Względą ekspresję oceniano metodą  $2^{-\Delta\Delta C_t}$ .

## **Wyniki:**

W pierwszej publikacji, obejmującej wyniki badania pilotażowego, oceniono wpływ wysiłku fizycznego na ekspresję wybranych krążących mikroRNA u 22 dzieci z nadciśnieniem tętniczym pierwotnym (NTP). Wykazano, że zmiany ekspresji mikroRNA po wysiłku fizycznym korelowały z parametrami nadciśnieniowego uszkodzenia narządowego (HMOD): Wzrost ekspresji mikroRNA-133a był związany z większą masą lewej komory serca (LVMI,  $r=0,491$ ,  $p=0,020$ ), natomiast zwiększenie ekspresji mikroRNA-145 korelowało z niższymi wartościami skurczowego ciśnienia tętniczego w 24-godzinnym pomiarze ( $r= -0,430$ ,  $p=0,046$ ) oraz mniejszym nasileniem zmian naczyniowych ocenianych między innymi za pomocą grubości kompleksu błona wewnętrzna–błona środkowa tętnic szyjnych wspólnych (cIMT,  $r=0,445$ ,  $p=0,033$ ) oraz prędkości fali tętna (cfPWV,  $r=-0,557$ ,  $p=0,007$ ).

W drugiej, głównej publikacji u 56 pacjentów z nadciśnieniem tętniczym pierwotnym oraz 59 zdrowych dzieci wykazano odmienny profil ekspresji wybranych krążących mikroRNA

u dzieci z NTP w porównaniu ze zdrowymi rówieśnikami. Ekspresja mikroRNA-27b oraz mikroRNA-133a była istotnie obniżona u pacjentów z NTP. Ekspresja mikroRNA-16, -21, -27a i -145 korelowała również z wartościami ciśnienia tętniczego u dzieci z NTP. Ponadto w modelu wieloczynnikowej regresji logistycznej wykazano, że wysoka wyjściowa ekspresja mikroRNA-16 była niezależnym czynnikiem różnicującym pacjentów z wyższymi ( $>2,1$ ) i niższymi wartościami DBP Z-score. Nie wykazano istotnych korelacji pomiędzy poziomem ekspresji badanych mikroRNA a wskaźnikami HMOD.

W tej samej pracy wykazano również istotne obniżenie wartości ciśnienia tętniczego po wysiłku fizycznym u dzieci z NTP, którego nie obserwowano w grupie kontrolnej, a także różnice między grupami w odpowiedzi wybranych mikroRNA na wysiłek fizyczny. W grupie kontrolnej po wysiłku obserwowano wzrost ekspresji mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145, natomiast u dzieci z NTP istotnemu zwiększeniu ulegała jedynie ekspresja mikroRNA-145.

W trzeciej publikacji u 50 pacjentów z nadciśnieniem tętniczym pierwotnym oraz 57 zdrowych rówieśników oceniono parametry echokardiograficzne w kontekście ekspresji krążących mikroRNA. Wykazano, że dzieci z NTP cechują się mniej ujemnymi wartościami globalnego odkształcenia podłużnego lewej komory (LV GLS,  $-19.28\% \pm 3.02$  vs.  $-20.99\% \pm 3.11$ ,  $p=0.002$ ). Dodatkowo stwierdzono istotną korelację pomiędzy ekspresją mikroRNA-16 a wartościami LV GLS ( $R = 0.305$ ;  $p = 0.031$ ).

### **Wnioski:**

1. Dzieci z nadciśnieniem tętniczym pierwotnym cechują się odmiennym profilem ekspresji wybranych krążących mikroRNA w porównaniu ze zdrowymi rówieśnikami, co może wskazywać na udział mechanizmów epigenetycznych w patogenezie nadciśnienia tętniczego w wieku rozwojowym. Ekspresja mikroRNA-27b oraz mikroRNA-133a jest obniżona u dzieci z nadciśnieniem tętniczym pierwotnym.
2. U nieleczonych dzieci z nadciśnieniem tętniczym pierwotnym ekspresja mikroRNA-16, mikroRNA-21, mikroRNA-27a i mikroRNA-145 koreluje dodatnio z wartościami ciśnienia tętniczego, co może sugerować udział tych cząsteczek w patogenezie nadciśnienia tętniczego pierwotnego. Wysoka ekspresja mikroRNA-16 jest związana z wyższymi wartościami ciśnienia tętniczego rozkurczowego i może stanowić potencjalny biomarker ciężkości nadciśnienia u dzieci.

3. U dzieci z nadciśnieniem tętniczym pierwotnym brak jest korelacji pomiędzy klasycznymi parametrami uszkodzenia narządowego a ekspresją cząsteczek mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a i mikroRNA-145.
4. MikroRNA-16 może stanowić potencjalny biomarker wczesnego uszkodzenia skurczowego lewej komory serca, co może wskazywać na udział tej cząsteczki we wczesnych etapach przebudowy układu sercowo-naczyniowego u dzieci z nadciśnieniem tętniczym pierwotnym.
5. Pojedyncza sesja standaryzowanego wysiłku aerobowego prowadzi, inaczej niż u zdrowych rówieśników, do istotnego obniżenia wartości ciśnienia tętniczego u dzieci z nadciśnieniem tętniczym pierwotnym oraz wpływa na ekspresję wybranych mikroRNA, co podkreśla potencjalne znaczenie aktywności fizycznej w modulacji mechanizmów związanych z rozwojem nadciśnienia tętniczego w wieku rozwojowym.

### **3. Streszczenie w języku angielskim**

#### **ATHENA Study - ArTerial HypErtension PAtogenesis - microRNA as a missing link in the disease development in children.**

##### **Introduction:**

Arterial hypertension (HTN) occurs in 3–5% of children, and approximately half of the cases are primary hypertension (PH). Most cases of HTN are asymptomatic. Serious complications are rare in children and adolescents with HTN. Subclinical organ damage, such as left ventricular hypertrophy, is more common. The pathogenesis of PH is multifactorial (genetic and environmental factors, the sympathetic nervous system, the renin–angiotensin–aldosterone system, and substances produced by the endothelium, e.g., nitric oxide).

MicroRNAs are small, single-stranded ribonucleic acid molecules that are formed in the cell nucleus in the process of transcription from a DNA template. These molecules bind to specific mRNAs, leading to their degradation or inhibition of translation - this phenomenon is known as post-transcriptional regulation of gene expression. Disturbances in the above process may impair the function of individual cells in the body, leading to the development of many diseases. MicroRNA molecules that have been well studied in adults with HTN include microRNA-16, microRNA-21, microRNA-27a/b, microRNA-133a, and microRNA-145. Regular physical activity is one of the non-pharmacological methods of preventing and treating HTN. Both short-term and regular aerobic exercise reduce blood pressure in adults. There is limited data on this phenomenon in pediatric patients. There are no data on the relationship between microRNAs and PH and hypertension-mediated organ damage (HMOD) in children, nor on the relationship between microRNAs and blood pressure after a single session of aerobic exercise in patients with HTN.

**Objectives:**

1. Assessment of the expression of microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145 in children and adolescents with PH and in healthy peers.
2. Assessment of the relationship between the expression levels of the above microRNAs and blood pressure in pharmacologically untreated children with primary hypertension.
3. Assessment of the relationship between microRNA expression levels and hypertension-mediated organ damage: left ventricular mass index (LVMI) assessed by echocardiography, common carotid artery intima-media thickness (cIMT), and arterial stiffness in children with primary hypertension.
4. Assessment of circulating microRNAs as potential biomarkers of impaired cardiac systolic function expressed as left ventricular global longitudinal strain (LV GLS) in children with primary hypertension.
5. Assessment of the effect of a single session of standardized aerobic exercise on blood pressure and microRNA expression in children and adolescents with PH and in healthy peers.

**Materials and Methods:**

The studies were observational, partly cross-sectional and partly pre-post studies following a single session of standardized aerobic exercise. The analyses included children and adolescents with newly diagnosed, pharmacologically untreated PH and healthy peers constituting the control group. The study included 56 children with primary hypertension ( $15.4 \pm 1.9$  years, 42 boys, 14 girls) and 59 healthy peers ( $15.3 \pm 1.6$  years, 41 boys, 18 girls). The diagnosis of arterial hypertension was established according to the current European Society of Hypertension guidelines and confirmed by ABPM.

Basic anthropometric parameters, including height, body weight, body mass index (BMI), and their Z-score values, were assessed in all participants. Office blood pressure measurements were performed using the oscillometric method, and ABPM was performed with simultaneous analysis of hemodynamic parameters and central blood pressure. The analyzed parameters included mean systolic, diastolic, and mean peripheral blood pressure values (SBP, DBP, MAP) and central blood pressure (AoSBP, AoDBP, AoMAP).

As part of the assessment of HMOD, echocardiographic examinations were performed with assessment of left ventricular mass (LVM) and left ventricular mass index (LVMI). In

addition, left ventricular global longitudinal strain (LV GLS) was assessed using speckle-tracking echocardiography. Common carotid artery intima–media thickness (cIMT), carotid–femoral pulse wave velocity (cfPWV), and the albumin-to-creatinine ratio in a morning urine sample (ACR) were also measured.

The main study analyzed the effect of a single standardized session of aerobic exercise on microRNA expression and blood pressure parameters. For this purpose, all participants underwent cardiopulmonary exercise testing (CPET) to individually determine exercise intensity. Subsequently, after 2–6 weeks, they performed 60 minutes of supervised exercise on a cycle ergometer at an intensity corresponding to 90–110% of the workload achieved at the anaerobic threshold (AT), in accordance with the American College of Sports Medicine recommendations concerning moderate-intensity exercise. Blood samples were collected in the morning, after fasting, before and after physical exercise.

For molecular analysis, venous blood plasma was collected using Vacutainer systems. MicroRNA isolation was performed using commercially available mirVana PARIS diagnostic kits, followed by reverse transcription and quantitative real-time PCR (qPCR) using TaqMan assays and probes. The expression of selected microRNAs was analyzed: microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145. Relative expression was assessed using the  $2^{-\Delta\Delta C_t}$  method.

## **Results:**

In the first publication, which included the results of the pilot study, the effect of physical exercise on the expression of selected circulating microRNAs was assessed in 22 children with primary hypertension (PH). It was demonstrated that changes in microRNA expression after physical exercise correlated with parameters of HMOD: an increase in microRNA-133a expression was associated with greater left ventricular mass (LVMI,  $r = 0.491$ ,  $p = 0.020$ ), whereas an increase in microRNA-145 expression correlated with lower systolic blood pressure values in 24-hour monitoring ( $r = -0.430$ ,  $p = 0.046$ ) and with a lower severity of arterial damage as assessed, among others, by common carotid artery intima–media thickness (cIMT,  $r = 0.445$ ,  $p = 0.033$ ) and pulse wave velocity (cfPWV,  $r = -0.557$ ,  $p = 0.007$ ).

In the second, main publication, in 56 patients with primary hypertension and 59 healthy children, a different expression profile of selected circulating microRNAs was demonstrated in children with PH compared with healthy peers. The expression of microRNA-27b and microRNA-133a was significantly reduced in patients with PH. The expression levels of microRNA-16, -21, -27a, and -145 also correlated positively with blood pressure values.

Furthermore, in a multivariable logistic regression model, high baseline microRNA-16 expression was an independent factor distinguishing patients with higher (>2.1) versus lower DBP Z-score values. No significant correlations were found between the expression levels of the investigated microRNAs and markers of HMOD.

The same study also demonstrated a significant reduction in blood pressure following exercise in children with PH, which was not observed in the control group, as well as between-group differences in the exercise-induced responses of selected microRNAs. In the control group, exercise increased microRNA-27b, microRNA-133a, and microRNA-145 expression, whereas in children with PH, only microRNA-145 expression increased significantly.

In the third publication, echocardiographic parameters were assessed in relation to circulating microRNA expression levels in 50 patients with primary hypertension and 57 healthy controls. It was demonstrated that children with PH were characterized by less negative left ventricular global longitudinal strain values (LV GLS,  $-19.28\% \pm 3.02$  vs.  $-20.99\% \pm 3.11$ ,  $p = 0.002$ ). In addition, a significant correlation was found between microRNA-16 expression and LV GLS values ( $R = 0.305$ ;  $p = 0.031$ ).

### **Conclusions:**

1. Children with primary hypertension are characterized by a different expression profile of selected circulating microRNAs compared with healthy peers, which may indicate the involvement of epigenetic mechanisms in the pathogenesis of arterial hypertension in the pediatric population. The expression of microRNA-27b and microRNA-133a is reduced in children with primary hypertension.
2. In untreated children with primary hypertension, higher expression of microRNA-16, microRNA-21, microRNA-27a, and microRNA-145 correlates positively with blood pressure values, which may suggest the involvement of these molecules in the pathogenesis of primary hypertension. High microRNA-16 expression is associated with higher diastolic blood pressure values and may constitute a potential biomarker of hypertension severity in children.
3. In children with primary hypertension, there is no correlation between classical parameters of organ damage and the expression of microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145.
4. MicroRNA-16 may constitute a potential biomarker of early systolic damage of the left

ventricle, which may indicate the involvement of this molecule in the early stages of cardiovascular remodeling in children with primary hypertension.

5. A single session of standardized aerobic exercise, unlike in healthy peers, leads to a significant reduction in blood pressure values in children with primary hypertension and affects the expression of selected microRNAs, which highlights the potential importance of physical activity in the modulation of mechanisms associated with the development of arterial hypertension in the pediatric population.

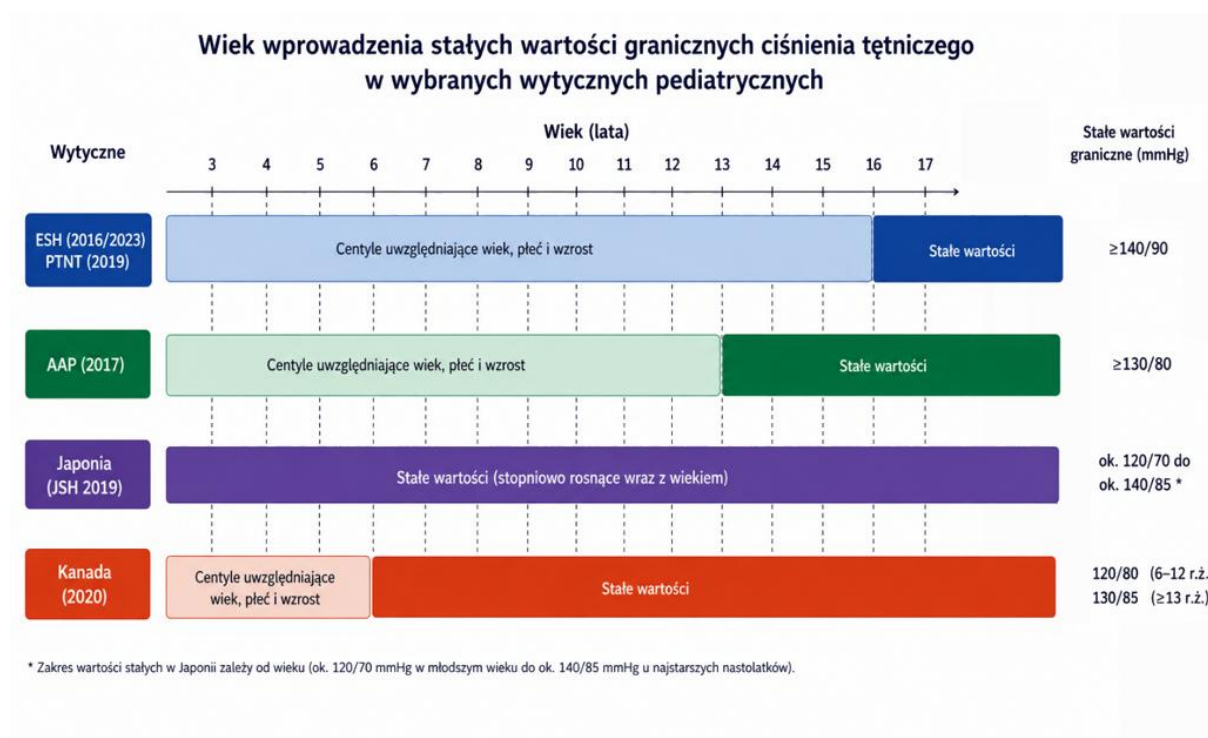
## 4. Wstęp

### 4.1. Nadciśnienie tętnicze u dzieci

Nadciśnienie tętnicze (NT) jest jedną z najczęściej występujących chorób przewlekłych na świecie oraz jednym z najważniejszych modyfikowalnych czynników ryzyka chorób sercowo-naczyniowych. Ze względu na etiologię NT możemy wyróżnić nadciśnienie tętnicze pierwotne (NTP) i wtórne. Większość przypadków NTP u dzieci i młodzieży przebiega bezobjawowo. Pomimo braku jawnych objawów klinicznych już we wczesnym okresie choroby mogą rozwijać się zmiany narządowe obejmujące serce, nerki oraz naczynia tętnicze. Wczesna identyfikacja mechanizmów odpowiedzialnych za rozwój NT oraz markerów pozwalających na wykrycie subklinicznych zmian narządowych pozostaje jednym z ważniejszych wyzwań współczesnej hipertensjologii dziecięcej.

Zarówno Polskie Towarzystwo Nadciśnienia Tętniczego (PTNT), jak i Europejskie Towarzystwo Nadciśnienia Tętniczego (ang. *European Society of Hypertension* – ESH) rekomendują rozpoznawanie nadciśnienia tętniczego u dzieci do ukończenia 16. roku życia na podstawie wartości centylowych uwzględniających płeć, wiek i wzrost, natomiast u młodzieży w wieku 16–17 lat stosowanie wartości granicznych obowiązujących u dorosłych ( $\geq 140/90$  mmHg). Rozpoznanie nadciśnienia wymaga stwierdzenia podwyższonych wartości ciśnienia tętniczego podczas co najmniej trzech niezależnych wizyt lekarskich, a do badań przesiewowych zaleca się stosowanie automatycznych aparatów oscylometrycznych z potwierdzeniem rozpoznania metodą osłuchową [1-4].

Należy jednak podkreślić, że nie wszystkie międzynarodowe wytyczne przyjmują takie samo podejście. Amerykańskie zalecenia (AAP) od 13. roku życia stosują stałą wartość graniczną 130/80 mmHg, natomiast wytyczne japońskie i kanadyjskie wprowadzają wartości bezwzględne już we wcześniejszym wieku. Różnice te mogą wpływać na częstość rozpoznawania nadciśnienia tętniczego, kwalifikację pacjentów do dalszej diagnostyki oraz interpretację wyników badań epidemiologicznych i klinicznych. Porównanie wybranych rekomendacji przedstawiono na Rycinie 1.



Rycina 1. Porównanie wybranych wytycznych dotyczących wieku, od którego wartości ciśnienia tętniczego są interpretowane na podstawie stałych progów diagnostycznych zamiast norm centylowych zależnych od wieku, płci i wzrostu. ESH – European Society of Hypertension; PTNT – Polskie Towarzystwo Nadciśnienia Tętniczego; AAP – American Academy of Pediatrics; JSH – Japanese Society of Hypertension. Opracowanie własne na podstawie wytycznych ESH/PTNT, AAP, JSH oraz Hypertension Canada’s.

## 4.2. Epidemiologia i etiologia nadciśnienia tętniczego w wieku rozwojowym

Według dostępnych danych epidemiologicznych NT występuje u ponad 40% dorosłych Polaków [5]. Globalną chorobowość NT WHO szacuje na około 1,4 miliarda osób w wieku 30–79 lat. Mimo że choroba jest zdecydowanie częstsza w populacji osób dorosłych, w ostatnich dekadach obserwuje się również wzrost częstości jej występowania wśród dzieci i młodzieży, co wiąże się między innymi z narastającym problemem nadwagi i otyłości. Zgodnie z wynikami metaanalizy przeprowadzonej przez Songa i wsp., obejmującej 47 badań z udziałem 186 630 dzieci i młodzieży, częstość występowania nadciśnienia tętniczego w populacji pediatrycznej wynosi około 3–5%. W okresie dojrzewania NT występuje 3–4 razy częściej u chłopców niż u dziewcząt, a najczęstszą postacią choroby w tej grupie wiekowej jest NTP.

Najczęściej spotykanym fenotypem NT u dzieci jest izolowane nadciśnienie skurczowe, którego częstość występowania wynosi około 1,5% w populacji ogólnej dzieci i młodzieży, co odpowiada 37,5% wszystkich przypadków nadciśnienia tętniczego. Kolejno, tylko nieco

rzadziej występuje nadciśnienie skurczowo-rozkurczowe (1,25% populacji; 31,25% przypadków nadciśnienia), natomiast najrzadziej rozpoznawane jest izolowane nadciśnienie rozkurczowe (0,75% populacji; 18,75% przypadków nadciśnienia tętniczego).

W cytowanej metaanalizie nie stwierdzono istotnych różnic w częstości występowania nadciśnienia tętniczego pomiędzy chłopcami a dziewczynkami; nie analizowano również zależności od wieku. Wykazano natomiast silny związek pomiędzy występowaniem NT, a nadmierną masą ciała. Chorobowość nadciśnienia była najwyższa w grupie dzieci z otyłością (15,27%), niższa wśród dzieci z nadwagą (4,99%), a najniższa wśród dzieci z prawidłową masą ciała (1,90%) [6].

Dostępne dane epidemiologiczne wskazują na stopniowy wzrost częstości występowania NT wraz z wiekiem, ze szczególnie dynamicznym wzrostem obserwowanym w okresie pokwitania. Zjawisko to dotyczy przede wszystkim chłopców, u których wartości ciśnienia tętniczego rosną szybciej niż u dziewcząt, co ma odzwierciedlenie w dostępnych siatkach centylowych. W polskim badaniu przeprowadzonym wśród 1472 osiemnastoletnich uczniów szkół średnich wykazano, że NT rozpoznawane według kryteriów dla dorosłych występowało u 9% badanych, w tym u 16,2% chłopców i jedynie 0,9% dziewcząt. Wyniki te wskazują, że pod koniec wieku rozwojowego częstość występowania nadciśnienia tętniczego osiąga poziom porównywalny z obserwowanym w populacji młodych dorosłych, przy znaczącej przewadze mężczyzn. Ponadto w pracy wykazano i podkreślono ścisły związek między nadmierną masą ciała a ryzykiem wystąpienia NT w tej grupie pacjentów [7].

W przeciwieństwie do populacji osób dorosłych, w której zdecydowaną większość przypadków stanowi NTP, przez wiele lat uważano, że w wieku rozwojowym dominuje nadciśnienie wtórne. Pogląd ten wynikał przede wszystkim z częstego występowania chorób nerek, wad układu moczowego oraz innych schorzeń mogących prowadzić do wtórnego wzrostu ciśnienia tętniczego już we wczesnym dzieciństwie. Wśród dzieci z nadciśnieniem wtórnym najczęstszą przyczynę stanowią choroby miększu nerek, odpowiadające za około 40–45% przypadków. Istotny odsetek stanowią również koarktacja aorty, nadciśnienie naczyniowo-nerkowe (w naszym rejonie świata przede wszystkim na tle dysplazji włóknisto-mięśniowej) oraz przyczyny polekowe (glikokortykosteroidy, inhibitory kalcyneuryny), natomiast przyczyny endokrynologiczne (m. in. hiperkortyzolemia, guzy chromochłonne/przyzwojaki), neurologiczne, nowotworowe czy NT monogenowe występują zdecydowanie rzadziej [8].

W ostatnich latach pogląd o dominacji nadciśnienia wtórnego w wieku rozwojowym uległ jednak istotnej zmianie. Współczesne dane epidemiologiczne wskazują, że NTP

odpowiada obecnie nawet za połowę przypadków nadciśnienia tętniczego w populacji pediatrycznej. Choć u najmłodszych dzieci nadal dominują wtórne postacie choroby, znaczenie nadciśnienia pierwotnego stopniowo wzrasta wraz z wiekiem. NTP może być rozpoznawane już u dzieci w wieku przedszkolnym, nawet około 3. roku życia, zwłaszcza u pacjentów obciążonych czynnikami ryzyka sercowo-naczyniowego, takimi jak otyłość, nadwaga oraz dodatni wywiad rodzinny w kierunku nadciśnienia tętniczego [9, 10].

Potwierdzają to również dane pochodzące z Polski. W analizie obejmującej 2008 pacjentów jednego ośrodka referencyjnego, spośród 1260 dzieci z potwierdzonym nadciśnieniem tętniczym u 49,3% rozpoznano nadciśnienie pierwotne, a u 50,7% nadciśnienie wtórne. Wykazano ponadto, że prawdopodobieństwo rozpoznania nadciśnienia pierwotnego było istotnie większe u chłopców, dzieci w wieku powyżej 12 lat, pacjentów z nadmierną masą ciała oraz osób z hiperurykemią [8]. Co istotne, u blisko co piątego nastolatka z pierwotnym nadciśnieniem tętniczym stwierdza się współistnienie zespołu metabolicznego [11].

Podsumowując, NT należy obecnie uznać za jedno z najważniejszych przewlekłych schorzeń wieku rozwojowego. Szczególne znaczenie kliniczne ma NTP, którego częstość występowania wzrasta równolegle z epidemią otyłości i innych zaburzeń kardiometabolicznych obserwowanych u dzieci i młodzieży. Coraz częstsze rozpoznawanie nadciśnienia pierwotnego już w młodszych grupach wiekowych, częste współwystępowanie nadwagi, otyłości, hiperurykemii oraz zespołu metabolicznego, a także obecność wczesnych zmian narządowych wskazują, że choroba ta stanowi ważny problem zdrowia publicznego. Wartości ciśnienia tętniczego w dzieciństwie wykazują silny związek z jego wartościami w wieku dorosłym, a podwyższone ciśnienie tętnicze w wieku rozwojowym stanowi istotny czynnik predykcyjny rozwoju NT w późniejszym życiu [12]. Co istotne, wykazano, że wartości ciśnienia tętniczego u młodzieży, nawet mieszczące się w zakresie normotensji, są liniowo związane z ryzykiem rozwoju NT u młodych dorosłych [13]. Wczesna identyfikacja dzieci obciążonych czynnikami ryzyka oraz skuteczne działania profilaktyczne i terapeutyczne mogą mieć kluczowe znaczenie w ograniczaniu ryzyka chorób sercowo-naczyniowych w późniejszym życiu.

#### **4.3. Patogeneza nadciśnienia tętniczego pierwotnego w wieku rozwojowym**

Pomimo intensywnych badań patogeneza NTP nadal nie została w pełni wyjaśniona i obecnie uważa się, że ma charakter wieloczynnikowy. W ujęciu hemodynamicznym wysokość ciśnienia tętniczego zależy od pojemności minutowej serca oraz całkowitego oporu obwodowego naczyń, co można przedstawić równaniem:  $MAP = CO \times TPR$ , gdzie pojemność minutowa serca (CO) jest wypadkową objętości wyrzutowej (SV) i częstości akcji serca (HR).

W warunkach fizjologicznych parametry te są ściśle regulowane przez współdziałające mechanizmy neurohormonalne, autonomiczny układ nerwowy, nerki oraz śródbłonek naczyniowy, których zadaniem jest utrzymanie prawidłowej perfuzji narządowej i stabilnych warunków hemodynamicznych. Rozwój nadciśnienia tętniczego jest konsekwencją zaburzenia równowagi pomiędzy tymi układami, prowadzącego do zwiększenia pojemności minutowej serca, wzrostu oporu naczyń obwodowych lub współwystępowania obu tych zjawisk [14].

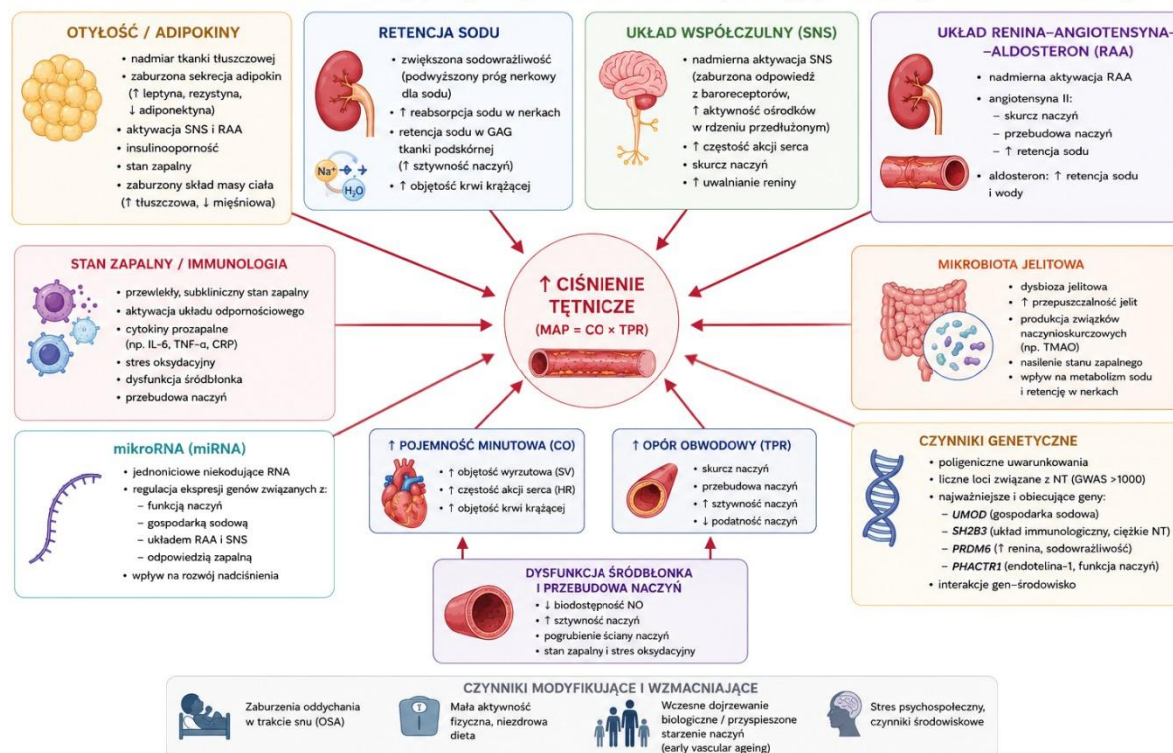
W świetle obecnej wiedzy patogeneza NTP w wieku rozwojowym obejmuje współdziałanie licznych mechanizmów genetycznych, metabolicznych, immunologicznych oraz środowiskowych. Szczególną rolę przypisuje się zaburzeniom gospodarki sodowej prowadzącym do zwiększonej sodowrażliwości i retencji sodu, nieprawidłowej aktywacji układów neurohormonalnych oraz zaburzeń funkcji śródbłonka naczyniowego. Istotne znaczenie mają również nieprawidłowości składu ciała związane z nadwagą i otyłością, obejmujące zwiększoną ilość tkanki tłuszczowej, względny niedobór masy mięśniowej, insulinooporność oraz zaburzoną sekrecję cytokin produkowanych przez tkankę tłuszczową (adipokiny). Czynniki te sprzyjają retencji sodu, nasileniu aktywności współczulnej oraz przebudowie naczyń, prowadząc do utrwalonego wzrostu ciśnienia tętniczego [15].

Coraz więcej dowodów wskazuje także na znaczenie przewlekłego stanu zapalnego o niewielkim nasileniu oraz aktywacji układu odpornościowego w rozwoju nadciśnienia tętniczego. W proces ten mogą być zaangażowane zaburzenia składu i funkcji mikrobioty jelitowej, a także czynniki epigenetyczne, w tym mikroRNA regulujące ekspresję genów związanych z funkcją naczyń, nerek oraz układów neurohormonalnych. Dodatkowo u dzieci z nadciśnieniem tętniczym pierwotnym obserwuje się cechy przyspieszonego dojrzenia biologicznego oraz przedwczesnego starzenia naczyń (ang. *early vascular ageing* – EVA), manifestujące się zwiększoną sztywnością tętnic i pogrubieniem ich ściany [10].

Znaczenie mają również czynniki genetyczne. Badania asocjacyjne całego genomu (ang. *genome-wide association studies* – GWAS) zidentyfikowały ponad tysiąc loci związanych z regulacją ciśnienia tętniczego, jednak wpływ pojedynczych wariantów genetycznych jest zazwyczaj niewielki. Szczególne zainteresowanie budzą obecnie geny związane z regulacją gospodarki sodowej, aktywnością układu renina–angiotensyna–aldosteron, funkcją śródbłonka oraz odpowiedzią immunologiczną [2, 14, 16, 17].

Główne i obiecujące czynniki wpływające na podwyższenie ciśnienia tętniczego krwi zostały przedstawione na Rycinie 2.

## Główne mechanizmy patogenetyczne nadciśnienia tętniczego pierwotnego u dzieci i młodzieży



Rycina 2. Główne mechanizmy patogenetyczne nadciśnienia tętniczego pierwotnego w wieku rozwojowym.

→ - mechanizm wpływający na ciśnienie tętnicze

Skróty: CO (ang. *cardiac output*) – pojemność minutowa serca; HR (ang. *heart rate*) – częstość akcji serca; MAP (ang. *mean arterial pressure*) – średnie ciśnienie tętnicze; mikroRNA (ang. *microRNA*) – mikroRNA; NO (ang. *nitric oxide*) – tlenek azotu; OSA (ang. *obstructive sleep apnea*) – obturacyjny bezdech senny; RAA (ang. *renin-angiotensin-aldosterone*) – renina-angiotensyna-aldosteron; SNS (ang. *sympathetic nervous system*) – układ współczulny; SV (ang. *stroke volume*) – objętość wyrzutowa serca; TMAO (ang. *trimethylamine N-oxide*) – N-tlenek trimetyloaminy; TPR (ang. *total peripheral resistance*) – całkowity opór obwodowy; GWAS (ang. *genome-wide association studies*) – badania asocjacyjne całego genomu; HPA (ang. *hypothalamic-pituitary-adrenal axis*) – oś podwzgórze-przysadka-nadnercza; IL-6 (ang. *interleukin 6*) – interleukina 6; TNF-α (ang. *tumor necrosis factor alpha*) – czynnik martwicy nowotworów α; CRP (ang. *C-reactive protein*) – białko C-reaktywne.

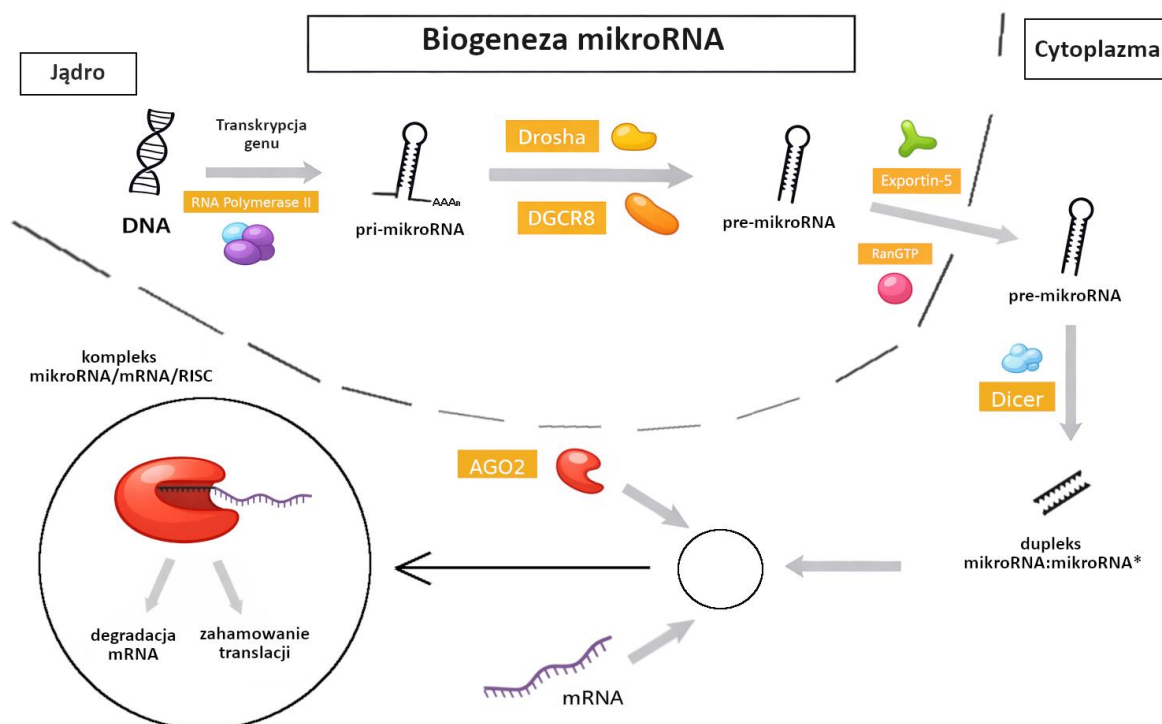
## 4.4. MikroRNA

### 4.4.1. MikroRNA jako posttranskrypcyjne regulatory ekspresji genów

MikroRNA stanowią grupę krótkich, jednoniciowych, ewolucyjnie konserwatywnych cząsteczek kwasu rybonukleinowego (RNA). Są one kodowane przez geny zlokalizowane zarówno w intronach, jak i w eksonach innych genów. Proces biogenezy mikroRNA rozpoczyna się od transkrypcji przez polimerazę RNA II, prowadzącej do powstania pierwotnego transkryptu (pri-mikroRNA) o charakterystycznej strukturze spinki do włosów. Następnie, z udziałem kompleksu białek Drosha i DGCR8, dochodzi do jego obróbki w jądrze komórkowym oraz utworzenia prekursorowego mikroRNA (pre-mikroRNA). Cząsteczka ta jest transportowana do cytoplazmy przez eksportynę 5, gdzie podlega dalszej obróbce przez enzym Dicer. W wyniku tego procesu powstaje dwuniciowy kompleks mikroRNA/mikroRNA\*, z którego jedna nić staje się dojrzałym, funkcjonalnym mikroRNA. Dojrzałe mikroRNA zawierają zwykle od 20 do 25 nukleotydów i wchodzi w skład kompleksu wyciszania indukowanego RNA (ang. *RNA-induced silencing complex* – RISC). Uproszczony schemat biosyntezy mikroRNA drogą kanoniczną został przedstawiony na rycinie 3.

MikroRNA należą do grupy RNA niekodujących, ponieważ nie ulegają translacji do białek. Ich podstawową funkcją jest regulacja ekspresji genów na poziomie potranskrypcyjnym. Kompleks RISC wiąże się z docelowymi cząsteczkami mRNA, co prowadzi do zahamowania translacji lub degradacji transkryptu. Dzięki temu pojedyncze mikroRNA może regulować ekspresję wielu genów uczestniczących w tych samych szlakach biologicznych. Zaburzenia ekspresji mikroRNA mogą prowadzić do nieprawidłowego funkcjonowania komórek i odgrywać istotną rolę w rozwoju wielu chorób.

Choć większość mikroRNA znajduje się wewnątrz komórek, część z nich występuje również w przestrzeni pozakomórkowej. Tak zwane krążące mikroRNA można wykryć w różnych płynach ustrojowych, w tym w surowicy i osoczu. Dzięki temu mogą one oddziaływać nie tylko na komórkę, w której zostały wytworzone, lecz także na komórki i tkanki położone w innych częściach organizmu.



**Rycina 3. Proces biogenezy mikroRNA.** MikroRNA powstają w wyniku transkrypcji genów przez polimerazę RNA II, co prowadzi do utworzenia pierwotnego transkryptu (pri-miRNA). W jądrze komórkowym pri-miRNA jest przetwarzane przez kompleks mikroprocesorowy złożony z białek Drosha i DGCR8 do postaci prekursorowej (pre-miRNA). Następnie pre-miRNA jest transportowane do cytoplazmy przez kompleks Exportin-5/RanGTP. W cytoplazmie enzym Dicer przetwarza pre-miRNA do dwuniciowego dupletu mikroRNA:mikroRNA\*. Jedna z nici (dojrzałe mikroRNA) zostaje włączona do kompleksu wyciszającego indukowanego przez RNA (ang. *RNA-induced silencing complex* – RISC), którego kluczowym składnikiem jest białko Argonaute (AGO2), natomiast druga nić zwykle ulega degradacji, choć w niektórych przypadkach może również pełnić funkcje regulatorowe. Kompleks mikroRNA–RISC wiąże się z docelowym mRNA, prowadząc do zahamowania translacji lub degradacji mRNA, a tym samym do regulacji ekspresji genów.

Skróty: AGO2 – Argonaute 2; DGCR8 – ang. *DiGeorge syndrome critical region gene 8*; mRNA – informacyjny RNA (ang. *messenger RNA*); pre-mikroRNA – prekursorowe mikroRNA; pri-mikroRNA – pierwotny transkrypt mikroRNA; RISC – kompleks wyciszający indukowany przez RNA (ang. *RNA-induced silencing complex*); RanGTP – białko Ran związane z GTP.

#### **4.4.2. MikroRNA w chorobach układu krążenia**

Dynamiczny rozwój biologii molekularnej i metod analitycznych w ostatnich latach znacząco poszerzył wiedzę na temat roli mikroRNA w rozwoju układu sercowo-naczyniowego, jego fizjologii oraz patofizjologii. Coraz więcej danych wskazuje na ich udział w regulacji ciśnienia tętniczego oraz w procesach prowadzących do rozwoju chorób układu krążenia. Mimo to dokładna rola mikroRNA w regulacji ciśnienia tętniczego oraz w patogenezie nadciśnienia tętniczego pozostaje przedmiotem intensywnych badań.

Najnowsze dane sugerują, że dysregulacja ekspresji mikroRNA jest związana nie tylko z rozwojem nadciśnienia tętniczego, ale również z nadciśnieniowym uszkodzeniem narządowym. Opisano ich związek z przerostem lewej komory, przebudową naczyń, przewlekłą chorobą nerek oraz angiopatią nadciśnieniową [18, 19]. Ponadto zmieniona ekspresja wybranych krążących mikroRNA może wiązać się ze zwiększonym ryzykiem wystąpienia incydentów sercowo-naczyniowych, takich jak zawał mięśnia sercowego, udar mózgu czy niewydolność serca oraz gorszym rokowaniem [18-20]. Z tego względu mikroRNA są obecnie intensywnie badane jako potencjalne biomarkery ryzyka sercowo-naczyniowego, jak również jako potencjalne cele nowych terapii [21].

W ostatnich latach zidentyfikowano liczne mikroRNA mogące uczestniczyć w regulacji ciśnienia tętniczego oraz rozwoju nadciśnienia tętniczego. Wykazano, że wpływają one między innymi na funkcję śródbłonna, proliferację komórek mięśni gładkich naczyń, aktywność układu renina–angiotensyna–aldosteron, gospodarkę sodową, procesy zapalne oraz przebudowę mięśnia sercowego i ściany naczyń [18, 19, 22]. Wśród najlepiej poznanych cząsteczek związanych z patogenezą nadciśnienia tętniczego u osób dorosłych znajdują się mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145. Z uwagi na ich potencjalne znaczenie biologiczne oraz dostępność danych eksperymentalnych i klinicznych, zostały one wybrane do dalszej analizy w ramach projektu ATHENA.

#### **4.4.3. Potencjalny udział mikroRNA w mechanizmach regulacji ciśnienia tętniczego i rozwoju nadciśnienia tętniczego**

##### **mikroRNA-16**

MikroRNA-16 należy do najlepiej poznanych cząsteczek mikroRNA i odgrywa istotną rolę w regulacji proliferacji komórek, apoptozy oraz odpowiedzi zapalnej. Do jego głównych genów docelowych należą między innymi cykliny (*CCND1*, *CCND3*, *CCNE1*) oraz gen kinazy *CDK6*, dzięki czemu uczestniczy w regulacji cyklu komórkowego [23, 24]. W badaniach eksperymentalnych wykazano również jego udział w regulacji angiogenezy oraz funkcji komórek mięśni gładkich naczyń [25].

Coraz więcej danych wskazuje na potencjalny udział mikroRNA-16 w patogenezie nadciśnienia tętniczego. W modelach zwierzęcych wykazano, że może ono wpływać na aktywność układu współczulnego poprzez regulację ekspresji genu receptora  $\alpha 1A$ -adrenergicznego (*ADRA1A*), a także uczestniczyć w procesach przebudowy naczyń związanych z aktywacją układu renina–angiotensyna–aldosteron [26]. Obniżenie ekspresji mikroRNA-16 prowadziło do zwiększonej proliferacji komórek mięśni gładkich naczyń, co sugeruje jego potencjalne działanie ochronne wobec niekorzystnej przebudowy ściany naczyniowej [25].

Znaczenie mikroRNA-16 w nadciśnieniu tętniczym potwierdzają również badania prowadzone w grupie osób dorosłych. Wykazano zmianę ekspresji tej cząsteczki zarówno u pacjentów z nadciśnieniem tętniczym, jak i z przewlekłą chorobą nerek [19, 27].

### **mikroRNA-21**

MikroRNA-21 należy do najlepiej poznanych mikroRNA uczestniczących w regulacji procesów zapalnych, proliferacji komórek oraz przebudowy tkanek. W układzie sercowo-naczyniowym wpływa na funkcję fibroblastów, komórek mięśni gładkich naczyń oraz kardiomiocytów, regulując między innymi szlaki sygnałowe TGF- $\beta$ , NF- $\kappa$ B i mTOR. Wykazano również, że zwiększona ekspresja mikroRNA-21 może sprzyjać rozwojowi włóknienia, przerostowi mięśnia sercowego oraz zwiększeniu sztywności naczyń [28-30].

Coraz więcej danych wskazuje na istotną rolę mikroRNA-21 w patogenezie nadciśnienia tętniczego pierwotnego. Cząsteczka ta wpływa na aktywność układu RAA, procesy zapalne oraz przebudowę ściany naczyń, uczestnicząc w rozwoju uszkodzenia narządowego zależnego od nadciśnienia [28, 31, 32]. Ponadto wykazano zwiększoną ekspresję mikroRNA-21 w blaszkach miażdżycowych oraz dodatnią korelację ekspresji tego mikroRNA z grubością kompleksu błona wewnętrzna–błona środkowa tętnic szyjnych wspólnych [29, 33].

W badaniach u dorosłych stwierdzano podwyższone stężenia krążącego mikroRNA-21 u pacjentów z nadciśnieniem tętniczym, szczególnie w przypadku nadciśnienia opornego [34]. Ekspresja tej cząsteczki korelowała również z wartościami ciśnienia tętniczego, stężeniem aldosteronu oraz wykładnikami uszkodzenia narządowego zależnego od nadciśnienia, takimi jak albuminuria [34, 35]. W metaanalizie obejmującej 12 badań wykazano istotnie wyższe poziomy ekspresji mikroRNA-21 u osób z nadciśnieniem tętniczym w porównaniu z osobami normotensyjnymi [36]. Wyniki te sugerują, że mikroRNA-21 może stanowić obiecującą cząsteczkę biorącą udział w aktywacji układu renina–angiotensyna–aldosteron oraz w przebudowie układu sercowo-naczyniowego związanej z NT.

### **mikroRNA-27a i mikroRNA-27b**

MikroRNA-27a i mikroRNA-27b należą do tej samej rodziny mikroRNA i uczestniczą w regulacji licznych procesów metabolicznych oraz procesów sercowo-naczyniowych. Obie cząsteczki wpływają między innymi na metabolizm lipidów, funkcję komórek mięśni gładkich naczyń oraz aktywność układu renina–angiotensyna–aldosteron. Wykazano również, że mogą regulować ekspresję genu konwertazy angiotensyny (*ACE*), co może sugerować ich potencjalny udział w regulacji ciśnienia tętniczego poprzez modulację układu RAA [37, 38].

MikroRNA-27a uczestniczy w regulacji funkcji kardiomiocytów, procesów apoptozy oraz proliferacji i migracji komórek mięśni gładkich naczyń krwionośnych [39]. Badania eksperymentalne wskazują ponadto, że może odgrywać rolę w ochronnym wpływie wysiłku fizycznego na układ sercowo-naczyniowy poprzez modulację aktywności układu renina–angiotensyna–aldosteron [40]. W badaniach u dorosłych obserwowano niższe stężenia krążącego mikroRNA-27a u osób z nowo rozpoznanym nadciśnieniem tętniczym w porównaniu z osobami normotensyjnymi [41].

MikroRNA-27b zaś jest uznawane za jeden z kluczowych regulatorów metabolizmu lipidów i procesów angiogenezy [22, 42, 43]. Wykazano również jego związek z otyłością i zespołem metabolicznym [22]. W układzie sercowo-naczyniowym mikroRNA-27b uczestniczy w regulacji przebudowy mięśnia sercowego oraz procesów włóknienia, choć wyniki badań dotyczących jego dokładnej roli pozostają niejednoznaczne [44, 45]. W części badań przeprowadzonych w grupie osób dorosłych stwierdzano podwyższone stężenia mikroRNA-27b u pacjentów z nadciśnieniem tętniczym, zwłaszcza w przypadku współistniejącego przerostu lewej komory [51], co może sugerować potencjalny związek tej cząsteczki z przebudową układu sercowo-naczyniowego obserwowaną w nadciśnieniu tętniczym.

### **mikroRNA-133a**

Cząsteczka ta należy do grupy tzw. miomiR (ang. *muscle-specific microRNAs*), których ekspresja jest szczególnie wysoka w mięśniu sercowym oraz mięśniach szkieletowych. Cząsteczka ta uczestniczy w regulacji proliferacji, różnicowania i przeżycia kardiomiocytów, a także w procesach przebudowy mięśnia sercowego [46]. W badaniach eksperymentalnych wykazano, że jednym z mechanizmów jej działania może być wpływ na aktywność układu RAA poprzez regulację ekspresji receptora proreninowego (PRR) oraz angiotensynogenu [47]. Ponadto mikroRNA-133a wykazuje działanie ochronne wobec rozwoju włóknienia i przerostu mięśnia sercowego [48].

W badaniach prowadzonych u osób dorosłych z nadciśnieniem tętniczym obserwowano obniżoną ekspresję mikroRNA-133a w porównaniu z osobami normotensyjnymi [41]. Część

autorów wykazała również zależność pomiędzy niższą ekspresją tej cząsteczki a obecnością przerostu lewej komory, co sugeruje jej potencjalny udział w przebudowie mięśnia sercowego związanej z nadciśnieniem tętniczym [49, 50]. Dotychczasowe wyniki sugerują udział mikroRNA-133a w patogenezie nadciśnienia tętniczego oraz związanej z nim przebudowie układu sercowo-naczyniowego, jednak znaczenie tej cząsteczki w przebiegu choroby pozostaje przedmiotem dalszych badań.

MikroRNA-133a jest również przedmiotem zainteresowania w fizjologii wysiłku fizycznego. Wykazano, że jego ekspresja wzrasta po wysiłku wytrzymałościowym, prawdopodobnie na skutek uwalniania cząsteczki z mięśni szkieletowych i mięśnia sercowego [22, 51]. Sugeruje to, że mikroRNA-133a może uczestniczyć w molekularnych mechanizmach adaptacji układu sercowo-naczyniowego do wysiłku fizycznego.

### **mikroRNA-145**

MikroRNA-145 należy do najlepiej poznanych mikroRNA związanych z funkcją komórek mięśni gładkich naczyń. Cząsteczka ta uczestniczy w regulacji ich proliferacji, różnicowania oraz utrzymania prawidłowego fenotypu kurczliwego. Do jej najważniejszych genów docelowych należą między innymi *ACE*, *MYOCD*, *KLF4* oraz *KLF5*, co wskazuje na potencjalny udział mikroRNA-145 zarówno w regulacji napięcia ściany naczyniowej, jak i w procesach jej przebudowy [48, 52, 53].

Ze względu na kluczową rolę w regulacji fenotypu komórek mięśni gładkich naczyń, mikroRNA-145 jest uznawane za jedną z cząsteczek mogących uczestniczyć w procesach przebudowy naczyń oraz wczesnego starzenia naczyniowego. Dotychczasowe dane sugerują zarówno potencjalny udział tej cząsteczki w rozwoju zmian naczyniowych związanych z nadciśnieniem tętniczym, jak i możliwe działanie kompensacyjne ograniczające ich progresję [54, 55].

Dostępne dane sugerują, że wpływ mikroRNA-145 na rozwój nadciśnienia tętniczego może być zależny od miejsca jego ekspresji oraz dominujących mechanizmów patofizjologicznych, co prawdopodobnie tłumaczy niejednoznaczne wyniki dotychczasowych badań.

## **4.5. Wysiłek fizyczny**

### **4.5.1. Wysiłek fizyczny a nadciśnienie tętnicze**

Regularna aktywność fizyczna stanowi jeden z podstawowych elementów postępowania nefarmakologicznego w nadciśnieniu tętniczym i jest rekomendowana zarówno w profilaktyce, jak i leczeniu choroby na każdym etapie jej zaawansowania [3]. U osób

dorosłych liczne badania wykazały, że systematyczny trening aerobowy prowadzi do trwałego obniżenia wartości ciśnienia tętniczego oraz redukcji ryzyka sercowo-naczyniowego [56]. Podobne obserwacje zauważono w grupie pediatrycznej – badanie przeprowadzone na 44 otyłych dzieciach w wieku 8-12 lat wykazało, że 3-miesięczny program regularnego wysiłku fizycznego prowadził do obniżenia wartości ciśnienia tętniczego nawet o 7 mmHg oraz poprawy funkcji śródbłonna, ocenianej na podstawie rozszerzalności naczyń zależnej od przepływu (ang. *flow-mediated dilation* – FMD), niezależnie od zmian masy ciała i zawartości tkanki tłuszczowej [57].

Korzystne działanie obserwuje się jednak nie tylko w następstwie długotrwałego programu treningowego. Już pojedyncza sesja wysiłku dynamicznego może wywołać przejściowe obniżenie ciśnienia tętniczego określane jako hipotensja powysiłkowa (ang. *post-exercise hypotension* – PEH) [58]. Zjawisko to może utrzymywać się nawet do 24 godzin po zakończeniu wysiłku, a jego nasilenie oraz czas trwania są zwykle większe u osób z nadciśnieniem tętniczym niż u osób normotensyjnych. Mechanizmy odpowiedzialne za PEH obejmują przede wszystkim zmniejszenie obwodowego oporu naczyniowego, poprawę funkcji śródbłonna, zwiększoną biodostępność tlenu azotu, zmniejszenie aktywności współczulnego układu nerwowego oraz przejściowe wyhamowanie aktywności układu renina–angiotensyna–aldosteron [59]. Chociaż większość danych pochodzi z badań prowadzonych u dorosłych, dostępne obserwacje wskazują, że podobne efekty występują również u dzieci i młodzieży [60]. Liczba badań oceniających hipotensję powysiłkową w populacji pediatrycznej pozostaje jednak ograniczona, szczególnie wśród dzieci i młodzieży z nadciśnieniem tętniczym pierwotnym.

#### **4.5.2. Wysiłek fizyczny a mikroRNA**

W ostatnich latach coraz większą uwagę zwraca się na rolę mikroRNA jako potencjalnych mediatorów adaptacji organizmu do wysiłku fizycznego. Wykazano, że zarówno pojedyncza sesja wysiłku fizycznego, jak i długotrwały trening mogą modulować ekspresję licznych mikroRNA zaangażowanych w regulację funkcji układu sercowo-naczyniowego. Badania u dorosłych wskazują, iż ekspresja wielu mikroRNA związanych z patogenezą nadciśnienia tętniczego może ulegać korzystnym zmianom pod wpływem aktywności fizycznej. Dotyczy to między innymi mikroRNA-21, mikroRNA-27a/b, mikroRNA-126, mikroRNA-133a oraz klastra mikroRNA-143/145, uczestniczących w regulacji funkcji śródbłonna, przebudowie naczyń, procesach angiogenezy oraz aktywności układu renina–angiotensyna–aldosteron [22]. Ponadto zmiany stężeń wybranych krążących mikroRNA obserwowano już po pojedynczym wysiłku aerobowym, przy czym część z nich ulegała szybkiej normalizacji w ciągu 24 godzin po zakończeniu wysiłku [61]. Szczególnie interesujące

są obserwacje dotyczące mikroRNA-133a, którego ekspresję opisywano jako obniżoną w nadciśnieniu tętniczym, natomiast poziomy ekspresji tej cząsteczki wzrastały po wysiłku fizycznym u osób zdrowych [22]. Pomimo rosnącej liczby danych nadal brakuje badań bezpośrednio oceniających zależność między zmianami ekspresji mikroRNA a hipotensją powysiłkową u pacjentów z NTP w populacji pediatrycznej.

#### **4.6. Braki w aktualnym stanie wiedzy i uzasadnienie podjętego tematu**

Pomimo dynamicznego rozwoju badań nad mikroRNA, ich znaczenie w patogenezie nadciśnienia tętniczego pozostaje nie w pełni poznane. Dotychczas zidentyfikowano liczne cząsteczki mogące uczestniczyć w regulacji ciśnienia tętniczego poprzez wpływ na funkcję śródbłonna, komórek mięśni gładkich naczyń, układu renina–angiotensyna–aldosteron, procesów zapalnych oraz przebudowy układu sercowo-naczyniowego. Wyniki dostępnych badań są jednak często niejednoznaczne, a obserwowane zależności mogą wynikać z różnic w rodzaju analizowanej tkanki, stadium choroby, wieku badanej populacji oraz współistniejących czynników środowiskowych i metabolicznych.

Większość dotychczasowych badań dotyczących mikroRNA w nadciśnieniu tętniczym przeprowadzono u osób dorosłych. Dane dotyczące dzieci i młodzieży pozostają bardzo ograniczone, mimo że NTP w wieku rozwojowym stanowi szczególnie interesujący model badawczy. W porównaniu z populacją dorosłych choroba charakteryzuje się zwykle krótszym czasem trwania, mniejszym nasileniem zmian narządowych oraz mniejszym wpływem wieloletniej farmakoterapii i chorób współistniejących. Dzięki temu możliwa jest ocena potencjalnych mechanizmów zaangażowanych we wczesne etapy rozwoju nadciśnienia tętniczego. Brakuje badań oceniających rolę krążących mikroRNA u dzieci z pierwotnym nadciśnieniem tętniczym. W szczególności nie ustalono dotychczas, czy i w jakim stopniu ekspresja mikroRNA wiąże się z wartościami ciśnienia tętniczego, obecnością wczesnych zmian sercowo-naczyniowych oraz odpowiedzią organizmu na wysiłek fizyczny u dzieci z nadciśnieniem tętniczym pierwotnym.

Lepsze poznanie znaczenia mikroRNA w nadciśnieniu tętniczym może w przyszłości ułatwić identyfikację pacjentów szczególnie narażonych na rozwój choroby oraz umożliwić zindywidualizowane podejście do diagnostyki i monitorowania leczenia już we wczesnym okresie choroby na etapie wieku rozwojowego.

Wreszcie poznanie roli mikroRNA w NTP u dzieci stwarza szansę na opracowanie nowych strategii terapeutycznych opartych na modulacji ekspresji genów. Chociaż terapie ukierunkowane na mikroRNA pozostają obecnie na etapie badań przedklinicznych i wczesnych

badan klinicznych, dynamiczny rozwój technologii wykorzystujących małe cząsteczki RNA, takie jak siRNA (ang. *small interfering RNA* – małe interferujące RNA), wskazuje na realny potencjał tej koncepcji. Przykładem jest zilebesiran – lek oparty na technologii siRNA hamujący syntezę angiotensynogenu w wątrobie, który w badaniach klinicznych u dorosłych wykazał działanie hipotensyjne utrzymujące się nawet do 6 miesięcy po pojedynczym podaniu podskórnym [62]. Sukces tego typu terapii sugeruje, że w przyszłości również modulacja ekspresji wybranych mikroRNA może znaleźć zastosowanie w leczeniu nadciśnienia tętniczego.

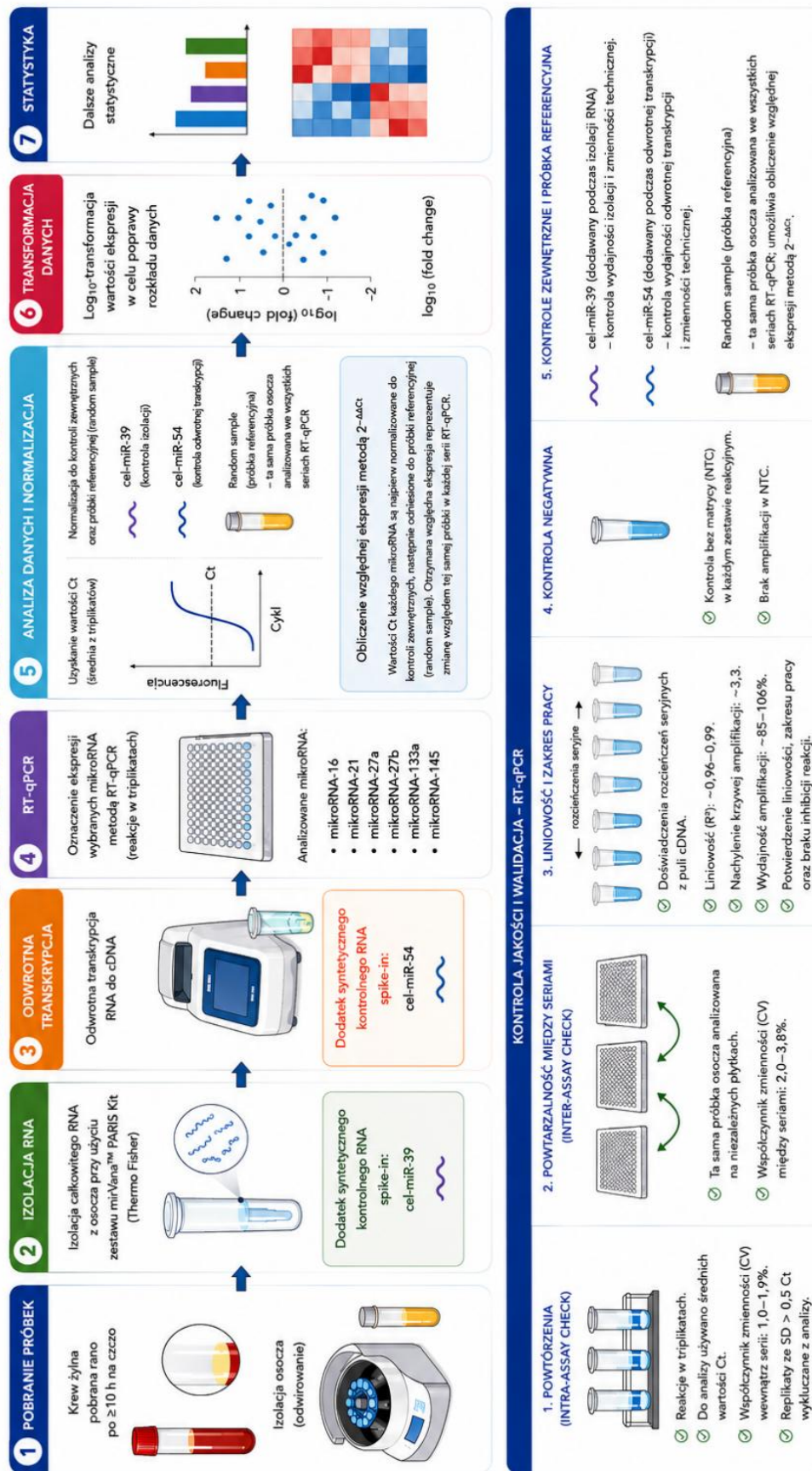
## **4.7. Najważniejsze elementy metodologii badań**

### **4.7.1. Oznaczanie ekspresji mikroRNA**

Próbki krwi żyłnej do oznaczeń cząsteczek mikroRNA pobierano rano, po co najmniej 10-godzinnym okresie pozostawania na czczo. Izolację całkowitego RNA z osocza przeprowadzono z wykorzystaniem zestawu mirVana™ PARIS Kit (Thermo Fisher Scientific). Następnie wykonano odwrotną transkrypcję oraz oznaczenie ekspresji wybranych mikroRNA metodą ilościowej reakcji łańcuchowej polimerazy w czasie rzeczywistym (RT-qPCR) z zastosowaniem zestawów TaqMan™ Advanced microRNA Assays oraz aparatu LightCycler® 480 II (Roche Diagnostics). Analizowano ekspresję sześciu mikroRNA: mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145.

Ze względu na brak powszechnie akceptowanej endogennej kontroli referencyjnej dla oznaczeń krążących mikroRNA w osoczu, do kontroli jakości zastosowano syntetyczne oligonukleotydy *Caenorhabditis elegans* (cel-mikroRNA-39 oraz cel-mikroRNA-54), dodawane odpowiednio na etapie izolacji RNA oraz odwrotnej transkrypcji. Wszystkie próbki analizowano w trzech powtórzeniach PCR. W celu zapewnienia porównywalności wyników pomiędzy kolejnymi seriami oznaczeń we wszystkich reakcjach RT-qPCR analizowano dodatkowo losowo wybraną próbkę kalibracyjną (ang. *reference sample*), względem której obliczano względny poziom ekspresji metodą  $2^{-\Delta\Delta C_t}$ . Szczegółowy opis procedury laboratoryjnej, zastosowanych odczynników, kontroli jakości, normalizacji wyników oraz analizy ekspresji mikroRNA przedstawiono w publikacjach stanowiących podstawę niniejszej rozprawy, natomiast na Rycinie 4 zaprezentowano uproszczony schemat oznaczania i analizy ekspresji mikroRNA obejmujący ich najważniejsze etapy.

# OZNACZANIE I ANALIZA EKSPRESJI mikroRNA



Rycina 4. Schemat postępowania podczas oznaczania i analizy ekspresji krążących mikroRNA. RT-qPCR – ilościowa reakcja łańcuchowa polimerazy w czasie rzeczywistym; RNA – kwas rybonukleinowy; DNA – kwas deoksyrybonukleinowy; cDNA – komplementarny DNA; Ct – próg cyklu (ang. *cycle threshold*); CV – współczynnik zmienności; NTC (ang. *no-template control*) – kontrola bez matrycy.

#### **4.7.2. Protokół wysiłku fizycznego**

U wszystkich uczestników badania, przed rozpoczęciem właściwego protokołu, wykonano próbę wysiłkową z oceną wydolności krążeniowo-oddechowej (ang. *cardiopulmonary exercise test* – CPET) na cykloergometrze w celu wyznaczenia indywidualnego progu beztlenowego. Po upływie 2–6 tygodni uczestnicy zostali poddani pojedynczej, standaryzowanej, 60-minutowej sesji wysiłku aerobowego na cykloergometrze o intensywności odpowiadającej 90–110% obciążenia osiąganego na progu beztlenowym, zgodnie z zaleceniami American College of Sports Medicine [63].

Oceny wyjściowe wykonywano rano na czczo. Bezpośrednio po zakończeniu wysiłku rozpoczynano kolejne 24-godzinne monitorowanie ciśnienia tętniczego metodą ABPM, natomiast ponowną ocenę laboratoryjną, pomiary ciśnienia tętniczego oraz badania naczyń przeprowadzano następnego dnia, 12–16 godzin po zakończeniu wysiłku, w identycznych warunkach jak podczas badania wyjściowego. Taki schemat badania umożliwiał ocenę utrzymujących się zmian wywołanych pojedynczą sesją wysiłku fizycznego przy jednoczesnym ograniczeniu wpływu ostrych zmian metabolicznych występujących bezpośrednio po zakończeniu ćwiczeń. Szczegółowy opis kwalifikacji uczestników, przebiegu testu CPET oraz protokołu wysiłkowego przedstawiono w publikacjach stanowiących podstawę niniejszej rozprawy.

## 5. Uzasadnienie połączenia publikacji w jeden cykl

Publikacje stanowiące cykl rozprawy doktorskiej dotyczą jednolitej grupy badanej – dzieci i młodzieży z nieleczonym dotychczas farmakologicznie nadciśnieniem tętniczym pierwotnym. Wszystkie prace koncentrują się na ocenie potencjalnej roli krążących mikroRNA w tej grupie pacjentów, ze szczególnym uwzględnieniem ich związku z wartościami ciśnienia tętniczego, a w kolejnych pracach skupiają się na odpowiedzi organizmu na wysiłek fizyczny oraz na wybranych parametrach funkcji i przebudowy układu sercowo-naczyniowego. Publikacje powstały w ramach projektu ATHENA (ang. *ArTerial HypErtension Pathogenesis — microRNA as a missing link in the disease development in children*) i stanowią kolejne etapy realizacji tego programu badawczego.

Pierwsza publikacja miała charakter pilotażowy i była poświęcona ocenie wykonalności przyjętego protokołu badawczego, obejmującego analizę ekspresji mikroRNA oraz odpowiedź na standaryzowany wysiłek fizyczny. Uzyskane wyniki pozwoliły na wstępną ocenę badanego modelu i stanowiły podstawę do realizacji kolejnych etapów projektu.

Druga publikacja przedstawia wyniki głównej części badania ATHENA przeprowadzonej w większej grupie dzieci z nieleczonym nadciśnieniem tętniczym pierwotnym oraz zdrowych rówieśników. W pracy tej oceniono wyjściową ekspresję wybranych mikroRNA oraz ich odpowiedź na wysiłek fizyczny, analizując potencjalny związek tych cząsteczek z nadciśnieniem tętniczym i HMOD.

Trzecia publikacja stanowi pogłębioną analizę znaczenia mikroRNA w kontekście subklinicznych zaburzeń funkcji mięśnia sercowego. Pozwoliła ona rozszerzyć wcześniejsze obserwacje o ocenę zależności pomiędzy ekspresją mikroRNA a parametrami odkształcenia podłużnego mięśnia sercowego ocenianymi metodą śledzenia markerów akustycznych (STE).

Ostatnia publikacja ma charakter przeglądowy i podsumowuje aktualny stan wiedzy dotyczący mikroRNA w nadciśnieniu tętniczym. Szczególną uwagę poświęcono ograniczeniom metodologicznym badań nad mikroRNA, przyczynom rozbieżności pomiędzy publikowanymi wynikami oraz kierunkom dalszych badań, które mogą przyczynić się do lepszego zrozumienia znaczenia tych cząsteczek w nadciśnieniu tętniczym.

Publikacje tworzą spójny cykl badawczy obejmujący kolejne etapy procesu naukowego: od opracowania i weryfikacji modelu badawczego, poprzez analizę ekspresji wybranych mikroRNA w większej kohorcie pacjentów i pogłębioną ocenę ich potencjalnego znaczenia klinicznego, aż do krytycznej syntezy dostępnej wiedzy i identyfikacji najważniejszych wyzwań badawczych w tej dziedzinie.

## **6. Założenia i cele pracy**

Celami pracy są:

1. Ocena ekspresji mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a i mikroRNA-145 u dzieci i młodzieży z nadciśnieniem tętniczym pierwotnym i u zdrowych rówieśników.
2. Ocena zależności pomiędzy poziomem ekspresji powyższych mikroRNA a ciśnieniem tętniczym u nieleczonych farmakologicznie dzieci z nadciśnieniem tętniczym pierwotnym.
3. Ocena zależności pomiędzy poziomem ekspresji mikroRNA a zmianami narządowymi: masą lewej komory w badaniu echokardiograficznym, grubością kompleksu błona wewnętrzna–błona środkowa tętnic szyjnych wspólnych (cIMT) oraz sztywnością tętnic u dzieci z nadciśnieniem tętniczym pierwotnym.
4. Ocena krążących mikroRNA jako potencjalnych biomarkerów zaburzeń funkcji skurczowej serca wyrażonych jako odkształcenie podłużne lewej komory (LV GLS) u dzieci z nadciśnieniem tętniczym pierwotnym.
5. Ocena wpływu pojedynczej sesji standaryzowanego wysiłku aerobowego na ciśnienie tętnicze i ekspresję mikroRNA u dzieci i młodzieży z NTP i u zdrowych rówieśników.

## **7. Kopie opublikowanych prac**

**7.1. Publikacja I: „MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension—A Preliminary Study”**

Article

# MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension—A Preliminary Study

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**Abstract: Background/Objectives:** Studies in adults have demonstrated the essential role of microRNAs in developing hypertension and their effect on hypertension sequelae. In this preliminary study, we aimed to investigate the expression of five miRNA particles, miRNA-21, miRNA-27a, miRNA-27b, miRNA-133a, and miRNA-145, in school-aged children with primary hypertension and to examine their correlations with blood pressure and arterial and heart properties. **Methods:** In 22 hypertensive children ( $15.1 \pm 1.9$  years), we measured blood pressure parameters (office, central, and 24 h), the urinary albumin/creatinine ratio, and the pulse wave velocity (PWV) before and after one hour of aerobic exercise. The left ventricular mass index (LVMI) and common carotid artery intima-media thickness (cIMT) were also assessed. The relative miRNA expression was calculated using the  $2^{-\Delta\Delta C_t}$  method with miRNA-16 as an endogenous control and the pre-exercise miRNA expression levels as the control (baseline). **Results:** We found a statistically significant decrease in both the office and 24 h ambulatory diastolic blood pressure after 1 h of exercise ( $82.2 \pm 8.5$  mm Hg versus  $78.6 \pm 8.8$  mm Hg,  $p = 0.01$  and  $75.0 \pm 8.3$  mm Hg versus  $73.0 \pm 7.4$  mm Hg,  $p = 0.02$ ). The increase in miRNA-133a expression after exercise correlated positively with the LVMI. Furthermore, the rise in miRNA-145 expression after exercise correlated negatively with the systolic and diastolic office and 24 h blood pressure and with markers of arterial damage: 24 h PWV and cIMT. **Conclusions:** In conclusion, miRNA-133a may be a biomarker of left ventricular hypertrophy in children with elevated blood pressure. Additionally, changes in miRNA-145 expression induced by exercise might reduce the blood pressure after exercise and protect against arterial damage. Both miRNA-133a and miRNA-145 may be involved in epigenetic alterations in children affected by primary hypertension that may contribute to the exacerbation of HMOD.

**Keywords:** microRNA; primary hypertension; adolescents; hypertension-mediated organ damage



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## 1. Introduction

MicroRNAs (miRNAs) are single-stranded, non-coding, evolutionarily conserved RNA molecules that regulate post-transcriptional gene expression in all eukaryotic cells [1]. It has been more than two decades since the discovery of the first two microRNA molecules, named lin-4 and let-7, in the nematode *C. elegans*. The latter was also found as the first known human miRNA. The discovery of these unique molecules was even awarded this year's Nobel Prize in Physiology or Medicine (2024); Victor Ambros and Gary Ruvkun were recognized for their discovery and for demonstrating their role in post-transcriptional gene regulation. In recent years, there has been growing interest in these peculiar molecules, which have been studied extensively in oncology, particularly for their emerging therapeutic applications. However, studies also point to the multiple effects of miRNAs on processes in cardiovascular diseases, including hypertension, as well as diabetes or kidney diseases [2]. There are promising results when looking at the adult population but there

are no data available for pediatric patients with primary hypertension. This is particularly important given the significant contribution of genetic factors to the development of primary hypertension, which is much greater in children and is not masked by as many environmental factors that accumulate over a lifetime.

Arterial hypertension (AH) is found in approximately 4% of pediatric patients, with a growing prevalence with age [3]. From puberty, AH is 3–4 times more frequent in boys than in girls. Primary hypertension (PH) is related to half of all cases of pediatric patients with high blood pressure and is the most common form of arterial hypertension in adolescents. The vast majority of primary hypertension cases are asymptomatic, and the first manifestations show up when organ damage, such as heart failure, myocardial infarction, stroke, atherosclerosis, renal failure, or vision impairment, has already developed. Arterial hypertension was the leading global contributor to premature death in 2015, accounting for almost 10 million deaths. Most of the deaths were due to ischemic heart disease (4.9 million), hemorrhagic stroke (2.0 million), and ischemic stroke (1.5 million) [4].

PH is a complex and multifactorial disease, and several mechanisms are involved in its development. Genetic and environmental factors, the sympathetic nervous system, the hormonal–enzymatic renin–angiotensin–aldosterone system, and substances produced by the endothelium—nitric oxide and endothelins—play an essential role in this process. Genes play quite a significant role in BP regulation as well. A parental history of AH increases the risk of the disease in offspring, especially if both parents are hypertensive. Epidemiological studies have suggested that in arterial hypertension, 60% of cases have a genetic background (association), and 40% are environmental [5]. However, the exact process of hypertension development is not fully explained, and molecular pathways still have unexplored gaps.

Regular physical activity is an essential aspect of one's lifestyle and is used as a non-pharmacological strategy for preventing and treating early-stage hypertension or as an adjunct to pharmacological intervention in more advanced cases. The American Heart Association recommends at least 30 min of aerobic exercise at least five days a week to promote cardiovascular risk reduction [6]. It is well established that acute and chronic aerobic exercise lowers blood pressure in adults. An immediate reduction in BP after a dynamic exercise session has been termed PEH (post-exercise hypotension); the effect is seen up to 24 h after the end of the exercise. Scientific data suggest that the reduction in BP is more significant in patients not on antihypertensive medications [7]. There are less data on this topic in the pediatric population. However, preliminary evidence suggests that aerobic training can reduce BP and significantly reverse endothelial damage also in this group [8]. A study in obese children showed that regular exercise can lower BP by as much as seven mmHg in 3 months [9]. This effect is independent of reducing body fat. As for microRNA, it has been found that the expression of various miRNAs was up- or downregulated in healthy volunteers up to 72 h after a single session of exercise [10]. Furthermore, some miRNAs show the opposite form of expression in hypertension and after exercise. There is a lack of data on the relationship between these miRNA particles and the occurrence of indicators of hypertension-mediated organ damage (HMOD), and none of these particles have been studied in the pediatric population.

Our preliminary project aimed to check the expression of five microRNA particles (miRNA-21, miRNA-27a, miRNA-27b, miRNA-133a, and miRNA-145) in pediatric patients with PH. After analyzing the available literature in this field, we selected the above five particles, which might be involved in the development of arterial hypertension and HMOD in children and adolescents.

## 2. Materials and Methods

### 2.1. Study Group

As a preliminary study, the aim was to establish a proper protocol before recruiting a larger group of patients for a more thorough investigation. All 22 patients were recruited for this single-center observational study from individuals with primary hypertension who

presented to the ward and outpatient clinic of our university hospital without previous treatment with antihypertensive drugs.

The inclusion criteria were as follows: not yet pharmacologically treated arterial hypertension diagnosed following up-to-date recommendations of the European Society of Hypertension [11] and confirmed in ambulatory blood pressure monitoring, height  $\geq 120$  cm, and signed informed consent to participate in the study.

Patients with secondary forms of hypertension, severe kidney, liver, or heart disease that could influence the study's results, or any inflammatory disease, e.g., acute infection at the time of the study (temporary exclusion criterion), were excluded from the study.

This preliminary study analyzed the first consecutive 22 patients who qualified for the final study.

## 2.2. Basic Clinical Parameters

Upon admission, all patients underwent an assessment of basic anthropometric parameters, including height (in centimeters), weight (in kilograms), and body mass index (BMI) (in kilograms per square meter). We compared these anthropometric measurements with Polish normative data and expressed them as Z-scores [12]. According to the World Health Organization definition, children with BMI Z-scores  $> 1$  and  $> 2$  were considered overweight and obese, respectively [13].

A series of interviews were conducted to gather information on the subjects' birth weight, gestational age, and family history of hypertension.

## 2.3. Carrying Out Aerobic Training

To ensure the same exercise load for each study patient, an ergospirometric test was performed before the standardized exercise session to determine the maximal oxygen uptake ( $\text{VO}_2$  max) for each participant (using METALYZER 3B ergospirometry device during the ramp exercise test with the protocol for children, Cortex Biophysics GMBH, Leipzig, Germany). Then, during the study, all participants performed 60 min of aerobic exercise on the CYCLUS 2 ergometer (RMB elektronik-automation GMBH, Leipzig, Germany), corresponding to 65% of their  $\text{VO}_2$  max (steady-state cycling exercise), under the supervision of the investigator (MS). Patients were instructed to maintain their usual physical activity and diet and not to eat 2 h before exercise. After the training session, they continued with their daily routine.

## 2.4. Parameters of Blood Pressure and Hypertension-Mediated Organ Damage

The methodology employed for BP measurement and the assessment of arterial damage was described in detail in our previous manuscripts [14–17]. In brief, peripheral office BP was evaluated oscillometrically by the Omron HBP-1320 (OMRON HEALTHCARE, Co., Ltd., Kyoto, Japan) (mmHg) and Z-scores [12]. The Mobil-O-Graph device with 24 h pulse wave analysis and pulse wave velocity (I.E.M. Industrielle Entwicklung Medizintechnik GmbH, Stolberg, Germany) was used to conduct 24 h ambulatory blood pressure monitoring (ABPM). The data were interpreted following the recommendations for pediatric patients outlined in the cited article [18]. The following parameters were included in the final analysis: systolic, diastolic, and mean arterial blood pressure (SBP, DBP, MAP, respectively, mmHg), as well as systolic and diastolic central blood pressure (cSBP, cDBP, respectively, mmHg). We also analyzed the following hemodynamic parameters: the augmentation index normalized to the heart rate of 75 beats per minute ( $\text{AIx}_{75\text{HR}}$ ) (%), total vascular resistance (TVR) ( $\text{dyn}\cdot\text{s}/\text{cm}^5$ ), stroke volume (SV) (mL), and pulse wave velocity (PWV) analysis conducted over 24 h. Furthermore, 24 h heart rate (beats per minute) and nocturnal blood pressure dip (%) were measured. We also evaluated carotid–femoral pulse wave velocity using applanation tonometry (the Sphygmocor device [AtCor Medical Pty Ltd., Sydney, Australia]). The Aloka Prosound Alpha 6 (Hitachi Aloka Medical, Mitaka, Japan), equipped with a 13 MHz linear transducer, was employed to measure common

carotid artery intima–media thickness (cIMT) (mm). The cPWV and cIMT were presented as numeric values and Z-scores, as detailed in the following references [19,20].

In addition to the assessment of artery-associated HMOD (cIMT, atPWV), the albumin-to-creatinine ratio (ACR) (mg/g) in the first-morning portion of urine and the hypertrophy of the left ventricle expressed as the left ventricular mass index (LVMI) ( $\text{g}/\text{m}^2$ , 7 or  $\text{g}/\text{m}^2$ ) were also evaluated. The ACR was measured by the hospital laboratory using standard laboratory techniques. An ACR greater than 30 mg/g was considered an indicator of renal damage caused by hypertension [11].

Echocardiography was conducted using the Philips iE33 xMATRIX (Echocardiography System, Philips Healthcare, Amsterdam, The Netherlands) and a wide-spectrum probe with frequency adjusted to the patient's body mass index. Following the guidelines set forth by the European Society of Hypertension and the Polish Society of Hypertension, the LVMI was indexed for height<sup>2.7</sup> in children aged  $\leq 15$ . Left ventricular hypertrophy (LVH) was identified if the left ventricular mass index (LVMI) was equal to or exceeded the 95th percentile for age and gender [21]. In adolescents aged  $\geq 16$  years, left ventricular mass was indexed for body surface area, calculated using the DuBois and DuBois formula. The body surface area (BSA) is calculated as follows:  $\text{BSA} = 0.20247 \times \text{height (m)}^{0.725} \times \text{weight (kg)}^{0.425}$ . Left ventricular mass index (LVMI) is defined as  $\text{LVM}/\text{BSA}$ . In adolescents aged  $\geq 16$  years, left ventricular hypertrophy will be identified if the left ventricular mass index (LVMI) is  $>115 \text{ g}/\text{m}^2$  for boys and  $>95 \text{ g}/\text{m}^2$  for girls [21].

## 2.5. Additional Biochemical Parameters

In all patients, serum creatinine (mg/dL), uric acid (mg/dL), serum sodium (mmol/L) and potassium (mmol/L), total, low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol (mg/dL), and triglycerides (mg/dL), were measured using standard laboratory procedures employed by the hospital department. Normal values of lipid parameters were taken from Stewart et al. [22]. In all the participants, we calculated the glomerular filtration rate (GFR) from the revised creatinine-based Schwartz formula in children under 16 and the CKD-EPI in those who were equal to or above the age of 16 [23].

## 2.6. Evaluation of miRNA

Blood for miRNA analysis was collected using a vacuum technique in K2-EDTA tubes (BD Vacutainer®, Beckton, Dickinson and Company, Franklin Lakes, NJ, US). Each blood sample was centrifuged within 10 min at  $3000 \times g$  for 15 min at  $4^\circ\text{C}$ , and the supernatant was centrifuged again under the same conditions to remove any trace of blood cells. In addition, a visual inspection was performed to detect hemolysis. Hemolyzed samples were excluded from further analysis. Plasma was transferred to cryotubes and frozen at  $-80^\circ\text{C}$  until laboratory analysis. To protect against the degradation of microRNAs, the plasma samples were handled only with extended, sterile, double-filtered pipette tips free of RNase, DNase, PCR inhibitors, endotoxin, and DNA. There was no additional freeze–thaw cycle; all samples were used only once. miRNA extraction was performed using commercially available mirVana PARIS kits (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions with modifications based on our experience. The isolated RNA's quantity, quality, and purity were measured by spectrophotometry using the NanoDrop Lite (Thermo Fisher Scientific, Waltham, MA, USA). For reverse transcription, commercially available TaqMan kits (Thermo Fisher Scientific, Waltham, MA, USA) were used following the manufacturer's protocol with modifications based on our experience. Real-time PCR was performed using commercially available TaqMan Advanced miRNA Assays (Thermo Fisher Scientific, Waltham, MA, USA) and the LightCycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany). According to the available literature on plasma miRNA in PH, miRNA-16 expression was chosen as the normalization standard (endogenous control) [24], exogenous cel-miRNA-39 as isolation control, and exogenous cel-miRNA-54 as reverse transcription control. All samples were run in triplicate. Data were analyzed using the LightCycler 480 II Software 1.5, and relative miRNA levels were

calculated using the  $2^{-\Delta\Delta C_t}$  method. As there was no control group in this preliminary study, the baseline microRNA expression level before exercise was used as the patient-specific control. All miRNA sequences are listed in Supplementary Material Table S1 with the corresponding accessions derived from miRbase accessed on 15 March 2023 (<https://www.mirbase.org/>) for clarity and possible further analysis, including others to replicate and build on the published results.

### 2.7. Statistical Methods

The authors archived the obtained data in an anonymized form (Excel 365, Microsoft 365, Microsoft, Redmond, WA, USA) with password-protected user-level access. Statistical analysis was performed with Dell Statistica 13.0 PL software (TIBCO Software Inc., Palo Alto, CA, USA). The normality of numeric datasets was tested using the Shapiro–Wilk test.

All continuous variables were presented as mean  $\pm$  standard deviation (SD) and interquartile range (IQ). Subsequently, the following tests were used, depending on the data distribution: Student's *t*-test for dependent variables, Wilcoxon's test, Spearman's rank correlation, and Pearson's correlation. Test results with a *p*-value  $< 0.05$  were considered of statistical significance.

## 3. Results

### 3.1. Clinical and Biochemical Data of the Studied Children

The baseline clinical and biochemical parameters in the studied children are shown in Table 1. Most of the study participants were boys (82%), and their mean age was 15 years. There were two premature children in the study group. The vast majority of the patients with PH were overweight or obese, and all the studied patients had normal renal function. Elevated or borderline cholesterol was found in 23% and 9%, elevated or borderline LDL-cholesterol in 9% and 14%, lowered or borderline HDL-cholesterol in 23% and 9%, and elevated or borderline triglycerides in 27% and 27% of the participants, respectively. Hyperuricemia was found in half of all the patients (50%). All the patients had normal serum sodium and potassium concentrations.

**Table 1.** Clinical and biochemical data of the study group.

| Parameter                                         | Value $\pm$ SD [Quartiles]                     |
|---------------------------------------------------|------------------------------------------------|
| Number of patients ( <i>n</i> )                   | 22                                             |
| Boys/girls ( <i>n</i> , %)                        | 18/4 (82%/18%)                                 |
| Age [years]                                       | 15.1 $\pm$ 1.9 (from 11.3 to 18.0) [14.0–16.8] |
| Duration of gestation [weeks]                     | 38.5 $\pm$ 2.2 [37–40]                         |
| Birth weight [g]                                  | 3343 $\pm$ 480 [3090–3600]                     |
| BMI Z-score                                       | 1.34 $\pm$ 0.93 [0.66–1.99]                    |
| Overweight patients (BMI 85–95 c) ( <i>n</i> , %) | 7 (32%)                                        |
| Obese patients (BMI $> 95$ c) ( <i>n</i> , %)     | 9 (41%)                                        |
| GFR [mL/min/1.73 m <sup>2</sup> ]                 | 124.0 $\pm$ 11.7 [114–134]                     |
| Total cholesterol [mg/dL]                         | 157.7 $\pm$ 40.2 [125–193]                     |
| LDL-cholesterol [mg/dL]                           | 88.5 $\pm$ 31.6 [64–103]                       |
| HDL-cholesterol [mg/dL]                           | 49.4 $\pm$ 13.4 [41–60]                        |
| Triglycerides [mg/dL]                             | 99 $\pm$ 40 [62–141]                           |
| Uric acid [mg/dL]                                 | 5.9 $\pm$ 1.1 [5.1–6.7]                        |
| Serum sodium [mmol/L]                             | 140.0 $\pm$ 1.5 [139–141]                      |
| Serum potassium [mmol/L]                          | 4.34 $\pm$ 0.22 [4.18–4.50]                    |

*n*—number of patients, BMI—body mass index, GFR—glomerular filtration rate, LDL—low-density lipoprotein, HDL—high-density lipoprotein.

### 3.2. Blood Pressure of the Studied Children

Table 2 shows the office peripheral, ambulatory peripheral, and central blood pressure before and after a single bout of aerobic exercise. All patients had elevated systolic or

diastolic office and ambulatory blood pressure. Based on the ABPM results, 59% of the patients had isolated systolic hypertension, and 41% of the subjects had systolic–diastolic hypertension. Seven patients were dippers, ten were non-dippers, four were extreme dippers, and one was a reverse dipper.

**Table 2.** Office and 24 h ambulatory blood pressure of the studied children.

| Parameter                           | Before Exercise            | After Exercise            | <i>p</i> |
|-------------------------------------|----------------------------|---------------------------|----------|
| SBP [mm Hg]                         | 139.1 ± 12.0 [133–148]     | 134.9 ± 12.7 [125–145]    | 0.073    |
| SBP Z-score                         | 2.01 ± 0.92 [1.6–2.5]      | 1.66 ± 0.94 [0.9–2.5]     | 0.072    |
| DBP [mm Hg]                         | 82.2 ± 8.5 [75–88]         | 78.6 ± 8.8 [70–87]        | 0.010    |
| DBP Z-score                         | 2.3 ± 1.15 [1.5–3.0]       | 1.78 ± 1.14 [0.5–2.8]     | 0.010    |
| 24 h SBP [mm Hg]                    | 133.1 ± 10.6 [124–141]     | 131.7 ± 9.9 [125–137]     | 0.296    |
| 24 h DBP [mm Hg]                    | 75.0 ± 8.3 [68–82]         | 73.0 ± 7.4 [68–79]        | 0.019    |
| 24 h HR [beats/min]                 | 74.2 ± 9.5 [68–80]         | 77.8 ± 10.3 [70–82]       | 0.004    |
| 24 h MAP [mm Hg]                    | 101.5 ± 8.9 [94–109]       | 100.0 ± 7.8 [95–104]      | 0.133    |
| 24 h cSBP [mm Hg]                   | 115.7 ± 10.2 [108–124]     | 114.3 ± 8.6 [109–118]     | 0.264    |
| 24 h cDBP [mm Hg]                   | 77.5 ± 8.5 [69–86]         | 75.7 ± 7.7 [69–82]        | 0.049    |
| SBP DIP [%]                         | 10.5 ± 6.6 [5.3–13.6]      | 10.0 ± 7.2 [4.5–15.7]     | 0.712    |
| DBP DIP [%]                         | 14.0 ± 8.3 [6.6–18.3]      | 13.5 ± 8.5 [7.6–18.3]     | 0.755    |
| 24 h AIx 75 HR [%]                  | 14.7 ± 6.58 [9–18]         | 15.7 ± 6.29 [11–18]       | 0.160    |
| 24 h TVR [dyn × s/cm <sup>5</sup> ] | 1557.8 ± 104.2 [1501–1619] | 1509.4 ± 92.2 [1430–1579] | 0.007    |
| 24 h PWV [m/s]                      | 5.2 ± 0.3 [4.9–5.5]        | 5.2 ± 0.3 [5.0–5.3]       | 0.734    |
| 24 h stroke volume [mL]             | 74.5 ± 8.1 [71–79]         | 72.2 ± 8.4 [66–78]        | 0.011    |

SBP—systolic blood pressure, DBP—diastolic blood pressure, HR—heart rate, c—central blood pressure, AIx 75 HR—augmentation index normalized to the heart rate of 75 beats per minute, TVR—total vascular resistance, PWV—pulse wave velocity.

After one hour of a standardized exercise, we observed no significant changes in the systolic blood pressure. Still, there was a significant decrease in the office and 24 h diastolic and 24 h central diastolic blood pressure. There were also significant reductions in the 24 h stroke volume and total vascular resistance. After 60 min of exercise, the children’s heart rate was significantly higher. The 24 h pulse wave velocity remained the same before and after the exercise, and we found no difference in the augmentation index.

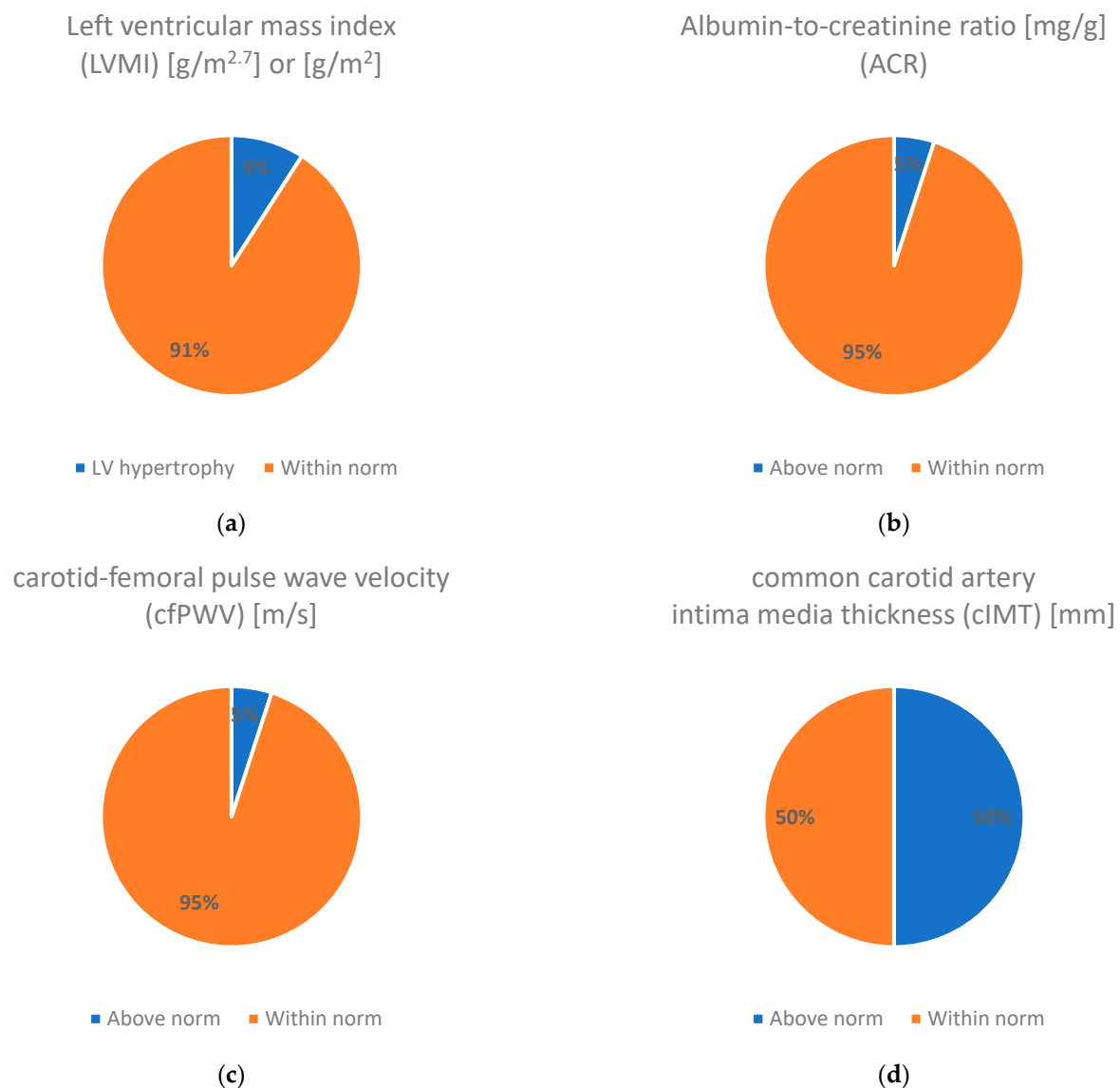
### 3.3. Hypertension-Mediated Organ Damage of the Studied Children

The HMOD parameters are shown in Table 3 and Figure 1. We observed no significant differences between the ACR and cfPWV measurements before and after the standardized aerobic exercise sessions. Half of the patients had an abnormally thickened carotid intima-media. Two patients had left ventricular hypertrophy (LVH), one had elevated albuminuria, and one had abnormal cfPWV at the time of the study.

**Table 3.** Hypertension-mediated organ damage indicators in the studied children.

| Parameter                  | Before Exercise            | After Exercise         | <i>p</i> |
|----------------------------|----------------------------|------------------------|----------|
| ACR [mg/g]                 | 7.0 ± 6.7 [3.5–7.3]        | 8.4 ± 11.3 [3.6–6.8]   | 0.606    |
| cfPWV [m/s]                | 5.2 ± 0.65 [4.8–5.6]       | 5.1 ± 0.75 [4.4–5.6]   | 0.255    |
| cfPWV Z-score              | −0.27 ± 0.9 [−0.9–0.3]     | −0.48 ± 1.0 [−1.4–0.2] | 0.234    |
| cIMT [mm]                  | 0.492 ± 0.05 [0.45–0.52]   |                        | NA       |
| cIMT Z-score               | 2.0 ± 1.0 [1.4–2.8]        |                        | NA       |
| LVM [g]                    | 145.7 ± 44.5 [114.5–162.2] |                        | NA       |
| LVMi [g/m <sup>2.7</sup> ] | 32.8 ± 8.6 [26.8–37.6]     |                        | NA       |
| LVMi [g/m <sup>2</sup> ]   | 74.9 ± 20.3 [65.6–80.8]    |                        | NA       |

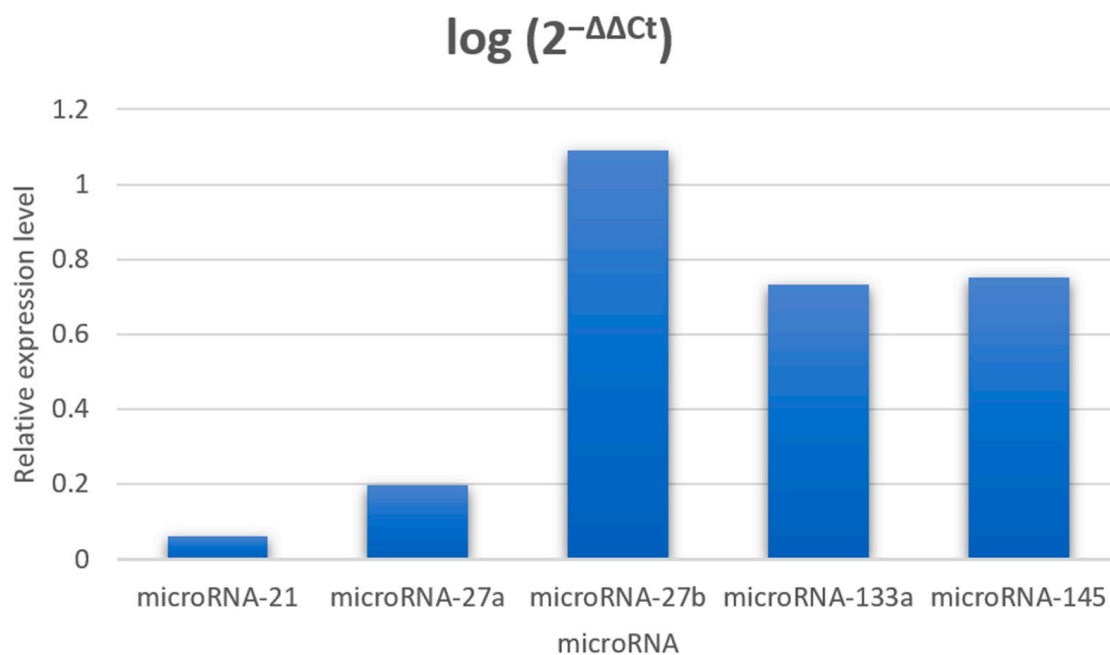
ACR—albumin-to-creatinine ratio; cfPWV—carotid–femoral pulse wave velocity; cIMT—common carotid artery intima–media thickness; LVM—left ventricular mass; LVMi—left ventricular mass index.



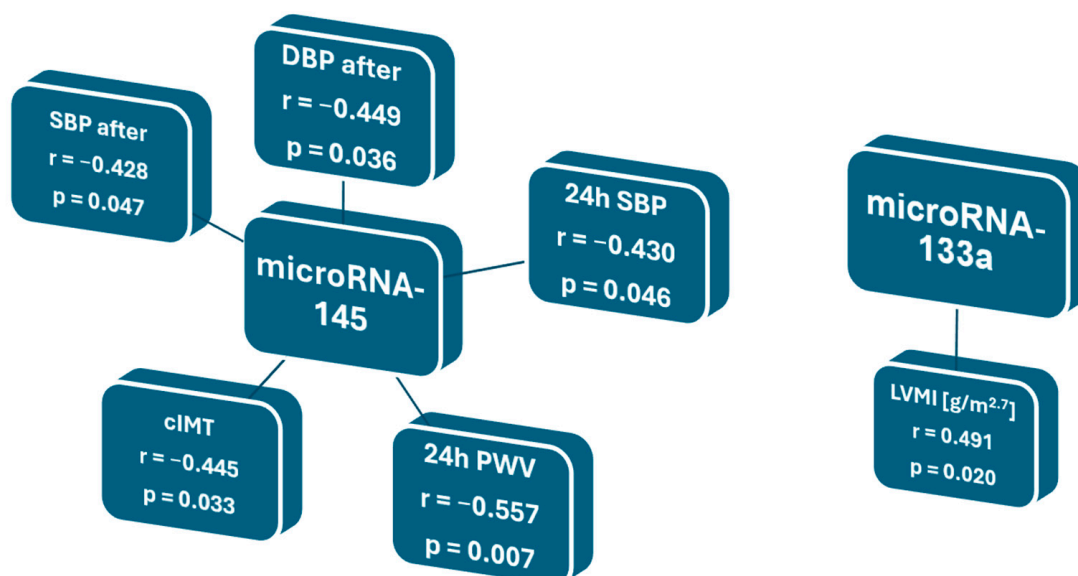
**Figure 1.** Hypertension-mediated organ damage indicators in the studied patients. (a) Left ventricular mass index ([g/m<sup>2.7</sup>] or [g/m<sup>2</sup>]) depending on age; (b) albumin-to-creatinine ratio (ACR) [mg/g]; (c) carotid–femoral pulse wave velocity (cfPWV) [m/s]; (d) common carotid artery intima–media thickness (cIMT) [mm].

### 3.4. MicroRNA of the Studied Children

An increase in the relative expression level of all the microRNA molecules was observed but was not statistically significant for the study group (Figure 2). the molecules numbered 27b had the highest increase in expression, followed by those numbered 145 and 133a. The post-exercise increase in the microRNA-133a expression correlated positively with the LVMI [g/m<sup>2.7</sup>] ( $r = 0.491$ ,  $p = 0.020$ ). The post-exercise increase in the microRNA-145 expression levels correlated negatively with the post-exercise systolic and diastolic blood pressure, 24 h systolic blood pressure, 24 h peripheral PWV, and cIMT ( $r = -0.428$ ,  $p = 0.047$ ,  $r = -0.449$ ,  $p = 0.036$ ,  $r = -0.430$ ;  $p = 0.046$ ,  $r = -0.557$ ,  $p = 0.007$  and  $r = -0.455$ ,  $p = 0.033$ , respectively) (Figure 3)—none of the tested microRNA molecules correlated with age.



**Figure 2.** Relative microRNA expression levels in plasma (before and after exercise session) were calculated by the  $2^{-\Delta\Delta C_t}$  method, with miRNA-16 expression level as endogenous control, and Ct before exercise as the norm.



**Figure 3.** Significant correlations of relative microRNA expression levels in plasma with blood pressure parameters and hypertension-mediated organ damage indicators. SBP—systolic blood pressure, DBP—diastolic blood pressure, 24 h SBP—24 h systolic blood pressure measured by ambulatory blood pressure monitoring; cIMT—common carotid artery intima-media thickness, 24 h PWV—pulse wave velocity measured by 24 h ambulatory blood pressure monitoring device, LVMI—left ventricular mass index.

#### 4. Discussion

Our observational preliminary study analyzed the selected microRNAs as potential biomarkers of blood pressure parameters and subclinical arterial damage in twenty-two previously untreated children with primary hypertension. All of the microRNA particles

were analyzed before and after a single 60 min standardized aerobic exercise. In addition, we evaluated the blood pressure parameters, the cfPWV, and the ACR before and after the exercise. We also looked at other indicators of hypertension-mediated organ damage, such as the LVMI and cIMT. Although the relative expression levels of the microRNAs measured did not increase significantly, all of them were overexpressed after the exercise session in this small group of patients. Interestingly, the study revealed significant correlations between microRNA-145 and blood pressure and arterial damage parameters. The microRNA-133a expression also correlated with the left ventricular mass index. The study also showed no difference between the PWV and ACR measured before and after the moderate-intensity exercise sessions and showed that the diastolic blood pressure decreased after exercise in both the office and 24 h measurements in the children with PH. After 60 min of exercise, the children's heart rate was higher, and there were also significant reductions in the 24 h stroke volume and total vascular resistance.

Regular physical activity is a well-established, essential component of a healthy lifestyle. It is used as a non-pharmacological strategy for prevention and treatment in the early stages of arterial hypertension or as an adjunct to pharmacological intervention in more advanced cases. The American Heart Association recommends at least 30 min of aerobic exercise five days a week to promote cardiovascular risk reduction [6]. The BP-lowering effects of exercise are much more pronounced in people with arterial hypertension than in healthy people [7]. However, most of the data are from adults, including older people, and there is little evidence of this phenomenon in children. One randomized controlled trial showed a reduction in the systolic and diastolic blood pressure of 10 and 6 mmHg, respectively, after plyometric exercise [25]. Another study in obese children shows that BP can be reduced by as much as 7 mmHg in 3 months with regular exercise, and this effect is independent of body fat reduction [9]. In our study, the SBP was reduced by 4 mmHg and the DBP by 3 mmHg after a single bout of aerobic exercise in hypertensive children, supporting the above findings. Despite the small number of studied children, we found significant differences in the central and peripheral diastolic blood pressure. The diastolic blood pressure mainly depends on the arterial elasticity and peripheral (total vascular) resistance, which decreased in our group after exercise. The systolic blood pressure, on the other hand, depends on the work of the heart (stroke volume and heart rate) and also on the elasticity of the large arteries. There were opposite changes in the HR and SV after exercise in the children studied, which may explain the lack of a significant change in systolic pressure.

Studies in recent years have linked exercise to changes in microRNA expression. A recently published systematic review of the literature by Kotewitsch et al. identified microRNA molecules whose change in expression was replicated in multiple studies in adults. In response to resistance exercise, the expressions of microRNA-133a and microRNA-206 were altered in most of the analyzed studies. In response to endurance exercise, microRNAs 15a, 29c, 30a, 30e, 103a, 130a, 132, 142, 143, 155, 181a, 181b, 338, 451a, and let-7e showed consistent elevation in 100% of all the included studies, whereas microRNAs 103a, 130a, and let-7e were consistently downregulated [26]. The review's authors linked the change in microRNA expression to the positive effects of exercise on elements of the innate and acquired immune system.

It also appears that, depending on the lifestyle and level of training, a single session of exercise may affect the expression of other microRNA molecules and thus have different clinical effects. Nair et al. showed that the profile of microRNAs altered by exercise in older adults is different in trained and sedentary individuals. This, in turn, may exert a different effect on the insulin-like growth factor 1 (IGF-1) signaling pathway and thus differently affect exercise-induced hypertrophy of skeletal muscle and cardiomyocytes [27]. Other studies, on the other hand, indicate that the dose of training may matter. Spanish authors have observed that different exercise doses induced specific microRNA profiles [28].

In many adult studies, the circulating levels of microRNA-133a were increased in healthy subjects after exercise training [29–33]. Interestingly, because miRNA-133a indi-

rectly targets antihypertrophic genes including guanosine triphosphate-guanosine diphosphate (GDP-GTP) exchange protein, Rhoa, signal transduction kinase cell division control protein 42 (Cdc42), and the nuclear factor negative elongation factor complex member A (Nelfa/Whsc2), miRNA-133a downregulation is involved in the process of cardiac hypertrophy [34]. Our study suggests this effect is also present in hypertensive children, in whom microRNA-133a exhibited an increased relative expression after a training bout. However, its level was positively correlated with the LVMI. We hypothesize that children with LVH already have a greater potential to reverse unfavorable changes in the heart, and microRNA-133a is more abundant in these patients after exercise. However, further studies with a control group and more detailed studies of the molecular pathways are needed.

Interestingly, we found numerous negative correlations of changes in the microRNA-145 expression levels with blood pressure and the arterial damage parameters cIMT and PWV. Our study sheds new light on the role of this molecule in generating hypertension and organ changes. Our result somewhat contradicts the result of the study by Santovito et al. The authors showed significantly higher expression of microRNA-145 in atherosclerotic plaque collected during endarterectomy in patients with hypertension [35]. Moreover, experimental work suggests an adverse role for microRNA-145 in the development of both systemic and pulmonary hypertension [36,37]. In our group, microRNA-145 behaved differently, indicating its protective role. The expression of the particle increased after exercise and showed a negative correlation with pressure and organ changes. Some experimental data suggest that microRNA-145 might be a negative regulator of the expression of GATA binding protein 6 (GATA6), thus attenuating cardiomyocyte hypertrophy in the experimental model [38]. Other correlations in our work compared to other authors may have been due to different protocols for determining microRNA expression (including different centrifugation conditions and use of probes from various companies) and determination of microRNA in different materials (serum, plasma, cells, atherosclerotic plaques). Obviously, our pilot study cannot unequivocally assess the role of microRNA145 in the development of hypertension and its sequelae in the pediatric population (small patient group, no comparison with healthy children, no evaluation of expression in target organs). Studies on larger groups of patients, studies with a control group, and prospective studies would be needed. We hope that the final results of our project will provide insight into the role of this molecule.

In our small group, we further observed a post-exercise increase in the expression of other microRNA molecules: microRNA-27a, microRNA-27b, and microRNA 21. However, we did not show any significant correlation between the change in the expression of these molecules and blood pressure or hypertension-mediated organ damage. We hope to confirm or exclude these relationships in the final work on a larger group of patients.

The expression of the angiotensin-converting enzyme gene is regulated by microRNA-27a and microRNA-27b [39]. In a five-year longitudinal population-based study from Japan, circulating microRNA-27a was negatively associated with incident hypertension [40]. In a rat model, exercise increased microRNA-27a and -27b [41,42]. A loss of microRNA-27a induced cardiac dysfunction [43], thus suggesting a cardioprotective role of this molecule. Conversely, the authors from China revealed that the serum microRNA-27b levels were significantly higher in hypertensive patients with left ventricular hypertrophy (LVH) than in hypertensive patients without LVH and in healthy volunteers [44].

MicroRNA-21 is a molecule closely related to primary hypertension. By affecting SMAD7, vascular endothelial growth factor, renin pathways, and subclinical inflammation, microRNA-21 is involved in the development and progression of hypertension and HMOD, including left ventricular hypertrophy and arterial damage measured by the cIMT and PWV [45]. A post-exercise decrease in microRNA-21 expression was revealed in an animal model [46] and in women with breast cancer [47], which may be another mechanism for the positive effects of exercise on human health.

A meta-analysis of fourteen trials (15 interventions, 642 adult participants with arterial hypertension), involving five aerobic, two dynamic resistance, six combined, and

two isometric resistance groups, showed that exercise interventions based on aerobic, combined, or isometric exercise were able to decrease the PWV [48]. Also, a meta-analysis of 26 studies in overweight and obese children revealed that aerobic exercise had a favorable effect on arterial stiffness, while the improvement was ineffective after resistance training and a combined training mode [49]. In our study, a single aerobic exercise session did not cause a significant change in any of the analyzed parameters of organ damage, including arterial stiffness. Nevertheless, we observed a substantially reduced total vascular resistance (TVR). According to Poiseuille's law, the TVR correlates positively with the blood viscosity and vessel length but is inversely related to the fourth power of the radius. Thus, reductions in the TVR are strongly associated with changes in the vessel diameter; even small changes profoundly affect the vascular resistance. A larger lumen diameter and greater distensibility of blood vessels are structural adaptations to exercise that allow for lower peripheral resistance. We want to underline that our study is preliminary, and the analysis will be repeated on a larger group of hypertensive children and healthy peers.

Our study has several obvious limitations. First, the study sample is small and does not allow us to draw definitive conclusions. Second, there is no control group, which affects the analysis of both clinical data and microRNA expression analysis. Hence, we used the microRNA expression before exercise (in a given patient) instead of the expression in the control group as the baseline expression. The group is not ethnically and age-diverse. Thus, it is not easy to transfer these results to other populations. On the other hand, despite these limitations, the study's strengths should be highlighted. First, it is the first pioneering analysis of microRNAs in children with hypertension. Second, in our study, we used (and described in detail in Section 2) an established method for assessing microRNA expression. All samples were analyzed by a single investigator (MS) previously trained by microRNA experts. Third, the patients' vascular phenotype and blood pressure were assessed in extraordinary detail. An additional strength is the inclusion of a standardized intervention and the evaluation of virtually all parameters before and after exercise. We are aware of the limitations and are confident that the final study will answer the question of the role of microRNA in pediatric primary hypertension, HMOD, and its relationship to exercise in this group of patients.

## 5. Conclusions

To conclude, our preliminary study in 22 pediatric patients with primary hypertension revealed a single bout of standardized aerobic exercise-induced changes in microRNA expression together with a decrease in blood pressure, stroke volume, and total vascular resistance. We hypothesize that microRNA-133a might be a marker of left ventricular hypertrophy in children with primary hypertension. Exercise-induced alteration of microRNA-145 expression may cause a post-exercise reduction in the blood pressure and have a protective effect on arterial parameters in children with primary hypertension. Both microRNA-133a and microRNA-145 may induce epigenetic modifications affecting hypertension-mediated organ damage in children with primary hypertension, but this requires further studies.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jcm13226929/s1>: Supplementary Table S1—microRNA names, miRbase accession name, and corresponding sequence.

**Author Contributions:** Conceptualization. M.S. and P.S.; methodology. M.S. and P.S.; software. M.S.; validation. P.S.; formal analysis. M.S. and P.S.; investigation. M.S. and P.S.; resources. M.S. and P.S.; data curation. M.S.; writing—original draft preparation. M.S. and P.S.; writing—review and editing. M.S. and P.S.; visualization. M.S.; supervision. P.S.; project administration. M.S.; funding acquisition. M.S. and P.S. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Prior to the start of the study, approval was obtained from the Bioethics Committee of Medical University of Warsaw (KB 154/2020; 14 September 2020). All procedures adhered to the highest ethical standards of the institutional research committee and were conducted in accordance with the tenets of the Declaration of Helsinki and its subsequent amendments.

**Informed Consent Statement:** Before the study began, patients and their representatives provided informed written and verbal consent.

**Data Availability Statement:** Data used to support the findings of this study are included within the Supplementary Information files (Dataset.xlsx).

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## References

- Huntzinger, E.; Izaurralde, E. Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* **2011**, *12*, 99–110. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rupaimoole, R.; Slack, F.J. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. *Nat. Rev. Drug Discov.* **2017**, *16*, 203–222. [\[CrossRef\]](#) [\[PubMed\]](#)
- Song, P.; Zhang, Y.; Yu, J.; Zha, M.; Zhu, Y.; Rahimi, K.; Rudan, I. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr.* **2019**, *173*, 1154–1163. [\[CrossRef\]](#)
- Forouzanfar, M.H.; Liu, P.; Roth, G.A.; Ng, M.; Biryukov, S.; Marczak, L.; Alexander, L.; Estep, K.; Hassen Abate, K.; Akinyemiju, T.F.; et al. Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990–2015. *JAMA* **2017**, *317*, 165–182. [\[CrossRef\]](#)
- Saxena, T.; Ali, A.O.; Saxena, M. Pathophysiology of essential hypertension: An update. *Expert. Rev. Cardiovasc. Ther.* **2018**, *16*, 879–887. [\[CrossRef\]](#)
- Whelton, P.K.; Carey, R.M.; Aronow, W.S.; Casey, D.E., Jr.; Collins, K.J.; Dennison Himmelfarb, C.; DePalma, S.M.; Gidding, S.; Jamerson, K.A.; Jones, D.W.; et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Hypertension* **2018**, *71*, e13–e115. [\[CrossRef\]](#) [\[PubMed\]](#)
- Carpio-Rivera, E.; Moncada-Jiménez, J.; Salazar-Rojas, W.; Solera-Herrera, A. Acute Effects of Exercise on Blood Pressure: A Meta-Analytic Investigation. *Arq. Bras. Cardiol.* **2016**, *106*, 422–433. [\[CrossRef\]](#)
- Clarke, M.M.; Zannino, D.; Stewart, N.P.; Glenning, J.P.; Pineda-Guevara, S.; Kik, J.; Mynard, J.P.; Cheung, M.M.H. Normative blood pressure response to exercise stress testing in children and adolescents. *Open Heart* **2021**, *8*, e001807. [\[CrossRef\]](#) [\[PubMed\]](#)
- Farpour-Lambert, N.J.; Aggoun, Y.; Marchand, L.M.; Martin, X.E.; Herrmann, F.R.; Beghetti, M. Physical activity reduces systemic blood pressure and improves early markers of atherosclerosis in pre-pubertal obese children. *J. Am. Coll. Cardiol.* **2009**, *54*, 2396–2406. [\[CrossRef\]](#)
- Widmann, M.; Nieß, A.M.; Munz, B. Physical Exercise and Epigenetic Modifications in Skeletal Muscle. *Sports Med.* **2019**, *49*, 509–523. [\[CrossRef\]](#)
- Lurbe, E.; Agabiti-Rosei, E.; Cruickshank, J.K.; Dominiczak, A.; Erdine, S.; Hirth, A.; Invitti, C.; Litwin, M.; Mancia, G.; Pall, D.; et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J. Hypertens.* **2016**, *34*, 1887–1920. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kułaga, Z.; Litwin, M.; Tkaczyk, M.; Palczewska, I.; Zajaczkowska, M.; Zwolińska, D.; Krynicki, T.; Wasilewska, A.; Moczulska, A.; Morawiec-Knysak, A.; et al. Polish 2010 growth references for school-aged children and adolescents. *Eur. J. Pediatr.* **2011**, *170*, 599–609. [\[CrossRef\]](#) [\[PubMed\]](#)
- de Onis, M. 4.1 The WHO Child Growth Standards. *World Rev. Nutr. Diet.* **2015**, *113*, 278–294. [\[CrossRef\]](#)
- Skrzypczyk, P.; Ozimek, A.; Ofiara, A.; Szyszka, M.; Sołtyski, J.; Stelmaszczyk-Emmel, A.; Górka, E.; Pańczyk-Tomaszewska, M. Markers of endothelial injury and subclinical inflammation in children and adolescents with primary hypertension. *Cent. Eur. J. Immunol.* **2019**, *44*, 253–261. [\[CrossRef\]](#) [\[PubMed\]](#)
- Skrzypczyk, P.; Ofiara, A.; Szyszka, M.; Dziedzic-Jankowska, K.; Sołtyski, J.; Pańczyk-Tomaszewska, M. Vitamin D in children with primary hypertension. *Arter. Hypertens.* **2018**, *22*, 127–134. [\[CrossRef\]](#)
- Skrzypczyk, P.; Przychodzień, J.; Mizerska-Wasiak, M.; Kuźma-Mroczkowska, E.; Okarska-Napierała, M.; Górka, E.; Stelmaszczyk-Emmel, A.; Demkow, U.; Pańczyk-Tomaszewska, M. Renalase in Children with Glomerular Kidney Diseases. *Adv. Exp. Med. Biol.* **2017**, *1021*, 81–92. [\[CrossRef\]](#)
- Skrzypczyk, P.; Przychodzień, J.; Mizerska-Wasiak, M.; Kuźma-Mroczkowska, E.; Stelmaszczyk-Emmel, A.; Górka, E.; Pańczyk-Tomaszewska, M. Asymmetric dimethylarginine is not a marker of arterial damage in children with glomerular kidney diseases. *Cent. Eur. J. Immunol.* **2019**, *44*, 370–379. [\[CrossRef\]](#)

18. Flynn, J.T.; Daniels, S.R.; Hayman, L.L.; Maahs, D.M.; McCrindle, B.W.; Mitsnefes, M.; Zachariah, J.P.; Urbina, E.M. Update: Ambulatory blood pressure monitoring in children and adolescents: A scientific statement from the American Heart Association. *Hypertension* **2014**, *63*, 1116–1135. [\[CrossRef\]](#)
19. Reusz, G.S.; Cseprekal, O.; Temmar, M.; Kis, E.; Cherif, A.B.; Thaleb, A.; Fekete, A.; Szabó, A.J.; Benetos, A.; Salvi, P. Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension* **2010**, *56*, 217–224. [\[CrossRef\]](#)
20. Doyon, A.; Kracht, D.; Bayazit, A.K.; Deveci, M.; Duzova, A.; Krmar, R.T.; Litwin, M.; Niemirska, A.; Oguz, B.; Schmidt, B.M.; et al. Carotid artery intima-media thickness and distensibility in children and adolescents: Reference values and role of body dimensions. *Hypertension* **2013**, *62*, 550–556. [\[CrossRef\]](#)
21. Khoury, P.R.; Mitsnefes, M.; Daniels, S.R.; Kimball, T.R. Age-specific reference intervals for indexed left ventricular mass in children. *J. Am. Soc. Echocardiogr.* **2009**, *22*, 709–714. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Stewart, J.; McCallin, T.; Martinez, J.; Chacko, S.; Yusuf, S. Hyperlipidemia. *Pediatr. Rev.* **2020**, *41*, 393–402. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Stevens, P.E.; Ahmed, S.B.; Carrero, J.J.; Foster, B.; Francis, A.; Hall, R.K.; Herrington, W.G.; Hill, G.; Inker, L.A.; Kazancioğlu, R.; et al. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int.* **2024**, *105*, S117–S314. [\[CrossRef\]](#)
24. Solayman, M.H.; Langaee, T.; Patel, A.; El-Wakeel, L.; El-Hamamsy, M.; Badary, O.; Johnson, J.A. Identification of Suitable Endogenous Normalizers for qRT-PCR Analysis of Plasma microRNA Expression in Essential Hypertension. *Mol. Biotechnol.* **2016**, *58*, 179–187. [\[CrossRef\]](#)
25. Góis Leandro, C.; Arnaut Brinco, R.; Góes Nobre, G.; Góes Nobre, I.; Silva-Santiago, L.C.; Aires-Dos-Santos, B.R.; Marinho-Dos-Santos, R.; Rodrigues-Ribeiro, M.; Marinho-Barros, M.R.; Alves-Macedo, F.; et al. Post-exercise hypotension effects in response to plyometric training of 7- to 9-year-old boys with overweight/obesity: A randomized controlled study. *J. Sports Med. Phys. Fit.* **2021**, *61*, 1281–1289. [\[CrossRef\]](#)
26. Kotewitsch, M.; Heimer, M.; Schmitz, B.; Mooren, F.C. Non-coding RNAs in exercise immunology: A systematic review. *J. Sport. Health Sci.* **2024**, *13*, 311–338. [\[CrossRef\]](#)
27. Nair, V.D.; Ge, Y.; Li, S.; Pincas, H.; Jain, N.; Seenarine, N.; Amper, M.A.S.; Goodpaster, B.H.; Walsh, M.J.; Coen, P.M.; et al. Sedentary and Trained Older Men Have Distinct Circulating Exosomal microRNA Profiles at Baseline and in Response to Acute Exercise. *Front. Physiol.* **2020**, *11*, 605. [\[CrossRef\]](#)
28. Fernández-Sanjurjo, M.; Úbeda, N.; Fernández-García, B.; Del Valle, M.; Ramírez de Molina, A.; Crespo, M.C.; Martín-Hernández, R.; Casas-Agustench, P.; Martínez-Camblor, P.; de Gonzalo-Calvo, D.; et al. Exercise dose affects the circulating microRNA profile in response to acute endurance exercise in male amateur runners. *Scand. J. Med. Sci. Sports* **2020**, *30*, 1896–1907. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Improtá Caria, A.C.; Nonaka, C.K.V.; Pereira, C.S.; Soares, M.B.P.; Macambira, S.G.; Souza, B.S.F. Exercise Training-Induced Changes in MicroRNAs: Beneficial Regulatory Effects in Hypertension, Type 2 Diabetes, and Obesity. *Int. J. Mol. Sci.* **2018**, *19*, 3608. [\[CrossRef\]](#)
30. Nielsen, S.; Åkerström, T.; Rinnov, A.; Yfanti, C.; Scheele, C.; Pedersen, B.K.; Laye, M.J. The miRNA plasma signature in response to acute aerobic exercise and endurance training. *PLoS ONE* **2014**, *9*, e87308. [\[CrossRef\]](#)
31. Baggish, A.L.; Park, J.; Min, P.K.; Isaacs, S.; Parker, B.A.; Thompson, P.D.; Troyanos, C.; D’Hemecourt, P.; Dyer, S.; Thiel, M.; et al. Rapid upregulation and clearance of distinct circulating microRNAs after prolonged aerobic exercise. *J. Appl. Physiol.* **2014**, *116*, 522–531. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Gomes, C.P.; Oliveira, G.P., Jr.; Madrid, B.; Almeida, J.A.; Franco, O.L.; Pereira, R.W. Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run. *Biomarkers* **2014**, *19*, 585–589. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Min, P.K.; Park, J.; Isaacs, S.; Taylor, B.A.; Thompson, P.D.; Troyanos, C.; D’Hemecourt, P.; Dyer, S.; Chan, S.Y.; Baggish, A.L. Influence of statins on distinct circulating microRNAs during prolonged aerobic exercise. *J. Appl. Physiol.* **2016**, *120*, 711–720. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Wang, J.; Liew, O.W.; Richards, A.M.; Chen, Y.T. Overview of MicroRNAs in Cardiac Hypertrophy, Fibrosis, and Apoptosis. *Int. J. Mol. Sci.* **2016**, *17*, 749. [\[CrossRef\]](#)
35. Santovito, D.; Mandolini, C.; Marcantonio, P.; De Nardis, V.; Bucci, M.; Paganelli, C.; Magnacca, F.; Uchino, S.; Mastroiacovo, D.; Desideri, G.; et al. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. *Expert. Opin. Ther. Targets* **2013**, *17*, 217–223. [\[CrossRef\]](#)
36. Wang, Y.; Jin, L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp. Ther. Med.* **2018**, *15*, 548–552. [\[CrossRef\]](#)
37. Ogorodnikova, N.; Arenz, C. MicroRNA-145-targeted drug and its preventive effect on pulmonary arterial hypertension (patent WO2012153135 A1). *Expert. Opin. Ther. Pat.* **2015**, *25*, 723–727. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Li, R.; Yan, G.; Zhang, Q.; Jiang, Y.; Sun, H.; Hu, Y.; Sun, J.; Xu, B. miR-145 inhibits isoproterenol-induced cardiomyocyte hypertrophy by targeting the expression and localization of GATA6. *FEBS Lett.* **2013**, *587*, 1754–1761. [\[CrossRef\]](#)
39. Goyal, R.; Goyal, D.; Leitzke, A.; Gheorghe, C.P.; Longo, L.D. Brain renin-angiotensin system: Fetal epigenetic programming by maternal protein restriction during pregnancy. *Reprod. Sci.* **2010**, *17*, 227–238. [\[CrossRef\]](#)
40. Suzuki, K.; Yamada, H.; Fujii, R.; Munetsuna, E.; Yamazaki, M.; Ando, Y.; Ohashi, K.; Ishikawa, H.; Mizuno, G.; Tsuboi, Y.; et al. Circulating microRNA-27a and -133a are negatively associated with incident hypertension: A five-year longitudinal population-based study. *Biomarkers* **2022**, *27*, 496–502. [\[CrossRef\]](#)

41. Fernandes, T.; Hashimoto, N.Y.; Magalhães, F.C.; Fernandes, F.B.; Casarini, D.E.; Carmona, A.K.; Krieger, J.E.; Phillips, M.I.; Oliveira, E.M. Aerobic exercise training-induced left ventricular hypertrophy involves regulatory MicroRNAs, decreased angiotensin-converting enzyme-angiotensin ii, and synergistic regulation of angiotensin-converting enzyme 2-angiotensin (1-7). *Hypertension* **2011**, *58*, 182–189. [[CrossRef](#)] [[PubMed](#)]
42. Gu, Q.; Wang, B.; Zhang, X.F.; Ma, Y.P.; Liu, J.D.; Wang, X.Z. Contribution of renin-angiotensin system to exercise-induced attenuation of aortic remodeling and improvement of endothelial function in spontaneously hypertensive rats. *Cardiovasc. Pathol.* **2014**, *23*, 298–305. [[CrossRef](#)] [[PubMed](#)]
43. Qin, X.D.; Liu, L. Loss of microRNA-27a induces cardiac dysfunction through activating FoxO1. *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 5941–5948. [[CrossRef](#)] [[PubMed](#)]
44. Wang, Y.; Chen, S.; Gao, Y.; Zhang, S. Serum MicroRNA-27b as a Screening Biomarker for Left Ventricular Hypertrophy. *Tex. Heart Inst. J.* **2017**, *44*, 385–389. [[CrossRef](#)]
45. Li, X.; Wei, Y.; Wang, Z. microRNA-21 and hypertension. *Hypertens. Res.* **2018**, *41*, 649–661. [[CrossRef](#)]
46. Hamid, R.R.; Hoseini, R.; Rahim, H.A. Impact of Combined Aerobic Training and Magnesium Supplementation on Serum Biomarkers and microRNA-155 and microRNA-21 Expression in Adipose Tissue of Type 2 Diabetic Rats: An Eight-Week Interventional Study. *Biol. Trace Elem. Res.* **2024**. *online ahead of print*. [[CrossRef](#)]
47. Isanejad, A.; Alizadeh, A.M.; Amani Shalamzari, S.; Khodayari, H.; Khodayari, S.; Khor, V.; Khojastehnejad, N. MicroRNA-206, let-7a and microRNA-21 pathways involved in the anti-angiogenesis effects of the interval exercise training and hormone therapy in breast cancer. *Life Sci.* **2016**, *151*, 30–40. [[CrossRef](#)]
48. Lopes, S.; Afreixo, V.; Teixeira, M.; Garcia, C.; Leitão, C.; Gouveia, M.; Figueiredo, D.; Alves, A.J.; Polonia, J.; Oliveira, J.; et al. Exercise training reduces arterial stiffness in adults with hypertension: A systematic review and meta-analysis. *J. Hypertens.* **2021**, *39*, 214–222. [[CrossRef](#)]
49. Cheng, Y.; Sun, Z.; Ya, X.; Zhou, L.; Wang, M.; Wang, X.; Liu, Y. Effect of exercise training on arterial stiffness in obese and overweight children: A meta-analysis. *Eur. J. Pediatr.* **2022**, *181*, 2633–2642. [[CrossRef](#)]

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**7.2. Publikacja II: „ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes”**

# ATHENA Study – The Role of Circulating Plasma MicroRNAs in Paediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes

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## Keywords

MicroRNA · Primary hypertension · Adolescents · Hypertension-mediated organ damage · Aerobic exercise

## Abstract

**Introduction:** The role of microRNA in primary hypertension (PH) remains unclear. This study examined the expression of microRNA-16, -21, -27a, -27b, -133a, and -145 in untreated hypertensive children, their response to aerobic exercise, and correlations with blood pressure (BP) and related parameters. **Methods:** A pre-post observational, hypothesis-generating study included 56 hypertensive (study group [SG]) and 59 healthy children (control group [CG]). Plasma microRNA levels and clinical, biochemical, BP, vascular, and hypertension-mediated

organ damage (HMOD) parameters were assessed before and after one session of standardized aerobic exercise. **Results:** The baseline relative microRNA-27b ( $p < 0.001$ ) and microRNA-133a ( $p = 0.018$ ) expressions were significantly lower in the SG; similarly, after an exercise bout. After an exercise bout, the expression of microRNA-27b, -133a, and -145 increased in CG, whereas only the expression of microRNA-145 increased in SG. In SG, microRNA-16, -21, -27a, and -145 correlated positively with pre-exercise BP. There were no correlations between the analysed microRNA particles and parameters of HMOD. ROC analysis identified the best prognostic profile for relative microRNA-27b expression as a potential biomarker of the absence of PH. Multivariate analysis revealed the relative microRNA-16 expression as the only significant predictor of elevated diastolic BP.

**Conclusions:** MicroRNA-27b and microRNA-133a are underexpressed in children with PH. These particles may represent potential markers of hypertension in children and may also be associated with a lower burden of hypertension-related alterations. MicroRNA-16 may serve as a marker of diastolic hypertension.

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## Introduction

Arterial hypertension (AH) affects 3–5% of children globally [1]. While secondary hypertension (SH) is more common in early childhood, the rising obesity epidemic has led to an increasing prevalence of primary hypertension (PH), now the dominant form in adolescents [2]. Recent studies suggest that PH accounts for approximately half of all cases of AH during the developmental period [3]. Children diagnosed with AH have a higher risk of cardiovascular events compared with normotensive peers [4]. Thus, improved detection, follow-up, and control of paediatric hypertension might reduce the risk of adult cardiovascular disease.

MicroRNAs have recently gained attention for their presumed role in hypertension pathogenesis [5]. Their discovery was recognized with a Nobel Prize (2024), and emerging therapies, such as zilebesiran, show promise in lowering blood pressure (BP) for up to 24 weeks after injection [6]. Despite significant research in adults, studies of microRNAs in paediatric PH are scarce [7].

Many complex factors and interactions influence the development of PH: genetic and environmental factors, the renin-angiotensin-aldosterone system, the sympathetic nervous system, and substances produced by the vascular endothelium [8]. However, the exact process of hypertension development is not yet fully understood, and the molecular pathways remain to be elucidated [2]. One of the fascinating clues that have yet to be fully explored in the pathogenesis of hypertension is epigenetic changes, including microRNA-mediated gene regulation. They might be a key but an underexplored aspect [9]. MicroRNA expression also changes with exercise [10], which is a well-established non-pharmacological approach to BP control [11]. Post-exercise hypotension, observed for up to 24 h after physical activity, further underscores the role of exercise in hypertension management [12].

MicroRNA-16 is recognized as an essential regulator of vascular smooth muscle cell (VSMC) growth and migration and participates in angiotensin II-mediated signalling in these

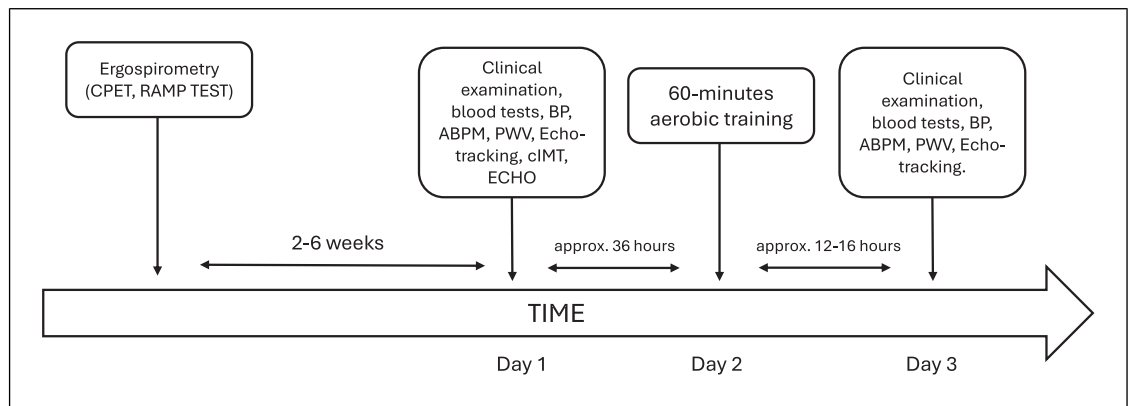
cells. Its overexpression is also thought to inhibit VSMC proliferation [13]. Notably, microRNA-16 appears to exert a bidirectional influence on inflammatory processes – acting in an anti-inflammatory manner by promoting the secretion of IL-10 and TGF- $\beta$ , or conversely, enhancing inflammation by stimulating NF- $\kappa$ B activation and increasing IL-8 production [14, 15]. Several studies have demonstrated elevated circulating plasma levels of microRNA-21 in hypertensive patients with hypertension-mediated organ damage (HMOD) [16, 17]. Other clinical investigations have reported a positive correlation between microRNA-21 levels and both BP values [18] as well as carotid intima-media thickness [19], and a negative association with arterial stiffness [20]. MicroRNA-27a and microRNA-27b have emerged as potent molecules of interest, acting as positive regulators of angiogenesis [21] and contributing to the regulation of endothelial cell repulsion – processes essential for the formation of a functional vascular network [22]. Furthermore, it has been suggested that both microRNA-27a and microRNA-27b may inhibit angiotensin-converting enzyme (ACE) expression by targeting specific binding sites within ACE transcripts [23]. MicroRNA-133a has similarly attracted attention, with studies reporting lower expression levels in patients with PH compared to healthy controls, as well as significant correlations with BP parameters [24]. Its expression has also been independently associated with urinary albumin excretion [25], and notably, circulating levels of microRNA-133a increase in healthy individuals following exercise [26]. Santovito et al. [27] reported significantly higher microRNA-145 levels within atherosclerotic plaques from hypertensive patients undergoing carotid endarterectomy. In contrast, Özkan et al. [28] observed significantly lower circulating microRNA-145 levels in newly diagnosed patients with PH during cohort follow-up, suggesting a potentially context-dependent role of this microRNA in hypertension.

Thus, we selected the six aforementioned microRNAs – microRNA-16, microRNA-21, microRNA-27a, microRNA-27b, microRNA-133a, and microRNA-145, as molecules with a potentially promising role in the development of PH. The study aimed to examine the expression of the six microRNAs in untreated adolescents with PH compared with healthy peers, assess changes in expression after exercise, and evaluate their correlations with BP and HMOD.

## Materials and Methods

### Study Design

The study employed a controlled single exercise session as an experimental intervention and standardized pre- and post-exercise analyses and measurements in a group of



**Fig. 1.** Summarized protocol of patients' examinations. Cardiopulmonary exercise test (CPET) was performed 2–6 weeks before the study to determine individualized exercise intensity. Baseline assessments were conducted on the morning of day 1. A 60-min aerobic exercise session was performed approximately 36 h later. Second ambulatory blood pressure monitoring (ABPM) was

initiated immediately after exercise. The remaining post-exercise assessments were performed on the morning of day 3 (12–16 h after exercise). Blood samples were collected after  $\geq 10$  h of fasting. BP, blood pressure; PWV, pulse wave velocity; cIMT, common carotid artery intima-media thickness; ECHO, echocardiography.

children with PH and their healthy peers. Since random allocation to groups was not feasible – group membership being determined by the presence or absence of pre-existing hypertension – the design qualifies as quasi-experimental, combining a pretest-post-test design and a nonequivalent groups design. Accordingly, the study should also be regarded as an observational, hypothesis-generating investigation.

The study protocol was reviewed and accepted by the National Science Centre in Poland (grant No. 2021/41/N/NZ5/04194) and approved by the Bioethics Committee of the Medical University of Warsaw (KB 154/2020). All procedures adhered to the highest ethical standards set by the Institutional Research Committee and were conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. The study protocol is summarized in Figure 1 and described in detail in online supplementary Table 1 (for all online suppl. material, see <https://doi.org/10.1159/000552984>). In brief, patients enrolled in the study underwent a cardiopulmonary exercise test (CPET) to determine the anaerobic threshold, which was used to standardize the training session. Two to 6 weeks after this test – to avoid the influence of maximal exertion on microRNA levels (see circulating microRNA kinetics and justification of the CPET interval in online supplementary Methods Section) – participants were invited for the first part of the study (day 1). The following day (day 2), participants performed a 60-min cycle ergometer

exercise session in the afternoon, using a workload determined by the CPET results. After the exercise test, a new ambulatory blood pressure monitoring (ABPM) measurement was initiated. The next morning (day 3), blood, urine, and arterial examinations were repeated. All participants underwent the same protocol.

#### Study Groups

Participants were recruited from a single paediatric nephrology centre between November 2021 and June 2023. The study group (SG) included children with untreated PH diagnosed at our university hospital. The control group (CG) consisted of age- and sex-matched children without hypertension, who met the same exclusion criteria.

Inclusion criteria for the SG were as follows: a diagnosis of untreated AH based on current European guidelines [29], confirmed by ABPM [30]; height  $\geq 120$  cm; and written informed consent from a parent or legal guardian. Exclusion criteria included SH; severe renal, hepatic, or cardiac disease; inflammatory conditions; contraindications to physical exercise; and acute infections (temporary 2-week exclusion). Children with behavioural disorders, disabilities, or noncompliance with study procedures were also excluded. Secondary causes of hypertension were excluded based on clinical evaluation and a standardized diagnostic workup, including laboratory testing and imaging, as described in detail in the online supplementary Methods.

### General Laboratory Procedures

All blood samples collected during the study (days 1 and 3 morning) were taken after at least 10 h of fasting (no meal) between 7 and 11 a.m. Blood sampling was conducted 12–16 h following the exercise session, not immediately after exertion. First, baseline blood was collected under controlled fasting and circadian conditions. Then, after the exercise, blood was drawn in the same conditions the next morning to assess sustained changes in circulating microRNA levels while maintaining comparability with baseline measurements. The supplementary materials describe the exact centrifugation conditions and the various sample processing steps (see supplementary methodology: evaluation of microRNA). All urine samples were the first morning samples. BP measurements and vascular tests were performed on each patient in the morning after fasting. Immediately after the exercise session, 24 h ABPM was initiated.

### Basic Clinical, Biochemical, BP, and Arterial Parameters

To avoid residual exertional effects on baseline biochemical or molecular analyses, a CPET was conducted at least 2 weeks before blood, urine, and BP assessments (see circulating microRNA kinetics and justification of the CPET interval in online supplementary Methods Section). Our previous manuscripts provide a detailed description of the methodology used for these parameters, including the preliminary study conducted to ensure proper study protocol management [7, 31]. Among measured parameters were as follows: NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; SBP, systolic blood pressure; office: DBP, diastolic blood pressure; MAP, mean arterial pressure; AoSBP, aortic systolic blood pressure; AoDBP, aortic diastolic blood pressure; AoMAP, aortic mean arterial pressure; AIx75-HR, augmentation index at a heart rate of 75/min; Buckberg SEVR, subendocardial viability ratio; ESP, end systolic pressure; 24 h-hour, 24 h; ABPM [32]: SBP 24 h, DBP 24 h, MAP 24 h (and their Z-score values according to paediatric normalized reference values [32]); cSBP 24 h, central systolic blood pressure; cDBP 24 h, central diastolic blood pressure; PP 24 h, pulse pressure; PWV 24 h, pulse wave velocity; TVR 24 h, total vascular resistance, AIx-24 h, augmentation index; CO 24 h, cardiac output; SV 24 h, stroke volume; RM 24 h, reflection magnitude; ET, ECHO tracking; beta, stiffness index; Ep, pressure strain elasticity modulus; AC, arterial compliance; AI, augmentation

index;  $D_{\max}$ , maximal diameter of common carotid artery;  $D_{\min}$ , minimal diameter of common carotid artery;  $DAT_{\max}$ , acceleration time to common carotid artery maximal diameter. Additional details can be found in the online supplementary Materials.

### Exercise Session

All participants underwent CPET (Cyclus 2, RMB elektronik-automation GmbH, Leipzig, Germany) to determine individualized exercise intensity. Subsequently, after 2–6 weeks, participants performed a 60-min supervised cycling session at 90–110% of the workload corresponding to each participant's anaerobic threshold (AT), in accordance with ACSM recommendations for moderate-intensity exercise [33]. A detailed description of CPET and the standardized exercise protocol is provided in the online supplementary Methods.

### HMOD and Vascular Parameters

Four indicators of HMOD were measured during the study: left ventricular mass index (LVMI) ( $\text{g}/\text{m}^{2.7}$  or  $\text{g}/\text{m}^2$ ) [34, 35], urinary albumin excretion, common carotid artery intima-media thickness (cIMT), and carotid-femoral pulse wave velocity (cfPWV). cIMT and cfPWV equal to or greater than the 95th percentile for age and height were considered abnormal [36, 37]. An exact description of the technique was included in the online supplementary material.

All remaining vascular and haemodynamic parameters – including central BP indices, augmentation index, parameters derived from 24 h ABPM, as well as ultrasound- and applanation tonometry-derived measures of vascular stiffness – were treated as exploratory variables and used for comprehensive characterization of cardiovascular function in the study population; accordingly, results derived from these exploratory variables should be interpreted as hypothesis-generating.

### Evaluation of microRNA

MicroRNA extraction was performed using mir-Vana PARIS kits (Thermo Fisher Scientific, Waltham, MA, USA) per the manufacturer's instructions. *C. elegans* miRNAs, cel-microRNA-39 and cel-microRNA-54 (synthetic RNA oligonucleotides synthesized by Thermo Fisher), were added in equal amounts to each sample: immediately after combining the plasma sample with the denaturing solution (cel-microRNA-39) and at the beginning of the poly(A) tailing reaction (cel-microRNA-54) [38]. Total RNA

was isolated from plasma samples without the use of an enrichment procedure for small RNAs. RNA quantity, quality, and purity were assessed via NanoDrop Lite (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription was conducted with TaqMan kits, followed by real-time PCR using TaqMan Advanced microRNA Assays (Thermo Fisher Scientific, Waltham, MA, USA) and the LightCycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany). All samples were run in triplicate. Each assay was run with 2 no-template controls to rule out nonspecific amplification. No sample included in the study demonstrated a Ct value near the no-template control or exceeded 40 cycles.

Data were analysed using LightCycler 480 II software, with relative microRNA levels calculated via the  $2^{-\Delta\Delta C_t}$  method [39]. In addition to two spike-in normalizers, one randomly selected sample was used as a calibrator (reference sample) across all qPCR runs to enable relative quantification using the  $2^{-\Delta\Delta C_t}$  method. qPCR runs to ensure inter-run comparability, and miRNA expressions were expressed as  $2^{-\Delta\Delta C_t}$  [40] (all the data normalization is present as online suppl. file 1). Expression levels were log 10-transformed for statistical analysis. All microRNA sequences and accession numbers (<https://www.mirbase.org/>) are listed in online supplementary Table 2 for reference and further study.

### Statistical Methods

A sample size assessment based on previous microRNA studies in adult hypertensive patients [16, 19, 20, 24, 28], indicated that 55 participants per group would be required (power 0.80,  $p = 0.05$ ,  $\Delta r = 0.4$ ) (online suppl. Fig. 1). Assuming a 20% dropout rate, we recruited 140 patients. Statistical analyses were performed using Dell Statistica 13.0 PL (TIBCO Software Inc.) and SPSS 22.0 (IBM Corp.). Data distribution was assessed using the Shapiro-Wilk test. Continuous variables were expressed as mean  $\pm$  SD and interquartile range, and categorical variables as percentages. All tests were two-sided with a significance level of  $p < 0.05$ .

Group comparisons were performed using Student's  $t$  test or Mann-Whitney U test and paired comparisons using paired  $t$  test or Wilcoxon test, as appropriate. Correlations were assessed using Spearman's or Pearson's coefficients. Multivariable regression and ROC analyses were performed to identify predictors of hypertension. Detailed further statistical procedures are described in the online supplementary Methods.

## Results

Five participants, including three from the study group and two from the control group, were unable to complete CPET despite repeated attempts (3) and were excluded prior to the intervention (online suppl. S2 Figure). During the exercise protocol, 3 participants, 2 from the study group and 1 from the control group, did not complete the session at the first attempt due to transient factors. All subsequently repeated the protocol after the predefined interval of 2–6 weeks, resulting in a final sample of 56 participants in the SG and 59 in the CG who completed all study phases and had complete data (online suppl. S2 Figure). Tables 1 and 2 and online supplementary Table 3 summarize the data obtained during both groups' measurements and the parameters' values tested before and after the standardized training session.

### Basic Clinical, Blood, and Urine Parameters

The groups did not differ in age, sex, height Z-scores, gestational age, or birth weight. However, the SG had significantly higher weight Z-scores, body mass index (BMI) Z-scores, waist and hip circumferences, and waist-hip-ratio. Kidney function and most biochemical parameters (total cholesterol, sodium, potassium, renin, aldosterone, aldosterone/renin ratio, and inflammatory markers) were similar. Significant differences were found in uric acid, HDL cholesterol, triglycerides, and NLR. After exercise, the SG had lower sodium-to-creatinine ( $p = 0.029$ ) and phosphorus-to-creatinine ( $p = 0.001$ ) excretion. Before exercise, the SG showed lower calciuria. Following exercise, the urinary sodium-to-creatinine ratio showed negative correlations with renin ( $R = -0.471$ ,  $p < 0.001$ ) and aldosterone levels ( $R = -0.465$ ,  $p < 0.001$ ) in the study group. In contrast, the urinary potassium-to-creatinine ratio exhibited a positive correlation with renin ( $R = 0.286$ ,  $p = 0.028$ ) in the control group.

### BP and Vascular Parameters, Including HMOD

There was a significant difference in BP parameters between hypertensive and normotensive children, in terms of SBP, DBP, and their Z-scores, as well as parameters measured during 24 h: SBP 24 h, DBP 24 h, MAP 24 h, PP 24 h, cSBP 24 h, cDBP 24 h, PWV 24 h, and TVR 24 h before exercise. The groups did not differ in HR at the 24 h measurement.

In the SG, all BP indices, except for 24 h pulse pressure, decreased significantly after an exercise session. Conversely, in the CG, BP indices did not change except

**Table 1.** Comparison of the studied parameters in the study and control group (baseline value)

| Parameter                        | Study group $\pm$ SD (IQR)        | Control group $\pm$ SD (IQR)     | p value |
|----------------------------------|-----------------------------------|----------------------------------|---------|
| Number of patients (n)           | 56                                | 59                               | N/A     |
| Age, years                       | 15.4 $\pm$ 1.9 (14.3–16.9)        | 15.3 $\pm$ 1.6 (13.8–16.6)       | 0.435   |
| Boys/girls                       | 42/14                             | 41/18                            | 0.378   |
| Hypertensive mother, %           | 29%                               | 8%                               | 0.053   |
| Hypertensive father, %           | 50%                               | 29%                              | 0.020   |
| Height, cm                       | 173.1 $\pm$ 8.8 (167.0–179.0)     | 171.7 $\pm$ 10.5 (165.0–180.0)   | 0.731   |
| Height Z-score                   | 0.64 $\pm$ 1.04 (–0.05–1.30)      | 0.57 $\pm$ 1.00 (–0.24–1.20)     | 0.461   |
| Weight, kg                       | 80.2 $\pm$ 19.7 (66.5–87.5)       | 61.7 $\pm$ 11.5 (55.0–69.0)      | <0.001  |
| Weight Z-score                   | 1.51 $\pm$ 1.10 (0.73–2.27)       | 0.43 $\pm$ 0.84 (0.01–1.03)      | <0.001  |
| BMI, kg/m <sup>2</sup>           | 26.8 $\pm$ 6.6 (22.5–30.1)        | 20.9 $\pm$ 3.4 (18.7–22.4)       | <0.001  |
| BMI Z-score                      | 1.39 $\pm$ 1.1 (0.68–2.22)        | 0.16 $\pm$ 1.1 (–0.42–0.83)      | <0.001  |
| Waist circumference, cm          | 84.9 $\pm$ 15.0 (74.5–90.4)       | 71.1 $\pm$ 6.7 (67.5–74.5)       | <0.001  |
| Hip circumference, cm            | 100.8 $\pm$ 12.0 (92.8–106.5)     | 91.4 $\pm$ 8.2 (86.5–96.0)       | <0.001  |
| WHR                              | 0.84 $\pm$ 0.11 (0.79–0.88)       | 0.78 $\pm$ 0.04 (0.75–0.81)      | <0.001  |
| Duration of hypertension, months | 10.2 $\pm$ 14.0 (1.0–12.5)        | 0                                | N/A     |
| Duration of pregnancy, weeks     | 38.5 $\pm$ 2.5 (37.3–40.0)        | 38.7 $\pm$ 1.9 (38.0–40.0)       | 0.797   |
| Birth weight, g                  | 3,252 $\pm$ 550 (3,073–3,500)     | 3,324 $\pm$ 583 (3,160–3,650)    | 0.185   |
| Sodium serum, mmol/L             | 139.3 $\pm$ 2.0 (138.0–140.5)     | 139.33 $\pm$ 2.1 (138.0–140.0)   | 0.950   |
| Potassium serum, mmol/L          | 4.28 $\pm$ 0.23 (4.10–4.43)       | 4.243 $\pm$ 0.30 (4.05–4.50)     | 0.460   |
| Uric acid, mg/dL                 | 5.8 $\pm$ 1.1 (5.1–6.6)           | 5.0 $\pm$ 0.9 (4.5–5.7)          | <0.001  |
| Cholesterol, mg/dL               | 163.3 $\pm$ 34.2 (137.0–187.0)    | 155.4 $\pm$ 30.3 (136.0–168.00)  | 0.154   |
| LDL cholesterol, mg/dL           | 91.1 $\pm$ 26.9 (74.0–104.0)      | 83.6 $\pm$ 25.4 (67.0–95.0)      | 0.091   |
| HDL cholesterol, mg/dL           | 52.3 $\pm$ 15.0 (42.5–59.5)       | 57.6 $\pm$ 11.7 (49.0–65.3)      | 0.009   |
| Triglycerides, mg/dL             | 99.8 $\pm$ 46.0 (64.5–126.5)      | 71.1 $\pm$ 24.8 (57.0–80.0)      | <0.001  |
| eGFR, mL/min/1.73 m <sup>2</sup> | 108.7 $\pm$ 18.3 (97.5–121.8)     | 107.0 $\pm$ 17.3 (92.4–117.0)    | 0.603   |
| Renin, mU/L                      | 34.4 $\pm$ 21.6 (18.8–44.7)       | 35.8 $\pm$ 25.0 (21.0–44.1)      | 0.838   |
| Aldosterone, ng/dL               | 12.1 $\pm$ 7.8 (7.4–13.8)         | 12.8 $\pm$ 6.6 (7.6–16.9)        | 0.383   |
| PAC/PRC                          | 0.40 $\pm$ 0.2 (0.25–0.52)        | 0.45 $\pm$ 0.3 (0.26–0.57)       | 0.467   |
| NLR                              | 1.65 $\pm$ 0.8 (1.09–1.93)        | 1.79 $\pm$ 3.2 (0.95–1.57)       | 0.014   |
| PLR                              | 130.12 $\pm$ 38.7 (100.43–150.89) | 118.91 $\pm$ 37.7 (93.75–139.26) | 0.082   |
| MLR                              | 0.26 $\pm$ 0.1 (0.20–0.28)        | 0.28 $\pm$ 0.3 (0.20–0.29)       | 0.889   |
| MNR                              | 0.18 $\pm$ 0.08 (0.12–0.22)       | 0.19 $\pm$ 0.06 (0.15–0.22)      | 0.086   |
| ACR, mg/g                        | 12.32 $\pm$ 22.0 (2.77–9.22)      | 8.12 $\pm$ 8.0 (3.58–9.10)       | 0.332   |
| U. Na/creat, mmol/mg             | 0.75 $\pm$ 0.46 (0.39–1.00)       | 0.75 $\pm$ 0.42 (0.49–0.92)      | 0.714   |
| U. K/creat, mmol/mg              | 0.23 $\pm$ 0.14 (0.16–0.26)       | 0.21 $\pm$ 0.19 (0.13–0.24)      | 0.119   |
| U. UA/creat, mg/mg               | 0.28 $\pm$ 0.10 (0.21–0.34)       | 0.33 $\pm$ 0.16 (0.23–0.37)      | 0.109   |
| U. Ca/creat, mg/mg               | 0.07 $\pm$ 0.05 (0.03–0.10)       | 0.09 $\pm$ 0.06 (0.05–0.13)      | 0.017   |
| U. P/creat, mg/mg                | 0.68 $\pm$ 0.17 (0.53–0.78)       | 0.78 $\pm$ 0.43 (0.60–0.86)      | 0.098   |

**Table 1** (continued)

| Parameter                   | Study group $\pm$ SD (IQR)            | Control group $\pm$ SD (IQR)          | <i>p</i> value |
|-----------------------------|---------------------------------------|---------------------------------------|----------------|
| SBP, mm Hg                  | 136.8 $\pm$ 10.4 (131.0–145.5)        | 118.8 $\pm$ 9.6 (113.0–126.0)         | <0.001         |
| SBP Z-score                 | 1.82 $\pm$ 0.86 (1.34–2.38)           | 0.25 $\pm$ 0.81 (–0.34–0.85)          | <0.001         |
| DBP, mm Hg                  | 82.1 $\pm$ 8.1 (76.0–88.0)            | 72.9 $\pm$ 6.3 (69.0–77.0)            | <0.001         |
| DBP Z-score                 | 2.24 $\pm$ 1.13 (1.44–3.06)           | 1.00 $\pm$ 0.81 (0.44–1.52)           | <0.001         |
| MAP, mm Hg                  | 98.3 $\pm$ 7.7 (92.9–103.0)           | 86.8 $\pm$ 6.6 (81.7–91.7)            | <0.001         |
| HR, 1/min                   | 75.9 $\pm$ 13.2 (66.9–84.3)           | 68.8 $\pm$ 11.0 (62.2–74.0)           | 0.001          |
| SBP 24 h, mm Hg             | 131.2 $\pm$ 9.5 (124.0–137.5)         | 114.9 $\pm$ 6.9 (109.0–121.0)         | <0.001         |
| DBP 24 h, mm Hg             | 73.6 $\pm$ 6.6 (68.0–78.0)            | 66.1 $\pm$ 5.1 (64.0–69.0)            | <0.001         |
| MAP 24 h, mm Hg             | 100.0 $\pm$ 7.3 (94.0–105.0)          | 88.5 $\pm$ 5.1 (85.0–92.0)            | <0.001         |
| PP 24 h, mm Hg              | 57.1 $\pm$ 6.9 (53.0–60.0)            | 48.3 $\pm$ 6.7 (43.0–52.0)            | <0.001         |
| HR 24 h, 1/min              | 73.5 $\pm$ 9.1 (67.0–79.5)            | 71.2 $\pm$ 9.7 (63.0–79.0)            | 0.146          |
| cSBP 24 h, mm Hg            | 114.5 $\pm$ 8.5 (108.0–119.5)         | 100.4 $\pm$ 5.5 (96.0–104.0)          | <0.001         |
| cDBP 24 h, mm Hg            | 76.0 $\pm$ 6.8 (70.0–80.5)            | 67.9 $\pm$ 5.2 (65.0–72.0)            | <0.001         |
| Alx@75-HR 24 h, %           | 15.0 $\pm$ 6.2 (10.0–18.5)            | 12.2 $\pm$ 6.2 (8.0–15.0)             | 0.013          |
| PWV 24 h, m/s               | 4.7 $\pm$ 0.46 (4.0–5.0)              | 4.0 $\pm$ 0.18 (4.0–4.0)              | <0.001         |
| SV, mL                      | 74.6 $\pm$ 7.7 (71.0–80.5)            | 73.6 $\pm$ 10.4 (65.0–82.0)           | 0.561          |
| CO, L/min                   | 4.79 $\pm$ 0.41 (5.00–5.00)           | 4.51 $\pm$ 0.50 (4.00–5.00)           | 0.010          |
| TVR, dyn* $s/cm^5$          | 1,552.5 $\pm$ 125.8 (1,447.0–1,612.5) | 1,461.5 $\pm$ 106.8 (1,375.0–1,540.0) | <0.001         |
| RM, %                       | 57 $\pm$ 6.4 (54–62)                  | 54 $\pm$ 5.4 (50–57)                  | 0.003          |
| Systolic DIP, %             | 11.0 $\pm$ 6.0 (7.1–14.8)             | 10.6 $\pm$ 4.6 (7.3–13.5)             | 0.676          |
| Diastolic DIP, %            | 14.0 $\pm$ 6.9 (8.4–19.1)             | 17.5 $\pm$ 6.0 (13.4–22.1)            | 0.005          |
| LVMI, g/m <sup>2.7</sup>    | 33.02 $\pm$ 8.17 (26.64–37.78)        | 28.12 $\pm$ 5.90 (22.68–34.09)        | <0.001         |
| LVMI, g/m <sup>2</sup>      | 74.59 $\pm$ 17.92 (63.19–85.44)       | 70.83 $\pm$ 13.98 (62.96–81.60)       | 0.210          |
| LVMI Z-score                | –0.554 $\pm$ 1.474 (–1.465–0.415)     | –1.334 $\pm$ 1.455 (–2.510 to –0.050) | 0.003          |
| cfPWV, m/s                  | 5.26 $\pm$ 0.71 (4.77–5.63)           | 4.69 $\pm$ 0.60 (4.20–5.07)           | <0.001         |
| cfPWV Z-score               | –0.19 $\pm$ 0.95 (–0.81–0.23)         | –0.96 $\pm$ 0.80 (–1.49 to –0.30)     | <0.001         |
| AoSBP, mm Hg                | 114.9 $\pm$ 8.6 (110.2–119.1)         | 100.7 $\pm$ 7.7 (94.0–107.0)          | <0.001         |
| AoDBP, mm Hg                | 83.5 $\pm$ 8.1 (77.1–89.0)            | 74.2 $\pm$ 6.4 (70.0–78.5)            | <0.001         |
| AoMAP, mm Hg                | 98.3 $\pm$ 7.7 (92.9–103.0)           | 86.8 $\pm$ 6.6 (81.7–91.7)            | <0.001         |
| Alx, %                      | –6.78 $\pm$ 11.86 (–15.84–1.10)       | –4.64 $\pm$ 10.77 (–13.50–1.00)       | 0.254          |
| Alx@HR75, %                 | –6.47 $\pm$ 11.85 (–14.75–2.53)       | –7.79 $\pm$ 10.09 (–13.75 to –2.20)   | 0.778          |
| Buckberg SEVR               | 184.7 $\pm$ 47.3 (151.5–209.1)        | 190.7 $\pm$ 33.5 (166.7–214.2)        | 0.147          |
| ESP, mm Hg                  | 105.5 $\pm$ 9.1 (100.2–109.5)         | 92.7 $\pm$ 7.7 (86.3–99.0)            | <0.001         |
| cIMT, mm                    | 0.48 $\pm$ 0.06 (0.45–0.52)           | 0.46 $\pm$ 0.04 (0.43–0.49)           | 0.067          |
| cIMT Z-score                | 1.77 $\pm$ 1.12 (1.04–2.58)           | 1.46 $\pm$ 0.89 (0.67–2.07)           | 0.108          |
| ET beta                     | 3.3 $\pm$ 0.7 (2.7–3.6)               | 3.0 $\pm$ 0.6 (2.7–3.3)               | 0.451          |
| ET Ep, kPa                  | 45.9 $\pm$ 11.5 (38.5–51.5)           | 37.5 $\pm$ 8.2 (32.0–42.5)            | <0.001         |
| ET AC, mm <sup>2</sup> /kPa | 1.30 $\pm$ 0.30 (1.11–1.46)           | 1.48 $\pm$ 0.36 (1.18–1.73)           | 0.007          |

**Table 1** (continued)

| Parameter                  | Study group $\pm$ SD (IQR)     | Control group $\pm$ SD (IQR)   | <i>p</i> value |
|----------------------------|--------------------------------|--------------------------------|----------------|
| ET AI, %                   | -4.8 $\pm$ 11.4 (-11.48–0.45)  | -6.0 $\pm$ 9.5 (-13.8 to -0.9) | 0.814          |
| ET PWV $\beta$ , m/s       | 4.1 $\pm$ 0.5 (3.8–4.3)        | 3.7 $\pm$ 0.4 (3.5–4.0)        | <0.001         |
| ET D <sub>max</sub> , mm   | 6.75 $\pm$ 0.62 (6.32–7.14)    | 6.46 $\pm$ 0.51 (6.07–6.76)    | 0.024          |
| ET D <sub>min</sub> , mm   | 5.81 $\pm$ 0.62 (5.33–6.10)    | 5.53 $\pm$ 0.48 (5.24–5.76)    | 0.066          |
| ET DAT <sub>max</sub> , ms | 134.0 $\pm$ 32.3 (116.0–142.8) | 134.2 $\pm$ 17.4 (120.5–142.0) | 0.102          |

SD, standard deviation; IQR, interquartile range; N/A, non-applicable; BMI, body mass index; WHR, waist-to-hip ratio; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; MNR, monocyte-to-neutrophil ratio; ACR, albumin-to-creatinine ratio; U, urine; Na, sodium; K, potassium; UA, uric acid; Ca, calcium; P, phosphorus; creat, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; PP, pulse pressure; 24 h, 24-hour; c, central blood pressure; AIx@75-HR, augmentation index normalized to heart rate of 75 beats per minute; PWV, pulse wave velocity; SV, stroke volume; CO, cardiac output; TVR, total vascular resistance; RM, reflection magnitude; DIP, blood pressure night dipping; LVMI, the left ventricular mass index; cf, carotid-femoral; Ao, central blood pressure (aortic); AIx, augmentation index; Buckberg SEVR, subendocardial viability ratio; ESP, end systolic pressure; cIMT, common carotid artery intima-media thickness; ET, ECHO tracking; beta, stiffness index; Ep, pressure strain elasticity modulus; AC, arterial compliance; AI, augmentation index; D<sub>max</sub>, maximal diameter of common carotid artery; D<sub>min</sub>, minimal diameter of common carotid artery; DAT<sub>max</sub>, acceleration time to common carotid artery maximal diameter.

for a significant rise in 24 h systolic BP and 24 h pulse pressure.

Concerning HMOD, the groups differed in LVMI [g/m<sup>2.7</sup>], LVMI Z-score, cfPWV and its Z-score, and central pressures AoSBP, AoDBP, AoMAP, and ESP, as well as in arterial parameters and local stiffness of the common carotid artery: ET Ep and ET-PVWB. No difference was found for AIx, AIx@75-HR, Buckberg SEVR index, ET beta, ET AC, ET AI, ET D<sub>min</sub>, ET D<sub>max</sub> and ET DAT<sub>max</sub> (using Bonferroni correction and Benjamini-Hochberg FDR corrections). There was a trend towards a higher mean cIMT value in the SG.

#### MicroRNA Expression

MicroRNA-27b expression was significantly higher ( $p < 0.001$ ) in the control group than in the study group both before and after exercise, and its expression after physical exercise increased significantly only in the control group (Fig. 2). Similar relationships were observed in microRNA-133a expression across the studied groups: it was higher in the control group both before and after exercise, and it increased after physical exercise in the control group (Fig. 2). In multivariable linear regression analyses, hypertension status remained independently associated with both microRNA-27b and microRNA-133a expression after adjustment for BMI Z-score, age, and sex. Specifically, hypertensive patients exhibited lower microRNA-133a and microRNA-27b levels compared to controls (online suppl. S4 Table).

These associations were consistent across models adjusted for different measures of body composition, including waist and hip circumference.

MicroRNA-27b was the best indicator for determining the presence or absence of hypertension among the subjects (Fig. 3). MicroRNA-133 was also a significant predictor of hypertension in the studied children, though with a lower AUC.

Among the six microRNAs tested, in multivariate analysis, microRNA-16 expression level was the best marker for predicting the 24 h DBP Z-score at the time of examination (Fig. 4, online suppl. Table S5). In the multivariate logistic regression model, high baseline expression of microRNA-16 was identified as an independent determinant differentiating patients with higher (>2.1) versus lower DBP Z-score values (Table 3).

The study also revealed that microRNA-145 expression increased after exercise in both groups; however, no statistically significant differences were observed between groups at baseline or post-exercise (Fig. 4). Circulating levels of microRNA-16, microRNA-21, and microRNA-27a were comparable between the groups and remained unchanged following exercise.

The most significant results of the univariate analysis of associations between relative microRNA expression levels in SG and clinical and biochemical parameters are presented in Figure 5 (the heatmap). MicroRNA-27a and microRNA-133a positively correlated with BMI Z-scores

**Table 2.** Comparison of the studied parameters in the study group before and after the aerobic exercise session

| Parameter                        | Before exercise                 | After exercise                 | <i>p</i> |
|----------------------------------|---------------------------------|--------------------------------|----------|
|                                  | mean±SD (IQR)                   |                                |          |
| Uric acid, mg/dL                 | 5.8±1.1 (5.1–6.6)               | 6.3±1.4 (5.2–7.3)              | <0.001   |
| Creatinine, mg/dL                | 0.80±0.2 (0.66–0.95)            | 0.81±0.2 (0.67–0.93)           | 0.378    |
| eGFR, mL/min/1.73 m <sup>2</sup> | 108.7±18.3 (97.5–121.8)         | 107.6±19.7 (93.4–123.4)        | 0.287    |
| Renin, mU/L                      | 34.4±21.6 (18.8–44.7)           | 36.1±21.3 (20.5–47.7)          | 0.324    |
| Aldosterone, ng/dL               | 12.1±7.8 (7.4–13.8)             | 13.8±6.5 (9.3–17.1)            | 0.054    |
| PAC/PRC                          | 0.40±0.2 (0.25–0.52)            | 0.45±0.2 (0.31–0.54)           | 0.081    |
| ACR, mg/g                        | 12.32±22.0 (2.77–9.22)          | 8.47±10.4 (2.87–8.01)          | 0.424    |
| U. Na/creat, mmol/mg             | 0.75±0.46 (0.39–1.00)           | 0.50±0.34 (0.26–0.60)          | 0.002    |
| U. K/creat, mmol/mg              | 0.23±0.14 (0.16–0.26)           | 0.20±0.08 (0.14–0.24)          | 0.071    |
| U. UA/creat, mg/mg               | 0.28±0.10 (0.21–0.34)           | 0.32±0.10 (0.25–0.38)          | 0.007    |
| U. Ca/creat, mg/mg               | 0.07±0.05 (0.03–0.10)           | 0.08±0.06 (0.04–0.11)          | 0.166    |
| U. P/creat, mg/mg                | 0.68±0.17 (0.53–0.78)           | 0.67±0.20 (0.53–0.79)          | 0.726    |
| SBP, mm Hg                       | 136.8±10.4 (131.0–145.5)        | 133.1±9.8 (127.0–140.0)        | 0.006    |
| SBP Z-score                      | 1.82±0.86 (1.34–2.38)           | 1.51±0.78 (1.02–2.04)          | 0.006    |
| DBP, mm Hg                       | 82.1±8.1 (76.0–88.0)            | 78.6±7.6 (72.5–84.5)           | <0.001   |
| DBP Z-score                      | 2.24±1.13 (1.44–3.06)           | 1.76±1.02 (0.98–2.44)          | <0.001   |
| MAP, mm Hg                       | 98.3±7.7 (92.9–103.0)           | 94.9±7.3 (89.7–100.0)          | <0.001   |
| HR, 1/min                        | 75.9±13.2 (66.9–84.3)           | 77.1±12.9 (68.4–83.1)          | 0.245    |
| SBP 24 h, mm Hg                  | 131.2±9.5 (124.0–137.5)         | 129.1±8.4 (123.5–135.0)        | 0.008    |
| DBP 24 h, mm Hg                  | 73.6±6.6 (68.0–78.0)            | 71.6±6.5 (67.0–75.5)           | <0.001   |
| MAP 24 h, mm Hg                  | 100.0±7.3 (94.0–105.0)          | 98.1±6.6 (93.5–102.0)          | 0.001    |
| PP 24 h, mm Hg                   | 57.1±6.9 (53.0–60.0)            | 57.0±7.1 (51.5–61.0)           | 0.851    |
| HR 24 h, 1/min                   | 73.5±9.1 (67.0–79.5)            | 78.0±9.3 (72.0–82.5)           | <0.001   |
| cSBP 24 h, mm Hg                 | 114.5±8.5 (108.0–119.5)         | 112.2±7.3 (107.5–116.5)        | 0.002    |
| cDBP 24 h, mm Hg                 | 76.0±6.8 (70.0–80.5)            | 74.2±6.7 (69.0–78.5)           | <0.001   |
| Alx@75-HR 24 h, %                | 15.0±6.2 (10.0–18.5)            | 16.3±5.9 (12.5–20.0)           | 0.002    |
| PWV 24 h, m/s                    | 4.7±0.46 (4.0–5.0)              | 4.6±0.48 (4.0–5.0)             | 0.370    |
| SV, mL                           | 74.6±7.7 (71.0–80.5)            | 71.8±8.2 (67.0–77.5)           | <0.001   |
| CO, L/min                        | 4.79±0.41 (5.00–5.00)           | 4.89±0.37 (5.00–5.00)          | 0.033    |
| TVR, dyn*s/cm <sup>5</sup>       | 1,552.5±125.8 (1,447.0–1,612.5) | 1,491.9±94.0 (1,418.0–1,551.5) | <0.001   |
| RM, %                            | 57±6.4 (54–62)                  | 55±6.6 (50–60)                 | <0.001   |
| Systolic DIP, %                  | 11.0±6.0 (7.1–14.8)             | 10.6±6.7 (5.5–15.4)            | 0.941    |
| Diastolic DIP, %                 | 14.0±6.9 (8.4–19.1)             | 15.4±7.2 (11.1–20.6)           | 0.112    |
| cfPWV, m/s                       | 5.26±0.71 (4.77–5.63)           | 5.22±0.68 (4.89–5.63)          | 0.388    |
| cfPWV Z-score                    | −0.19±0.95 (−0.81–0.23)         | −0.25±0.90 (−0.75–0.16)        | 0.366    |
| AoSBP, mm Hg                     | 114.9±8.6 (110.2–119.1)         | 111.2±7.8 (105.4–116.6)        | <0.001   |

**Table 2** (continued)

| Parameter                   | Before exercise           | After exercise            | <i>p</i> |
|-----------------------------|---------------------------|---------------------------|----------|
|                             | mean±SD (IQR)             |                           |          |
| AoDBP, mm Hg                | 83.5±8.1 (77.1–89.0)      | 80.1±7.8 (74.4–86.8)      | <0.001   |
| AoMAP, mm Hg                | 98.3±7.7 (92.9–103.0)     | 94.9±7.3 (89.7–100.0)     | <0.001   |
| Alx, %                      | –6.78±11.86 (–15.84–1.10) | –6.61±10.45 (–14.35–0.71) | 0.864    |
| Alx@HR75, %                 | –6.47±11.85 (–14.75–2.53) | –5.54±11.06 (–13.88–2.10) | 0.365    |
| Buckberg SEVR               | 184.7±47.3 (151.5–209.1)  | 181.0±50.7 (145.1–200.6)  | 0.291    |
| ESP, mm Hg                  | 105.5±9.1 (100.2–109.5)   | 101.6±7.8 (96.0–106.9)    | <0.001   |
| ET beta                     | 3.3±0.7 (2.7–3.6)         | 3.3±0.7 (2.7–3.6)         | 0.901    |
| ET Ep, kPa                  | 45.9±11.5 (38.5–51.5)     | 44.6±11.0 (36.5–50.5)     | 0.223    |
| ET AC, mm <sup>2</sup> /kPa | 1.30±0.30 (1.11–1.46)     | 1.35±0.27 (1.19–1.51)     | 0.141    |
| ET AI, %                    | –4.8±11.4 (–11.48–0.45)   | –6.8±8.7 (–12.8 to –4.0)  | 0.120    |
| ET PWVβ, m/s                | 4.1±0.5 (3.8–4.3)         | 4.0±0.5 (3.7–4.3)         | 0.124    |
| ET D <sub>max</sub> , mm    | 6.75±0.62 (6.32–7.14)     | 6.81±0.56 (6.44–7.13)     | 0.269    |
| ET D <sub>min</sub> , mm    | 5.81±0.62 (5.33–6.10)     | 5.84±0.56 (5.44–6.15)     | 0.426    |
| ET DAT <sub>max</sub> , ms  | 134.0±32.3 (116.0–142.8)  | 126.4±22.8 (111.9–135.6)  | 0.082    |

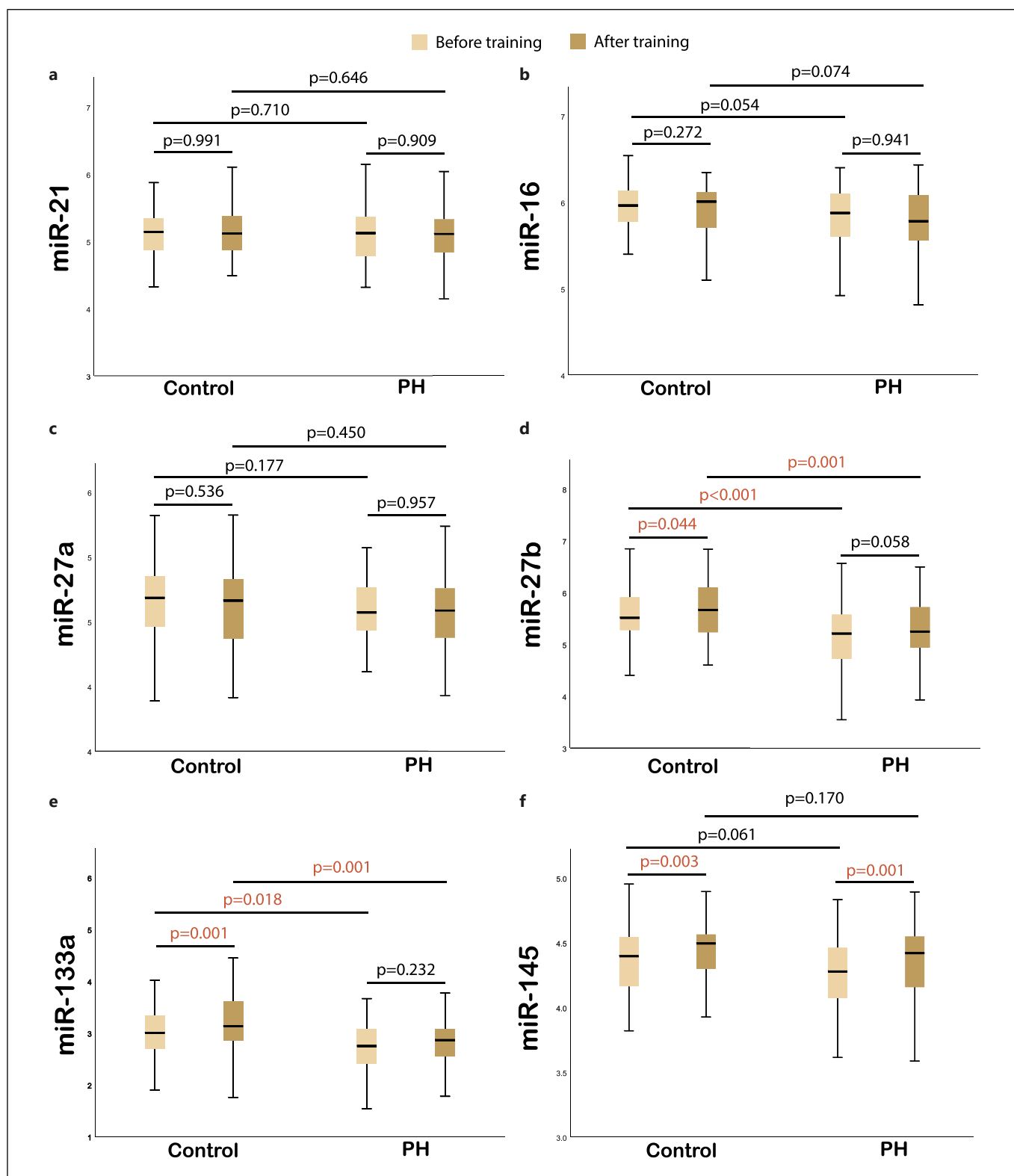
SD, standard deviation; IQR, interquartile range; eGFR, estimated glomerular filtration rate; PAC, plasma aldosterone concentration; PRC, plasma renin concentration; ACR, albumin-to-creatinine ratio; U, urine; Na, sodium; K, potassium; UA, uric acid; Ca, calcium; P, phosphorus; creat, creatinine; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; PP, pulse pressure; 24 h, 24-hour; c, central blood pressure; Alx@75-HR, augmentation index normalized to heart rate of 75 beats per minute; PWV, pulse wave velocity; SV, stroke volume; CO, cardiac output; TVR, total vascular resistance; RM, reflection magnitude; DIP, blood pressure night dipping; cf, carotid-femoral; Ao, central blood pressure (aortic); Alx, augmentation index; Buckberg SEVR, subendocardial viability ratio; ESP, end systolic pressure; ET, ECHO tracking; beta, stiffness index; Ep, pressure strain elasticity modulus; AC, arterial compliance; AI, augmentation index; D<sub>max</sub>, maximal diameter of common carotid artery; D<sub>min</sub>, minimal diameter of common carotid artery; DAT<sub>max</sub>, acceleration time to common carotid artery maximal diameter.

( $r = 0.313$ ,  $p = 0.019$ ;  $r = 0.485$ ,  $p < 0.001$ ), while microRNA-133a positively correlated with waist and hip circumference ( $r = 0.392$ ,  $p = 0.003$ ;  $r = 0.362$ ,  $p = 0.006$ ). As for biochemical parameters, we found the following significant correlations: between microRNA-133a and serum sodium before exercise bout ( $r = -0.283$ ,  $p = 0.034$ ); between microRNA-145 and MLR ( $r = -0.268$ ,  $p = 0.046$ ). MicroRNA-16, -27a, -27b, and -133a correlated negatively with aldosterone after an exercise bout. Regarding urinary indices, in the control group, post-exercise urinary sodium-to-creatinine ratio was negatively correlated with microRNA-16 ( $R = -0.311$ ,  $p = 0.016$ ) and microRNA-27a ( $R = -0.264$ ,  $p = 0.044$ ). At baseline, a positive correlation was observed between microRNA-133a and urinary uric acid-to-creatinine ratio ( $R = 0.258$ ,  $p = 0.049$ ). In contrast to the control group, no significant associations were found in the hypertensive group.

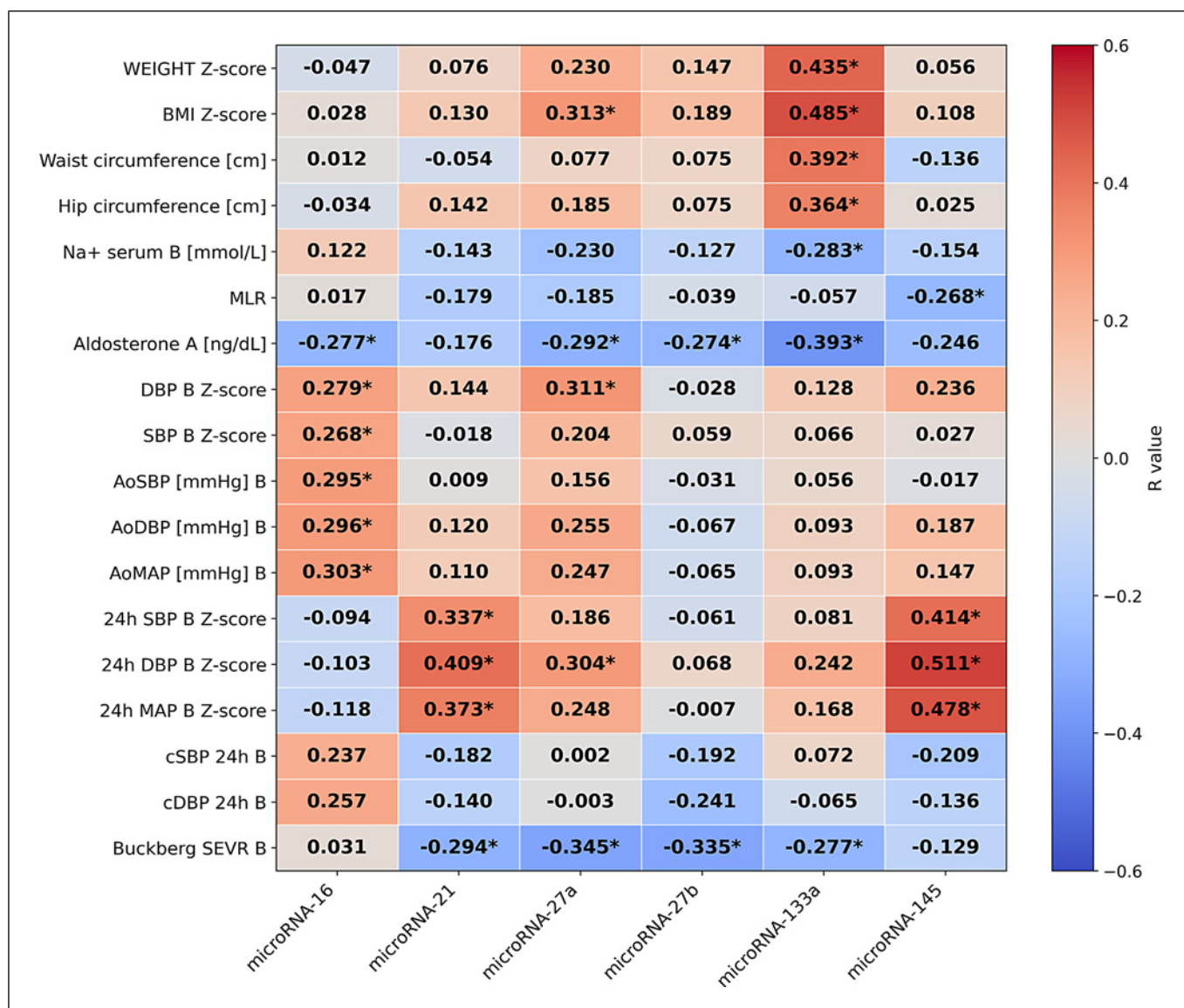
Regarding BP before the exercise session, microRNA-16 correlated positively with peripheral and central office BP. MicroRNA-21 and -145 correlated positively with ABPM BP values. MicroRNA-27a correlated positively with office and ambulatory DBP. Also, microRNA-21, -27a, -27b, and -133a correlated negatively with Buckberg SEVR. No significant correlations between microRNA particle expression levels and the analysed indices of HMOD were observed.

## Discussion

Our prospective, pre-post, observational, and hypothesis-generating study is the first to analyse microRNA expression in children with PH compared to healthy age- and sex-matched peers. We found lower expression levels of microRNA-27b and microRNA-133a in hypertensive



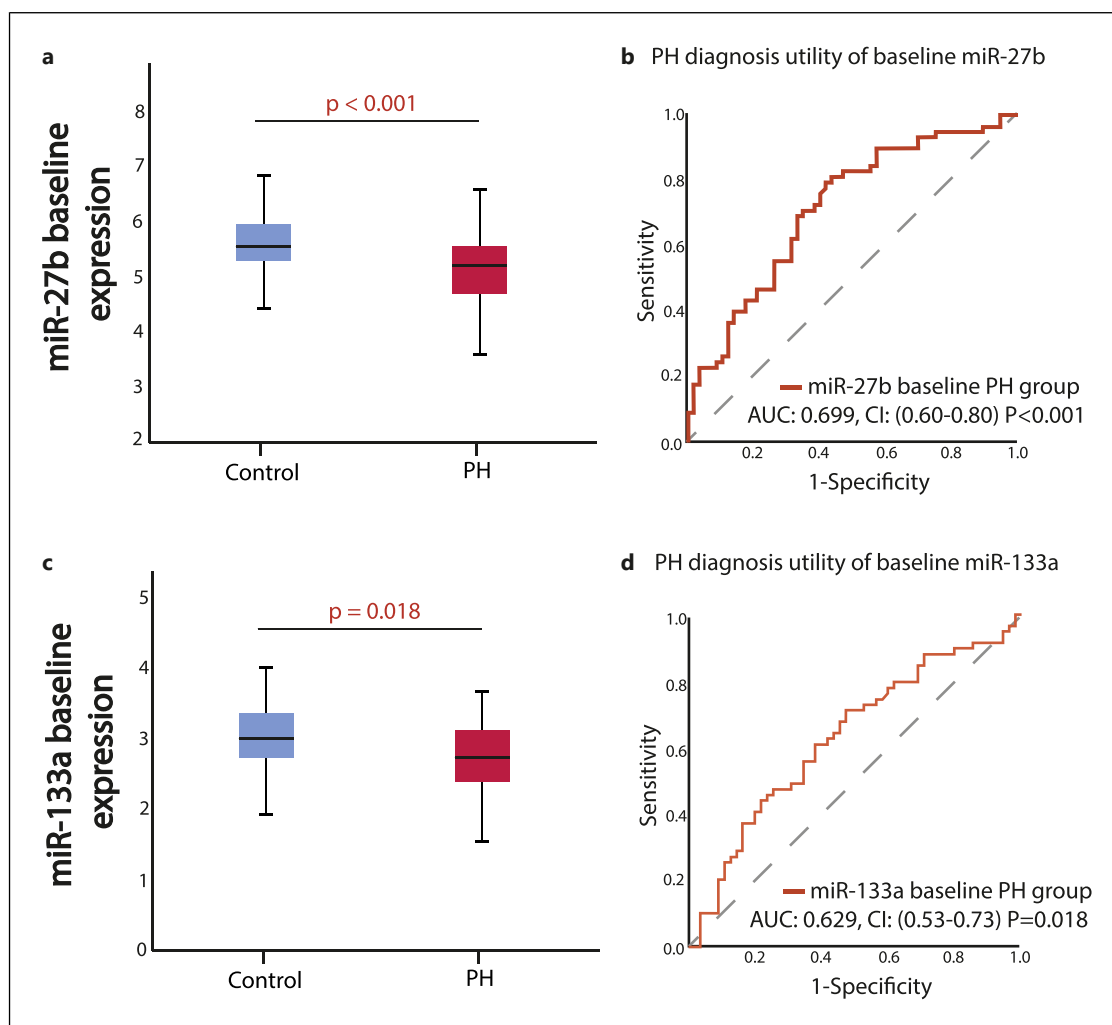
**Fig. 2.** Alteration of analysed microRNAs between the groups and after a single training session. Individual microRNAs are presented in the following subpanels: **(a)** microRNA-21, **(b)** microRNA-16, **(c)** microRNA-27a, **(d)** microRNA-27b, **(e)** microRNA-133a, and **(f)** microRNA-145. PH, primary hypertension.



**Fig. 3.** Heatmap (R-value) of correlations of microRNA expression levels in study group before exercise (baseline) and analyzed parameters. P-values < 0.05 are presented with \*. BMI – body mass index, MLR – monocyte-to-lymphocyte ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure, MAP – mean arterial pressure, Ao – central (aortic), Buckberg SEVR – subendocardial viability ratio, Na – sodium, 24h – 24-hour, c – central blood pressure, A – after exercise, B – before exercise.

children compared to the healthy group. In response to physical exertion, the expression of microRNA-27b, microRNA-133a, and microRNA-145 increased significantly in healthy children, whereas in hypertensive children, only microRNA-145 increased. Positive correlations were observed between the expression of microRNAs-16, -21, -27a, and -145 and numerous BP indices. ROC analysis identified baseline microRNA-27b expression as the best performing marker distinguishing

children with PH in our cohort. Multivariate analysis unmasked baseline microRNA-16 expression as the only significant predictor of elevated diastolic BP. Concerning HMOD, groups differed in LVMI, cPWV, ET PWV $\beta$ , and ET Ep. Hypertensive children showed a significant decrease in both office and ABPM measurements (peripheral and central) after exercise. In contrast, the CG showed no changes in BP, except for an increase in 24 h SBP.

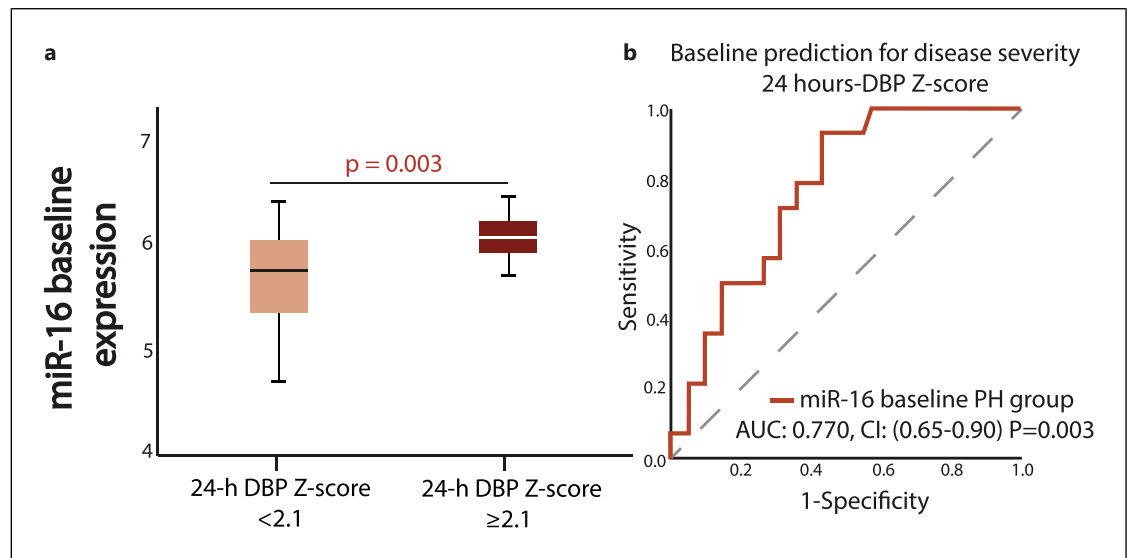


**Fig. 4.** Diagnostic utility of microRNAs based on baseline expression. Individual microRNAs are presented in the following subpanels: **(a)** baseline microRNA-27b expression, **(b)** receiver operating characteristic (ROC) curve for baseline microRNA-27b, **(c)** baseline microRNA-133a expression, and **(d)** receiver operating characteristic (ROC) curve for baseline microRNA-133a. PH, primary hypertension; AUC, area under the curve.

**Table 3.** Multivariate logistic regression model including high level of microRNA-16 on the baseline and clinical data for PH severity based on >2.1 DBP Z-score

| Variable                                        | OR    | Lower | Upper  | p value      |
|-------------------------------------------------|-------|-------|--------|--------------|
| MicroRNA-16 high vs. low expression on baseline | 4.556 | 1.130 | 18.376 | <b>0.033</b> |
| Age                                             | 1.187 | 0.809 | 1.742  | 0.380        |
| Gender                                          | 2.245 | 0.303 | 16.648 | 0.429        |
| BMI                                             | 1.006 | 0.888 | 1.140  | 0.921        |
| TVR 24 h                                        | 1.005 | 0.997 | 1.013  | 0.228        |

\*Bolded p value indicates <0.05.



**Fig. 5.** Boxplot and ROC curve for microRNA-16 baseline association on primary hypertension severity based on 24h DBP Z-score for a cut-off of 2.1. DBP - diastolic blood pressure; PH - primary hypertension; AUC - area under the curve.

When we launched in 2020 the ATHENA study, we made the decision, based on the literature available at the time, to select six microRNA molecules that, based on experimental data and studies in adults, were associated with the pathogenesis of hypertension and the development of HMOD, and for which there was also evidence suggesting that their expression levels may change in response to physical exercise.

MicroRNA-133a is a promising molecule in the pathogenesis of AH. It was found that the prorenin receptor and angiotensinogen are targets of microRNA-133a and that this microRNA may affect BP through this pathway [41]. Studies on microRNA-133a typically show reduced expression in hypertensive adults [42]. Our study found similar results: lower microRNA-133a expression in hypertensive children compared with healthy peers. Importantly, our cohort consisted of newly diagnosed patients without pharmacological treatment, suggesting that microRNA-133a expression is reduced in the early stages of hypertension and may be associated with pathways relevant to its pathogenesis. Moreover, microRNA-133a is highly expressed in the myocardium, and this particle appears to play an important role in hypertension-induced left ventricular hypertrophy (LVH) [43]. In adults, a negative correlation between left ventricular mass and risk of LVH was unmasked [17, 43]. MicroRNA-133a is also studied in exercise physiology.

Its expression increases in athletes after endurance exercise [44]. In our study, microRNA-133a expression increased after exercise, however, only in CG. MicroRNA-133a belongs to the group of muscle microRNAs (originating from cardiomyocytes and skeletal muscles), and its expression is thought to increase during exercise by release from skeletal muscle [10]. Due to its lower expression in PH patients and statistically significant increase after physical exercise only in healthy children, this molecule may be associated with a more favourable cardiovascular profile. Even more importantly, the expression level of this microRNA was significantly lower in the group of children with hypertension, even after the exercise session. It may serve as a marker for monitoring patient compliance with physical activity and may be a potential target for future anti-hypertensive drugs; however, further research and observation are certainly required to understand how this molecule changes over longer periods of regular physical exercise.

MicroRNA-27b is a key regulator of lipid metabolism and plays a role in obesity and metabolic syndrome, with reduced levels observed in obese patients [10]. It is also involved in angiogenesis and is highly expressed in the heart after myocardial infarction, influencing capillary formation and fibrosis [45]. MicroRNA-27b expression influences the angiogenic activity of endothelial cells by

regulating among others *Naa15* gene in a mouse model [46]. Zhu et al. [47] found increased serum microRNA-27b-3p expression in hypertensive and pre-eclamptic patients. Wang et al. [48] reported higher levels in hypertensive patients with LVH compared to those without LVH and healthy subjects. It was also shown that *ACE* gene expression and production of ACE are regulated by both microRNA-27a and microRNA-27b [23]. Our study revealed significantly higher microRNA-27b expression in healthy children compared to hypertensive children. In our cohort, expression of microRNA-27b was the strongest negative predictor of hypertension, also in the ROC analysis. This suggests impaired up-regulation in hypertensive children. Our findings align with most exercise-related studies but contrast with adult hypertension research showing lower microRNA-27b levels. The discrepancy between our findings and other studies may be due to differences in the age of study participants. Additionally, our study utilized plasma-based samples, whereas others used serum-based measurements, which may include more cell-derived microRNAs, such as those released from ruptured platelets. Given microRNA-27b's known role in angiogenesis, another possible explanation is that our hypertensive patients had relatively short disease duration and had not yet undergone pharmacological treatment. Another explanation may be that microRNA-27b levels decrease in the early stages of hypertension but significantly increase in later stages of disease progression. Studies on exercise-induced changes in microRNA-27b expression are limited. Some reported a twofold increase after regular aerobic exercise [10], while others found decreased levels after high-intensity interval training in women with polycystic ovary syndrome [49]. In our cohort, microRNA-27b expression increased after physical exercise in the control group. However, no such effect was observed in the group of children with PH. This may indicate an impairment in the mechanism connected with microRNA-27b production at the beginning of the development of PH. Further research is needed to explore microRNA-27b's role in hypertension.

Of note, we found no significant correlations between BP, HMOD, and microRNA-27a. This particle is a potent regulator of cardiomyocytes and VSMCs proliferation and apoptosis [50, 51]. In adults, expression of microRNA-27a was lower in newly recognized hypertensive patients compared to normotensive ones [42]. The fact that our study did not reveal such differences may be due to the slightly different pathophysiological basis of PH in adolescents and certainly warrants further investigation in prospective studies.

MicroRNA-145 is the most abundant microRNA in VSMCs, where it helps maintain their contractile phenotype, and is also found in plasma and the endothelium [52]. MicroRNA-145 targets numerous crucial pathways involved in BP regulation, including ACE [52, 53]. Targeting the *SLC7A1* gene, which is involved in arginine metabolism and nitric oxide production, is another possible pathway underlying this particle's role in the pathogenesis of PH [54]. Most of the microRNA-145 studies have been conducted in newly diagnosed adults with PH, showing decreased microRNA-145 expression [28]. Our study also found a trend towards lower microRNA-145 expression levels ( $p = 0.061$ ) in hypertensive children, which is consistent with the aforementioned studies. Its expression increased after an acute bout of bike exercise in both groups.

Numerous papers suggest the role of MicroRNA-16 in the pathogenesis of both systemic and pulmonary hypertension [55, 56]. One possible explanation is targeting by microRNA-16 *ADRA1A* gene, encoding the  $\alpha 1A$ -adrenergic receptor [57]. Although we did not demonstrate a correlation between microRNA-16 and the parameters studied in this study, another study from the ATHENA project revealed an association between increased microRNA-16 expression and impaired left ventricular global longitudinal strain [58]. This promising particle also requires further studies in a population of adolescents with PH.

MicroRNA-21 appears to be a key molecule in the pathogenesis of PH by influencing the TGF- $\beta 1$  pathway and the renin-angiotensin-aldosterone system, particularly in VSMCs [59]. A recently published meta-analysis of studies in adults clearly demonstrated that microRNA-21 expression is higher in patients with hypertension [60]. Furthermore, a positive correlation between its expression and cIMT was demonstrated [16]. Our study is negative in this regard—in our cohort, there was no association with BP or arterial damage. It is possible that our results can be explained by differences in the pathogenesis of PH in young people (most commonly isolated systolic hypertension, a greater role of sympathetic overdrive than activation of the RAA system, and established vascular changes) [8]. This difference also requires further investigation at the molecular and clinical levels.

In our hypertensive patients, we found an increase in both serum uric and uric acid urine excretion after an exercise bout. Rise in serum uric acid is a well-known phenomenon, reflecting increased purine metabolism and ATP turnover, revealed in many adults studies [61, 62]. Conversely, most studies show exercise-induced

inhibition of uric acid excretion caused by lactic acid accumulation [63, 64]. Therefore, the increased renal excretion of uric acid following exercise that we observed is surprising. It is possible that a well-designed exercise session did not generate excess lactic acid in our young patients, so the negative feedback mechanism was not triggered, and uric acid excretion increased simply as a result of its overproduction. It should be emphasized that, to the best of our knowledge, there are no reliable data in the literature on urinary uric acid excretion after exercise in children, let alone in children with hypertension.

Exercise may promote sodium conservation by increasing renal tubular sodium reabsorption and attenuating volume-regulatory responses, thereby reducing urinary sodium excretion in the post-exercise period [65]. The lower post-exercise urinary sodium excretion in our hypertensive group compared to controls may indicate more active sodium-retaining mechanisms (according to Guyton's classic hypothesis on the pathogenesis of PH) [8]. This view might be supported by the increase in aldosterone after exercise in SG, which approached statistical significance, and by the negative correlations between both renin and aldosterone, and urinary sodium excretion. Nevertheless, this finding should also be seen as exploratory and hypothesis-generating.

In our study, post-exercise phosphate excretion was significantly higher in the group of children with hypertension. However, as our data show, excretion is also higher (albeit not significantly,  $p = 0.098$ ) before exercise. Higher phosphate excretion may reflect, on the one hand, a less healthy diet rich in highly processed foods in patients with hypertension. On the other hand, the significant difference after exercise may indicate greater energy expenditure and higher ATP consumption in patients with hypertension. The relationship between BP and urinary phosphate excretion is complex and inconsistent across studies [66]. One mechanism by which a high phosphate diet negatively affects the cardiovascular system is the generation of FGF23, which activates the sympathetic nervous system and renin-angiotensin-aldosterone system, increases vascular stiffness, and causes LVH [67].

Previous research has demonstrated that microRNA-145 expression decreases, while microRNA-27b expression remains unchanged following a 10-day fasting period [68]. Another study reported upregulation of microRNA-133a expression after a 28-day reduction of total energy intake to 70% of daily requirements in overweight males aged 60–75 years [69]. Additionally, consuming a portion of extra-virgin olive oil was shown

to significantly decrease microRNA-21 levels 2 h after ingestion, with levels returning close to baseline after 6 h [70]. Notably, the majority of studies investigating the influence of diet on circulating microRNA levels have implemented a 12-h fasting period prior to blood sampling [71]. In line with this approach, our study adopted a similar fasting protocol to assess baseline and post-exercise changes in microRNA levels. However, it is important to emphasize that our investigation did not aim to evaluate diet-related changes in microRNA expression among children with PH and their healthy peers.

Last but not least, our study also provides valuable data on BP and vascular parameters before and after a standardized aerobic exercise session. The findings are particularly useful given the homogeneous nature of the SG (untreated children with PH without other comorbidities, e.g., diabetes mellitus, coronary heart disease) and the CG, which consists of healthy peers. A review by Falkner et al. [2] summarized that while physical activity is known to have beneficial health effects, its impact on BP in children remains unclear. It ranges from a drop of 12 mm Hg in SBP and 7 mm Hg in DBP to almost none, with an average of around 2 mm Hg [29]. Our study adds important knowledge about post-exercise BP changes, showing a significant reduction in SBP and DBP in hypertensive children (3.9 mm Hg and 3.3 mm Hg, respectively). At the same time, no such effect was seen in the CG. This might suggest different mechanisms of post-exercise BP changes in normotensive and hypertensive patients. It should also be emphasized that the observed effects are short-term and reflect a single exercise session. These results pertain solely to the effects of a single exercise session; therefore, no inferences regarding long-term BP regulation or the impact of habitual physical activity can be drawn from the current data.

### Limitations

We acknowledge a few limitations in our study. First, all patients were from a single centre, with limited ethnic and age diversity, making it difficult to generalize the results. Second, the study is limited by a narrow focus on a small set of preselected microRNAs (6) and by the inability to perform global normalization of microRNA expression. Therefore, future studies are necessary to identify and validate the most stable reference microRNAs in the paediatric population. Moreover, despite the strict pre-analytical procedures that were applied, haemolysis was assessed solely by visual inspection rather than by molecular indicators (e.g., the microRNA-451a/microRNA-23a-3p ratio) which should be acknowledged as a

limitation of the study. However, our study also has key strengths. It is the first to compare microRNA expression in children with PH with that of healthy peers. We used a well-established method for measuring microRNA expression, with all analyses conducted by a single investigator to reduce bias. Independent bodies reviewed the study protocol, and a preliminary study was done and published to validate the procedures. Additionally, we thoroughly assessed the patients' vascular phenotype and BP and included a standardized intervention based on a cardiopulmonary exercise test, with numerous parameters evaluated.

## Conclusions

MicroRNA-27b and microRNA-133a are underexpressed in children with PH. These molecules may be useful as potential markers of hypertension in children and may also be associated with a lower burden of hypertension-related alterations. Clarifying the exact place of these molecules in the pathogenesis of hypertension in children requires further research. MicroRNA-16 may serve as a marker of diastolic hypertension. Finally, our study demonstrates a significant decrease in BP after a standardized exercise session in hypertensive children, highlighting the importance of exercise interventions for managing hypertension in this population.

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## References

- 1 Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, et al. Global prevalence of hypertension in children: a systematic review and meta-analysis. *JAMA Pediatr.* 2019;173(12):1154–63. <https://doi.org/10.1001/jamapediatrics.2019.3310>
- 2 Falkner B, Gidding SS, Baker-Smith CM, Brady TM, Flynn JT, Malle LM, et al. Pediatric primary hypertension: an under-recognized condition: a scientific statement from the American heart association. *Hypertension.* 2023;80(6):e101–11. <https://doi.org/10.1161/hyp.0000000000000228>
- 3 Obrycki Ł, Skoczyński K, Sikorski M, Koziej J, Mitoraj K, Pilip J, et al. Current etiology of hypertension in European children-factors associated with primary hypertension. *Pediatr Nephrol.* 2025; 40(10):3233–44. <https://doi.org/10.1007/s00467-025-06761-x>

## Statement of Ethics

The study approved by the Bioethics Committee of the Medical University of Warsaw (KB 154/2020). All procedures adhered to the highest ethical standards set by the Institutional Research Committee and were conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. Written informed consent was obtained from their parent or legal guardian to participate in the study.

## Conflict of Interest Statement

Jolanta Malyszko was a member of the journal's Editorial Board at the time of submission. Other authors declare no conflicts of interest.

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## Author Contributions

Conceptualization and data curation: M.S. and P.S.; methodology: M.S., P.S., C.E., and H.K.; investigation: M.S., P.S., R.P., and K.S.; formal analysis and visualization: M.S., P.S., and C.E.; resources: M.S., P.S., and H.K.; original draft preparation: M.S., P.S., C.E., and J.M.; review and editing: M.S., P.S., C.E., J.M., K.S., R.P., and H.K.; and supervision: P.S. All authors have read and agreed to the published version of the manuscript. Drs Szyszka and Skrzypczyk had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

## Data Availability Statement

The data that support the findings of this study are not publicly available due to privacy reasons but are available from the corresponding author upon request.

- 4 Robinson CH, Hussain J, Jeyakumar N, Smith G, Birken CS, Dart A, et al. Long-term cardiovascular outcomes in children and adolescents with hypertension. *JAMA Pediatr.* 2024;178(7):688–98. <https://doi.org/10.1001/jamapediatrics.2024.1543>
- 5 Huntzinger E, Izaurralde E. Gene silencing by microRNAs: contributions of translational repression and mRNA decay. *Nat Rev Genet.* 2011;12(2):99–110. <https://doi.org/10.1038/nrg2936>
- 6 Desai AS, Webb DJ, Taubel J, Casey S, Cheng Y, Robbie GJ, et al. Zilebesiran, an RNA interference therapeutic agent for hypertension. *N Engl J Med.* 2023;389(3):228–38. <https://doi.org/10.1056/nejmoa2208391>
- 7 Szyszka M, Skrzypczyk P. MicroRNA-133a and MicroRNA-145 may be involved in the development of hypertension-mediated organ damage in children with primary Hypertension-A preliminary study. *J Clin Med.* 2024;13(22):6929. <https://doi.org/10.3390/jcm13226929>
- 8 Litwin M. Pathophysiology of primary hypertension in children and adolescents. *Pediatr Nephrol.* 2024;39(6):1725–37. <https://doi.org/10.1007/s00467-023-06142-2>
- 9 He L, Liu Y, Widlansky ME, Kriegl AJ, Qiu Q, Liang M. microRNA and hypertension. *Hypertension.* 2025;82(2):181–4. <https://doi.org/10.1161/hypertensionaha.124.21728>
- 10 Improta Caria AC, Nonaka KKV, Pereira CS, Soares MBP, Macambira SG, Souza BSDF. Exercise training-induced changes in MicroRNAs: beneficial regulatory effects in hypertension, type 2 diabetes, and obesity. *Int J Mol Sci.* 2018;19(11):3608. <https://doi.org/10.3390/ijms19113608>
- 11 Whelton PK, Carey RM, Aronow WS, Casey DE Jr, Collins KJ, Dennison Himmelfarb C, et al. ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American college of cardiology/American Heart Association task force on clinical practice guidelines. *Hypertension.* 2018;71(6):e13–115.
- 12 Pescatello LS, Buchner DM, Jakicic JM, Powell KE, Kraus WE, Bloodgood B, et al. Physical activity to prevent and treat hypertension: a systematic review. *Med Sci Sports Exerc.* 2019;51(6):1314–23. <https://doi.org/10.1249/mss.0000000000001943>
- 13 Gu Q, Zhao G, Wang Y, Xu B, Yue J. Silencing miR-16 expression promotes angiotensin II stimulated vascular smooth muscle cell growth. *Cell Dev Biol.* 2017;06(01):181. <https://doi.org/10.4172/2168-9296.1000181>
- 14 Zhou R, Li X, Hu G, Gong AY, Drescher KM, Chen XM. miR-16 targets transcriptional co-repressor SMRT and modulates NF-kappaB-Regulated transactivation of Interleukin-8 gene. *PLoS One.* 2012;7(1):e30772. <https://doi.org/10.1371/journal.pone.0030772>
- 15 Wang M, Li J, Cai J, Cheng L, Wang X, Xu P, et al. Overexpression of MicroRNA-16 alleviates atherosclerosis by inhibition of inflammatory pathways. *Biomed Res Int.* 2020;2020(1):8504238. <https://doi.org/10.1155/2020/8504238>
- 16 Yildirim E, Ermis E, Allahverdiyev S, Ucar H, Yavuzer S, Cengiz M. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. *Medicine.* 2019;98(39):e17297. <https://doi.org/10.1097/md.00000000000017297>
- 17 Kontaraki JE, Marketou ME, Parthenakis FI, Maragkoudakis S, Zacharis EA, Petousis S, et al. Hypertrophic and antihypertrophic microRNA levels in peripheral blood mononuclear cells and their relationship to left ventricular hypertrophy in patients with essential hypertension. *J Am Soc Hypertens.* 2015;9(10):802–10. <https://doi.org/10.1016/j.jash.2015.07.013>
- 18 Cengiz M, Karatas OF, Koparir E, Yavuzer S, Ali C, Yavuzer H, et al. Differential expression of hypertension-associated microRNAs in the plasma of patients with white coat hypertension. *Medicine.* 2015;94(13):e693. <https://doi.org/10.1097/md.0000000000000693>
- 19 Cengiz M, Yavuzer S, Kılıçkiran Avcı B, Yürüyen M, Yavuzer H, Dikici SA, et al. Circulating miR-21 and eNOS in subclinical atherosclerosis in patients with hypertension. *Clin Exp Hypertens.* 2015;37(8):643–9. <https://doi.org/10.3109/10641963.2015.1036064>
- 20 Parthenakis F, Marketou M, Kontaraki J, Patrianakos A, Nakou H, Touloupaki M, et al. Low levels of MicroRNA-21 are a marker of reduced arterial stiffness in well-controlled hypertension. *J Clin Hypertens.* 2017;19(3):235–40. <https://doi.org/10.1111/jch.12900>
- 21 Zhou Q, Gallagher R, Ufret-Vincenty R, Li X, Olson EN, Wang S. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters. *Proc Natl Acad Sci USA.* 2011;108(20):8287–92. <https://doi.org/10.1073/pnas.1105254108>
- 22 Urbich C, Kaluza D, Frömel T, Knau A, Bennewitz K, Boon RA, et al. MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A. *Blood.* 2012;119(6):1607–16. <https://doi.org/10.1182/blood-2011-08-373886>
- 23 Goyal R, Goyal D, Leitzke A, Gheorghe CP, Longo LD. Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. *Reprod Sci.* 2010;17(3):227–38. <https://doi.org/10.1177/1933719109351935>
- 24 Kontaraki JE, Marketou ME, Zacharis EA, Parthenakis FI, Vardas PE. Differential expression of vascular smooth muscle-modulating microRNAs in human peripheral blood mononuclear cells: novel targets in essential hypertension. *J Hum Hypertens.* 2014;28(8):510–6. <https://doi.org/10.1038/jhh.2013.117>
- 25 Parthenakis FI, Marketou ME, Kontaraki JE, Maragoudakis F, Maragkoudakis S, Nakou H, et al. Comparative microRNA profiling in relation to urinary albumin excretion in newly diagnosed hypertensive patients. *J Hum Hypertens.* 2016;30(11):685–9. <https://doi.org/10.1038/jhh.2016.15>
- 26 Ramos AE, Lo C, Estephan LE, Tai YY, Tang Y, Zhao J, et al. Specific circulating microRNAs display dose-dependent responses to variable intensity and duration of endurance exercise. *Am J Physiol Heart Circ Physiol.* 2018;315(2):H273–83. <https://doi.org/10.1152/ajpheart.00741.2017>
- 27 Santovito D, Mandolini C, Marcantonio P, De Nardis V, Bucci M, Paganelli C, et al. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. *Expert Opin Ther Targets.* 2013;17(3):217–23. <https://doi.org/10.1517/14728222.2013.745512>
- 28 Özkan G, Ulusoy Ş, Geyik E, Erdem Y. Down-regulation of miRNA 145 and up-regulation of miRNA 4516 may be associated with primary hypertension. *J Clin Hypertens.* 2019;21(11):1724–31. <https://doi.org/10.1111/jch.13704>
- 29 Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, et al. European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J Hypertens.* 2016;34(10):1887–920. <https://doi.org/10.1097/hjh.0000000000001039>
- 30 Flynn JT, Urbina EM, Brady TM, Baker-Smith C, Daniels SR, Hayman LL, et al. Ambulatory blood pressure monitoring in children and adolescents: 2022 update: a scientific statement from the American Heart Association. *Hypertension.* 2022;79(7):e114–24. <https://doi.org/10.1161/hyp.0000000000000215>
- 31 Skrzypczyk P, Przychodzień J, Mizerska-Wasiak M, Kuźma-Mroczkowska E, Stelmazczyk-Emmel A, GóRSKA E, et al. Asymmetric dimethylarginine is not a marker of arterial damage in children with glomerular kidney diseases. *Cent Eur J Immunol.* 2020;44(4):370–9. <https://doi.org/10.5114/ceji.2019.92788>
- 32 Wühl E, Witte K, Soergel M, Mehls O, Schaefer F, German Working Group on Pediatric Hypertension. Distribution of 24-h ambulatory blood pressure in children: normalized reference values and role of body dimensions. *J Hypertens.* 2002;20(10):1995–2007. <https://doi.org/10.1097/00004872-200210000-00019>
- 33 Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc.* 2011;43(7):1334–59. <https://doi.org/10.1249/mss.0b013e318213f8fb>

- 34 Foster BJ, Mackie AS, Mitsnefes M, Ali H, Mamber S, Colan SD. A novel method of expressing left ventricular mass relative to body size in children. *Circulation*. 2008; 117(21):2769–75. <https://doi.org/10.1161/circulationaha.107.741157>
- 35 Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. *Am J Cardiol*. 1986;57(6):450–8. [https://doi.org/10.1016/0002-9149\(86\)90771-x](https://doi.org/10.1016/0002-9149(86)90771-x)
- 36 Reusz GS, Csepkeal O, Temmar M, Kis E, Cherif AB, Thaleb A, et al. Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension*. 2010;56(2):217–24. <https://doi.org/10.1161/hypertensionaha.110.152686>
- 37 Doyon A, Kracht D, Bayazit AK, Deveci M, Duzova A, Krmar RT, et al. Carotid artery intima-media thickness and distensibility in children and adolescents: reference values and role of body dimensions. *Hypertension*. 2013;62(3):550–6. <https://doi.org/10.1161/hypertensionaha.113.01297>
- 38 Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA*. 2008;105(30):10513–8. <https://doi.org/10.1073/pnas.0804549105>
- 39 Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc*. 2008;3(6):1101–8. <https://doi.org/10.1038/nprot.2008.73>
- 40 Eyileten C, Fitas A, Jakubik D, Czajka P, Mróz A, Czajkowska A, et al. Alterations in circulating MicroRNAs and the relation of MicroRNAs to maximal oxygen consumption and intima-media thickness in ultramarathon runners. *Int J Environ Res Public Health*. 2021;18(14):7234. <https://doi.org/10.3390/ijerph18147234>
- 41 Hao S, Salzo J, Zhao H, Hao M, Darzynkiewicz Z, Ferreri NR. MicroRNA-133a-Dependent inhibition of proximal tubule angiotensinogen by renal TNF (tumor necrosis factor). *Hypertension*. 2020;76(6):1744–52. <https://doi.org/10.1161/hypertensionaha.120.15435>
- 42 Suzuki K, Yamada H, Fujii R, Munetsuna E, Yamazaki M, Ando Y, et al. Circulating microRNA-27a and -133a are negatively associated with incident hypertension: a five-year longitudinal population-based study. *Biomarkers*. 2022;27(5):496–502. <https://doi.org/10.1080/1354750x.2022.2070281>
- 43 Koval SM, Snihurska IO, Yushko KO, Mysnychenko OV, Penkova MY, Lytvynova OM, et al. Circulating microRNA-133a in patients with arterial hypertension, hypertensive heart disease, and left ventricular diastolic dysfunction. *Front Cardiovasc Med*. 2020;7:104. <https://doi.org/10.3389/fcvm.2020.00104>
- 44 Gomes CPC, Oliveira-Jr GP, Madrid B, Almeida JA, Franco OL, Pereira RW. Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run. *Biomarkers*. 2014;19(7):585–9. <https://doi.org/10.3109/1354750x.2014.952663>
- 45 Fu Q, Lu Z, Fu X, Ma S, Lu X. MicroRNA 27b promotes cardiac fibrosis by targeting the FBW7/Smad pathway. *Aging*. 2019;11(24):11865–79. <https://doi.org/10.18632/aging.102465>
- 46 Qun L, Wenda X, Weihong S, Jianyang M, Wei C, Fangzhou L, et al. miRNA-27b modulates endothelial cell angiogenesis by directly targeting Naa15 in atherosclerosis. *Atherosclerosis*. 2016;254:184–92. <https://doi.org/10.1016/j.atherosclerosis.2016.10.007>
- 47 Zhu L, Liu Z. Serum from patients with hypertension promotes endothelial dysfunction to induce trophoblast invasion through the miR-27b-3p/ATPase plasma membrane Ca transporting 1 axis. *Mol Med Rep*. 2021;23(5):319. <https://doi.org/10.3892/mmr.2021.11958>
- 48 Wang Y, Chen S, Gao Y, Zhang S. Serum MicroRNA-27b as a screening biomarker for left ventricular hypertrophy. *Tex Heart Inst J*. 2017;44(6):385–9. <https://doi.org/10.14503/thij-16-5955>
- 49 Lionetti S, Kiel IA, Camera DM, Vanky E, Parr EB, Lydersen S, et al. Circulating and adipose tissue miRNAs in women with polycystic ovary syndrome and responses to high-intensity interval training. *Front Physiol*. 2020;11:904. <https://doi.org/10.3389/fphys.2020.00904>
- 50 Yeh CH, Chen TP, Wang YC, Lin YM, Fang SW. MicroRNA-27a regulates cardiomyocyte apoptosis during cardioplegia-induced cardiac arrest by targeting interleukin 10-related pathways. *Shock*. 2012; 38(6):607–14. <https://doi.org/10.1097/shk.0b013e318271f944>
- 51 Xu MM, Deng HY, Li HH. MicroRNA-27a regulates angiotensin II-induced vascular smooth muscle cell proliferation and migration by targeting  $\alpha$ -smooth muscle-actin in vitro. *Biochem Biophys Res Commun*. 2019;509(4):973–7. <https://doi.org/10.1016/j.bbrc.2019.01.047>
- 52 Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 2009;460(7256):705–10. <https://doi.org/10.1038/nature08195>
- 53 Sawant D, Lilly B. MicroRNA-145 targets in cancer and the cardiovascular system: evidence for common signaling pathways. *Vasc Biol*. 2020;2(1):R115–28. <https://doi.org/10.1530/vb-20-0012>
- 54 Wang Y, Jin L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp Ther Med*. 2018;15(1):548–52.
- 55 Liu JJ, Tang MM, Zhu ML, Xie CX, Kang PF, Ling X, et al. Docosahexaenoic acid inhibits Ca influx and downregulates CaSR by up-regulating microRNA-16 in pulmonary artery smooth muscle cells. *J Biochem Mol Toxicol*. 2020;34(11):e22573. <https://doi.org/10.1002/jbt.22573>
- 56 Cheng B, Yang R, Xu H, Wang L, Jiang N, Song T, et al. Peripheral blood miRNA expression in patients with essential hypertension in the Han Chinese population in Hefei, China. *Biochem Genet*. 2025;63(4):3170–86. <https://doi.org/10.1007/s10528-024-10867-6>
- 57 He L, Huang C. MiR-19b and miR-16 cooperatively signaling target the regulator ADRA1A in Hypertensive heart disease. *Biomed Pharmacother*. 2017;91:1178–83. <https://doi.org/10.1016/j.biopha.2017.04.041>
- 58 Szyszka M, Pietrzak R, Obszajczyk K, Skubisz K, Eyileten C, Skrzypczyk P. Circulating miR-16 as an early biomarker of subclinical myocardial strain impairment in pediatric primary hypertension. *Int J Mol Sci*. 2026;27(6):2806. <https://doi.org/10.3390/ijms27062806>
- 59 Jafari-Nozad AM, Rostami N, Esmaeili M, Vahdati H, Hosseini S, Farkhondeh T, et al. A review of the dual role of MicroRNA-21 in cardiovascular diseases: risk factor or a potential therapeutic target. *Curr Mol Pharmacol*. 2024;17:e18761429287057. <https://doi.org/10.2174/0118761429287057240116040703>
- 60 Xin C, He W, Sun X, Li M, Wang H. The Association of Micro-RNA 21 and hypertension: a meta-analysis. *Glob Heart*. 2024; 19(1):41. <https://doi.org/10.5334/gh.1325>
- 61 Zhang T, Liu W, Gao S. Exercise and hyperuricemia: an opinion article. *Ann Med*. 2024;56(1):2396075. <https://doi.org/10.1080/07853890.2024.2396075>
- 62 Wołyniec W, Ratkowski W, Kasprzowicz K, Małgorzewicz S, Aleksandrowicz E, Witek K, et al. Changes in electrolytes and uric acid excretion during and after a 100 km run. *J Biol Regul Homeost Agents*. 2018;32(5):1205–10.
- 63 Kaya M, Moriwaki Y, Ka T, Inokuchi T, Yamamoto A, Takahashi S, et al. Plasma concentrations and urinary excretion of purine bases (uric acid, hypoxanthine, and xanthine) and oxypurinol after rigorous exercise. *Metabolism*. 2006;55(1):103–7. <https://doi.org/10.1016/j.metabol.2005.07.013>
- 64 Kaptanoğlu B, Turgut G, Genç O, Enli Y, Karabulut I, Zencir M, et al. Effects of acute exercise on the levels of iron, magnesium, and uric acid in liver and spleen tissues. *Biol Trace Elem Res*. 2003;91(2):173–8. <https://doi.org/10.1385/bter.91.2.173>
- 65 Nagashima K, Wu J, Kavouras SA, Mack GW. Increased renal tubular sodium reabsorption during exercise-induced hypervolemia in humans. *J Appl Physiol*. 2001; 91(3):1229–36. <https://doi.org/10.1152/jappl.2001.91.3.1229>
- 66 Kim HK, Mizuno M, Vongpatanasin W. Phosphate, the forgotten mineral in hypertension. *Curr Opin Nephrol Hypertens*. 2019;28(4):345–51. <https://doi.org/10.1097/mnh.0000000000000503>

- 67 Wong SK. A review of current evidence on the relationship between phosphate metabolism and metabolic syndrome. *Nutrients*. 2022;14(21):4525. <https://doi.org/10.3390/nu14214525>
- 68 Ravanidis S, Grundler F, de Toledo FW, Dimitriou E, Tekos F, Skaperda Z, et al. Fasting-mediated metabolic and toxicity reprogramming impacts circulating microRNA levels in humans. *Food Chem Toxicol*. 2021;152:112187. <https://doi.org/10.1016/j.fct.2021.112187>
- 69 Margolis LM, Rivas DA, Pasiakos SM, McClung JP, Ceglia L, Fielding RA. Upregulation of circulating myomiR following short-term energy restriction is inversely associated with whole body protein synthesis. *Am J Physiol Regul Integr Comp Physiol*. 2017;313(3):R298–304. <https://doi.org/10.1152/ajpregu.00054.2017>
- 70 Daimiel L, Micó V, Valls RM, Pedret A, Motilva MJ, Rubió L, et al. Impact of phenol-enriched virgin olive oils on the postprandial levels of circulating microRNAs related to cardiovascular disease. *Mol Nutr Food Res*. 2020;64(15):e2000049. <https://doi.org/10.1002/mnfr.202000049>
- 71 DeLucas M, Sánchez J, Palou A, Serra F. The impact of diet on miRNA regulation and its implications for health: a systematic review. *Nutrients*. 2024;16(6):770. <https://doi.org/10.3390/nu16060770>

**7.3. Publikacja III: „Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension”**



Article

# Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension

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## Abstract

The role of circulating microRNAs in the pathophysiology of cardiac remodeling in primary hypertension (PH) remains incompletely understood. Left ventricular global longitudinal strain (LV GLS) is a sensitive marker of subclinical systolic dysfunction and can be used to monitor early cardiac involvement in cardiovascular and renal diseases. To the best of our knowledge, this is the first study to demonstrate an association between circulating miR-16 and LV GLS in children. The study aimed to evaluate the expression levels of miR-16-5p, -21-5p, -27a-3p, -27b-3p, -133a-3p, and -145-5p in untreated children with PH and examine their associations with LV GLS. 50 children with PH and 57 normotensive controls were evaluated for circulating microRNA expression levels and echocardiographic parameters, including LV GLS. Comprehensive anthropometric, biochemical, blood pressure, and arterial indices were also assessed. Among the analyzed microRNAs, miR-16-5p exhibited a positive association with LV GLS ( $R = 0.305$ ,  $p = 0.031$ ), whereas miR-27b-3p demonstrated a negative association ( $R = -0.330$ ,  $p < 0.001$ ). Compared with controls, hypertensive children exhibited significantly higher (i.e., less negative) LV GLS ( $r = 0.29$ ,  $p = 0.002$ ), indicating early systolic dysfunction occurring already at an early stage of the disease. In conclusion, these findings support the idea that specific microRNAs might play a differential role in early myocardial functional alterations in pediatric PH. Higher miR-16 expression levels may be associated with impaired myocardial deformation, potentially reflecting its involvement in early maladaptive myocardial remodeling. Furthermore, LV GLS may represent a sensitive and clinically informative marker of early myocardial dysfunction beyond traditional echocardiographic parameters in this population.

**Keywords:** microRNA; miR-16-5p; miR-27b-3p; global longitudinal strain; primary hypertension; children; adolescents; cardiac alterations



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## 1. Introduction

MicroRNAs are small, non-coding RNAs that regulate gene expression and influence multiple, interconnected biological pathways [1,2]. Dysregulation of microRNAs has been implicated in cardiovascular remodeling and the development of related cardiovascular diseases [3]. Because they can be detected extracellularly, e.g., in plasma [4], circulating microRNAs have emerged as promising biomarkers of cardiovascular stress and early myocardial dysfunction [3].

Arterial hypertension (AH) in children is an increasing medical concern [5]. The prevalence of childhood AH increases during early adolescence, peaking around puberty (7.89% among teenagers aged 14 years) and subsequently declining in late adolescence (3.28% at 19 years) [5]. There is growing evidence that elevated blood pressure often begins in early life and continues into adulthood [6]. AH contributes to structural and functional changes in the heart, kidneys, and blood vessels [7]. Therefore, early identification and appropriate management are essential to prevent long-term cardiovascular complications. Unlike in adults, where hard endpoints such as strokes and myocardial infarctions are common, in children with primary hypertension, the presence of hypertension-mediated organ damage (HMOD) is the most important indicator for initiating pharmacological treatment [8]. Currently, left ventricular hypertrophy is considered the gold standard for assessing HMOD [9]. However, researchers are still seeking additional early markers of HMOD to improve prognosis and long-term treatment outcomes. One such marker may be global longitudinal strain (GLS) of the left ventricle (LV), as assessed by speckle tracking echocardiography (STE) [10].

MicroRNAs are increasingly recognized as key regulators and potential biomarkers of hypertensive heart disease [3,11]. Several circulating miRNAs have been associated with left ventricular hypertrophy (LVH) and subclinical myocardial remodeling in adults [12].

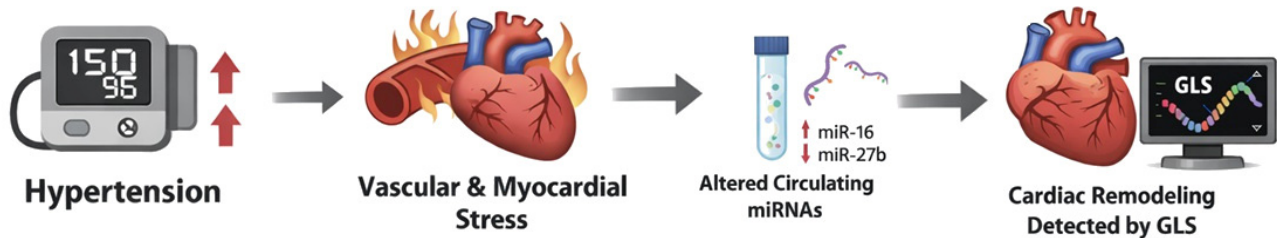
MiR-16-5p is a member of the miR-15 family. It is processed from the miR 15a/16-1 genomic cluster, which is located on human chromosome 13 [13]. Some studies have shown that it may be linked to mechanisms relevant to cardiovascular injury caused by hypertension, including the proliferation and migration of vascular smooth muscle cells related to angiotensin II [14]. Another study has shown that miR-16-3p can lead to the development of hypertensive heart disease by mediating sympathetic signaling in cardiomyocytes and vascular smooth muscle through the  $\alpha$ 1A-adrenergic receptor, which is encoded by the *ADRA1A* gene [15]. However, its exact role and whether it acts positively or negatively has not yet been fully elucidated.

MiR-27b-3p has been implicated in hypertensive cardiac remodeling. In adults, circulating miR-27b is elevated in patients with echocardiographic LVH and differentiates hypertensive individuals with and without LVH [16]. Experimental studies further demonstrate its involvement in pressure-overload-induced remodeling, although emerging data suggest that miR-27b may also exert context-dependent regulatory effects on endothelial function and myocardial adaptation [17].

Notably, miR-21 has been linked to interstitial fibrosis and, with miR-27a, to cardiac hypertrophy signaling [18,19]. MiR-133a was found to be involved in the regulation of cardiomyocyte hypertrophic responses, and miR-145 elicits a contribution to vascular smooth muscle remodeling [20,21].

Thus, microRNAs have been implicated in key mechanisms of cardiovascular remodeling, including inflammation, fibrosis, endothelial dysfunction, and myocardial structural adaptation. In the setting of chronic pressure overload, alterations in circulating microRNA profiles may reflect early maladaptive or compensatory responses to vascular and myocardial stress [22]. Such molecular changes may influence structural abnormalities and be detectable through sensitive functional markers such as LV GLS (Figure 1) [22]. The

relationship between circulating miR-16-5p and -27b-3p, and early myocardial dysfunction, particularly indices of myocardial deformation such as GLS, remains largely unexplored. Such data are virtually absent in children with PH. Given the increasing use of sensitive echocardiographic strain imaging, integrative studies combining circulating microRNAs with detailed myocardial phenotyping are urgently needed.



**Figure 1.** Conceptual model of hypertension-induced vascular stress, altered circulating microRNAs, and subclinical myocardial remodeling detected by LV GLS based on Sessen [22].

Thus, the aim of our study was to evaluate the expression levels of selected miRs (-16-5p, -21-5p, -27a-3p, -27b-3p, -133a-3p, and -145-5p) and the global longitudinal strain of the left ventricle in untreated pediatric patients with primary hypertension and to compare them with healthy peers.

## 2. Results

A total of 50 patients with primary hypertension and 57 healthy peers underwent complete microRNA evaluation and echocardiographic examinations and were included in the final analysis.

Table 1 summarizes the basic demographic, clinical, and biochemical parameters of both groups. Additional parameters and their comparison between the hypertensive and control groups, including *p*-values, are presented in Supplementary Table S1. The groups did not differ in terms of age or gender. However, significantly more patients in the study group had a family history of hypertension on their mother's side. While patients did not differ in height, significant differences were found in body weight, BMI, waist and hip circumference, waist-to-hip ratio, ambulatory blood pressure measurement, and serum uric acid concentration. All participants had normal kidney function, and there were no differences between groups in terms of creatinine-based estimated glomerular filtration rate.

Table 2 and Supplementary Table S1 present a comparison between the study and control groups for measurements obtained during 24 h automated monitoring of arterial pressure, central blood pressure, and vascular parameters. The groups differed significantly in terms of 24 h measurements: systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), pulse pressure (PP), central systolic blood pressure (cSBP), central diastolic blood pressure (cDBP), augmentation index corrected to 75 heartbeats (Aix@75HR), cardiac output (CO), total vascular resistance (TVR), reflection magnitude (RM), pulse wave velocity (PWV)—higher values in the group of patients with PH. No differences were found in 24 h heart rate (HR) and stroke volume (SV). The study group showed a significantly smaller nocturnal decrease in diastolic pressure, with no similar effect on systolic blood pressure. All ambulatory parameters of central pressure (AoSBP, AoDBP, AoMAP) were significantly higher in the group of patients with PH. In terms of vascular parameters, the study group had higher carotid–femoral PWV (cfPWV) and thicker carotid intima media thickness (cIMT); no differences between the groups were found in ambulatory augmentation index (Aix) measurements.

**Table 1.** Demographic, clinical, and basic biochemical characteristics of the study group and the control group, and comparison between them.

| Parameter                        | Study Group                    | Control Group                  | <i>p</i> |
|----------------------------------|--------------------------------|--------------------------------|----------|
| Age [years]                      | 15.39 ± 1.90 (14.36–16.84)     | 15.25 ± 1.55 (13.76–16.54)     | 0.424    |
| Sex [male/female]                | 80.0%/20.0%                    | 70.2%/29.8%                    | 0.243    |
| Hypertensive Mother              | 32.0%                          | 8.8%                           | 0.003    |
| Hypertensive Father              | 46.0%                          | 28.1%                          | 0.055    |
| Height [cm]                      | 173.74 ± 8.82 (168.00–180.00)  | 171.91 ± 10.61 (166.00–180.00) | 0.368    |
| Height Z-score                   | 0.71 ± 1.06 (0.07–1.42)        | 0.61 ± 0.99 (−0.07–1.20)       | 0.688    |
| Weight [kg]                      | 79.02 ± 19.60 (64.00–85.00)    | 61.77 ± 11.68 (55.00–69.00)    | <0.001   |
| Weight Z-score                   | 1.42 ± 1.10 (0.69–2.20)        | 0.44 ± 0.84 (0.02–1.03)        | <0.001   |
| BMI                              | 26.17 ± 6.22 (22.21–27.94)     | 20.83 ± 3.46 (18.67–22.35)     | <0.001   |
| BMI Z-score                      | 1.26 ± 1.06 (0.62–2.09)        | 0.16 ± 1.07 (−0.42–0.83)       | <0.001   |
| SPB [mmHg]                       | 136.34 ± 10.42 (131.00–144.00) | 118.68 ± 9.64 (113.00–125.00)  | <0.001   |
| SBP Z-score                      | 1.76 ± 0.84 (1.26–2.30)        | 0.23 ± 0.82 (−0.34–0.80)       | <0.001   |
| DBP [mmHg]                       | 82.21 ± 7.88 (76.00–88.00)     | 72.86 ± 6.35 (69.00–77.00)     | <0.001   |
| DBP Z-score                      | 2.25 ± 1.10 (1.40–3.07)        | 0.99 ± 0.82 (0.44–1.51)        | <0.001   |
| Waist circumference [cm]         | 83.8 ± 14.3 (73.6–88.8)        | 71.1 ± 6.8 (67.5–74.5)         | <0.001   |
| Hip circumference [cm]           | 100.3 ± 12.0 (92.0–104.3)      | 91.4 ± 8.3 (86.5–96.0)         | <0.001   |
| WtH ratio                        | 0.84 ± 0.11 (0.78–0.88)        | 0.78 ± 0.04 (0.75–0.81)        | <0.001   |
| Uric Acid [mg/dL]                | 5.81 ± 1.09 (5.10–6.30)        | 4.98 ± 0.88 (4.50–5.70)        | <0.001   |
| eGFR [mL/min/1.73 <sup>2</sup> ] | 107.58 ± 18.00 (96.01–121.32)  | 106.96 ± 17.51 (92.41–116.97)  | 0.854    |
| miR-16-5p expression             | 5.77 ± 0.43 (5.60–6.09)        | 5.93 ± 0.33 (5.77–6.13)        | 0.067    |
| miR-27b-3p expression            | 5.05 ± 0.65 (4.70–5.46)        | 5.58 ± 0.69 (5.28–5.96)        | <0.001   |

BMI—body mass index; SBP—systolic blood pressure; DBP—diastolic blood pressure; WtH ratio—waist-to-hip circumference ratio; eGFR—estimated glomerular filtration rate.

**Table 2.** 24 h blood pressure parameters and vascular parameters in the study group and the control group, and comparison between them.

| Parameter       | Study Group                   | Control Group                 | <i>p</i> |
|-----------------|-------------------------------|-------------------------------|----------|
| 24 h SBP [mmHg] | 131.60 ± 9.56 (124.00–138.00) | 114.91 ± 6.92 (109.00–121.00) | <0.001   |
| 24 h DBP [mmHg] | 74.06 ± 6.51 (70.00–78.00)    | 66.00 ± 5.14 (64.00–69.00)    | <0.001   |
| HR 24 h [1/min] | 73.24 ± 9.07 (67.00–79.00)    | 71.21 ± 9.82 (63.00–79.00)    | 0.221    |
| MAP 24 h [mmHg] | 100.38 ± 7.26 (95.00–105.00)  | 88.44 ± 5.11 (85.00–92.00)    | <0.001   |
| PP 24 h [mmHg]  | 57.02 ± 7.02 (53.00–59.00)    | 48.42 ± 6.57 (44.00–52.00)    | <0.001   |
| AoSBP [mmHg]    | 114.61 ± 8.50 (110.40–118.20) | 100.63 ± 7.83 (94.00–107.00)  | <0.001   |
| AoDBP [mmHg]    | 83.59 ± 7.94 (77.29–89.00)    | 74.12 ± 6.44 (70.00–78.33)    | <0.001   |
| AoMAP [mmHg]    | 98.18 ± 7.48 (93.17–103.20)   | 86.74 ± 6.73 (81.67–91.67)    | <0.001   |
| cfPWV [m/s]     | 5.26 ± 0.65 (4.78–5.63)       | 4.71 ± 0.60 (4.20–5.07)       | <0.001   |
| cfPWV Z-score   | −0.20 ± 0.85 (−0.80–0.19)     | −0.94 ± 0.80 (−1.44–−0.30)    | <0.001   |
| IMT mean [mm]   | 0.49 ± 0.05 (0.45–0.53)       | 0.46 ± 0.04 (0.43–0.49)       | 0.010    |
| IMT Z-score     | 1.87 ± 1.08 (1.27–2.71)       | 1.41 ± 0.85 (0.67–2.00)       | 0.020    |

24 h SBP—24 h mean systolic blood pressure; 24 h DBP—24 h mean diastolic blood pressure; HR 24 h—24 h mean heart rate; MAP 24 h—24 h mean arterial pressure; PP 24 h—24 h pulse pressure; AoSBP—central (aortic) systolic blood pressure; AoDBP—central (aortic) diastolic blood pressure; AoMAP—central (aortic) mean arterial pressure; cfPWV—carotid–femoral pulse wave velocity; cfPWV Z-score—Z-score of carotid–femoral pulse wave velocity adjusted for height and sex [23]; IMT mean—mean carotid intima–media thickness. IMT Z-score—carotid intima–media thickness adjusted for height and sex [24].

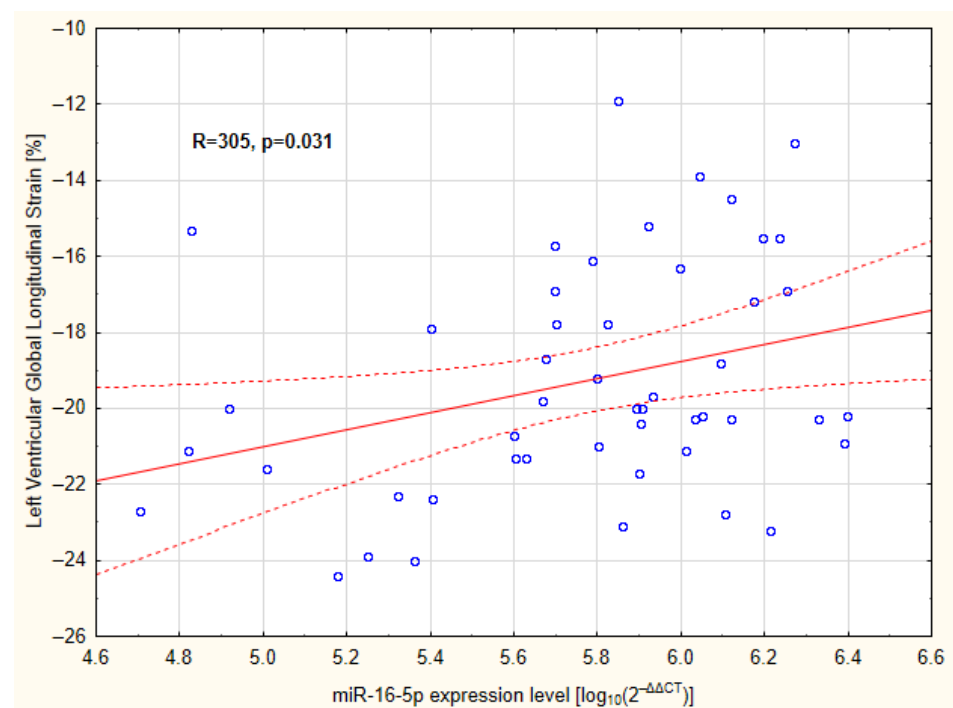
Table 3 and Supplementary Table S1 show the comparison of echocardiographic parameters between the control and study groups. Patients with PH were characterized by higher left ventricular mass (LVM) and left ventricular mass index (LVMI), expressed as g/m<sup>2.7</sup>, as well as a higher LVMI Z-score. This group was also characterized by higher LV GLS values and significantly lower early diastolic mitral annular velocity (E′) wave of the septum and lateral wall. Additionally, the PH group had higher isovolumetric relaxation time (IVRT). The ejection fraction of all subjects was normal, and the study group did not differ significantly from the control group in this respect. The analysis restricted to the study group revealed a significant positive correlation between miR-16-5p

and LV GLS ( $R = 0.305$ ;  $p = 0.031$ ; Figure 2). Patients with higher miR-16-5p levels showed correspondingly higher LV GLS values, suggesting a measurable association between this microRNA and myocardial deformation parameters in hypertensive pediatric patients.

**Table 3.** Echocardiography-derived parameters with extended indices of systolic cardiac function in the study and control groups, and a comparison between them.

| Parameter                  | Study Group                         | Control Group                       | <i>p</i>     |
|----------------------------|-------------------------------------|-------------------------------------|--------------|
| LVM [g]                    | 145.60 ± 44.12 (110.76–165.03)      | 122.07 ± 32.00 (103.40–142.60)      | 0.008        |
| LVMI [g/m <sup>2.7</sup> ] | 32.49 ± 8.45 (26.05–36.67)          | 28.00 ± 5.94 (22.68–32.30)          | 0.005        |
| LVMI [g/m <sup>2</sup> ]   | 74.59 ± 18.69 (62.88–87.87)         | 70.66 ± 14.05 (62.96–79.90)         | 0.342        |
| LVMI Z-score               | −0.66 ± 1.52 (−1.64–0.28)           | −1.36 ± 1.47 (−2.51–0.05)           | 0.014        |
| <b>LV GLS [%]</b>          | <b>−19.28 ± 3.02 (−21.30–16.90)</b> | <b>−20.99 ± 3.11 (−23.00–19.70)</b> | <b>0.002</b> |
| E-wave velocity [m/s]      | 90.19 ± 15.60 (79.30–100.00)        | 89.22 ± 13.09 (81.00–97.30)         | 0.776        |
| A-wave                     | 55.57 ± 9.67 (46.70–63.10)          | 53.05 ± 11.70 (45.50–58.20)         | 0.103        |
| E/A                        | 1.67 ± 0.39 (1.39–1.92)             | 1.72 ± 0.27 (1.53–1.93)             | 0.159        |
| E' sept [cm/s]             | 12.73 ± 2.34 (11.05–13.85)          | 14.46 ± 2.35 (13.00–15.70)          | <0.001       |
| A' sept [cm/s]             | 7.44 ± 1.74 (6.31–8.59)             | 7.56 ± 1.75 (6.22–8.70)             | 0.959        |
| E' lat                     | 17.25 ± 3.68 (14.50–18.90)          | 19.10 ± 2.95 (17.00–20.20)          | 0.004        |
| A' lat                     | 7.52 ± 1.90 (6.20–8.81)             | 7.26 ± 1.78 (5.91–8.27)             | 0.340        |
| E/E' sept                  | 7.23 ± 1.43 (6.12–7.95)             | 6.33 ± 1.28 (5.39–7.17)             | 0.001        |
| E/E' lat                   | 5.34 ± 0.96 (4.58–5.95)             | 4.77 ± 0.93 (4.03–5.34)             | <0.001       |
| IVCT [ms]                  | 69.64 ± 16.77 (58.00–79.00)         | 70.95 ± 15.38 (61.00–78.00)         | 0.965        |
| IVRT [ms]                  | 74.66 ± 13.34 (63.00–86.00)         | 65.71 ± 14.47 (58.00–69.00)         | <0.001       |

LVM—left ventricular mass; LVMI—left ventricular mass index LVMI; LV GLS—left ventricular global longitudinal strain; E-wave velocity—early diastolic transmitral inflow velocity; A-wave—late diastolic transmitral inflow velocity; E/A—ratio of early to late transmitral inflow velocities; E' sept—septal early diastolic mitral annular velocity; A' sept—septal late diastolic mitral annular velocity; E' lat—lateral early diastolic mitral annular velocity; A' lat—lateral late diastolic mitral annular velocity; E/E' sept—ratio of transmitral E-wave velocity to septal E' velocity; E/E' lat—ratio of transmitral E-wave velocity to lateral E' velocity; IVCT—iso-volumetric contraction time; IVRT—iso-volumetric relaxation time. Bold values indicate the primary study parameter (LV GLS).



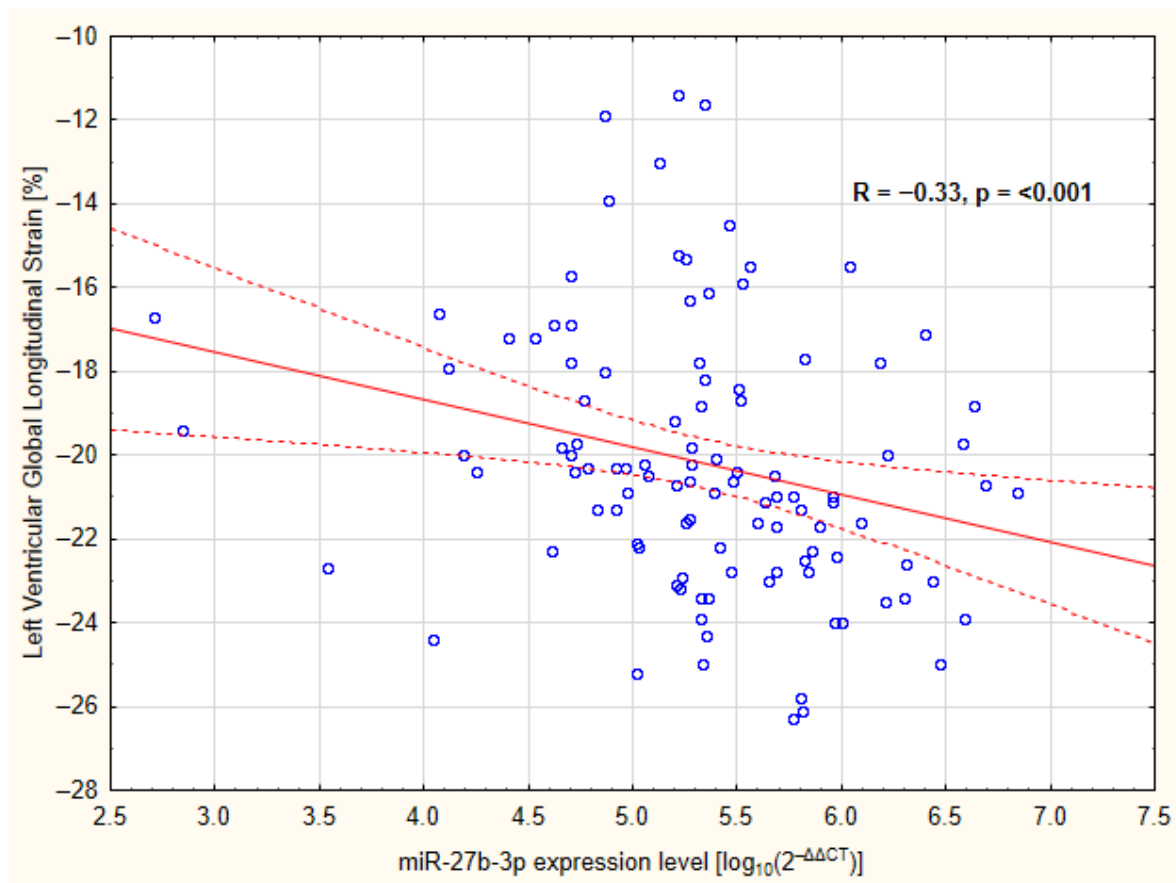
**Figure 2.** Relationship between global LV strain and relative miR-16-5p expression levels in children with primary hypertension. The figure shows the unadjusted Spearman correlation. The independent association was further confirmed in multivariable regression analysis (see Table 4). Blue circles represent individual study participants included in the analysis. Solid lines are linear regression lines, and dotted lines represent 95% confidence intervals.

**Table 4.** Multivariate analysis of factors influencing global left ventricle strain in the group of hypertensive children (generalized multiple regression; step-wise regression).

| Analyzed Parameter                | B (SE)       | t     | p      | 95% CI for B     | Standardized $\beta$ | 95% CI for $\beta$ |
|-----------------------------------|--------------|-------|--------|------------------|----------------------|--------------------|
| Intercept (constant)              | −51.7 (6.79) | −7.62 | <0.001 | −65.39 to −38.01 | −                    | −                  |
| RBC [ $\times 10^6/\mu\text{L}$ ] | 2.40 (0.79)  | 3.05  | 0.0039 | 0.81 to 3.98     | 0.375                | 0.13–0.62          |
| 24 h HR [1/min]                   | 0.09 (0.04)  | 2.41  | 0.0202 | 0.02 to 0.17     | 0.297                | 0.05–0.54          |
| miR-16-5p expression              | 2.24 (0.83)  | 2.70  | 0.0098 | 0.57 to 3.92     | 0.331                | 0.08–0.58          |

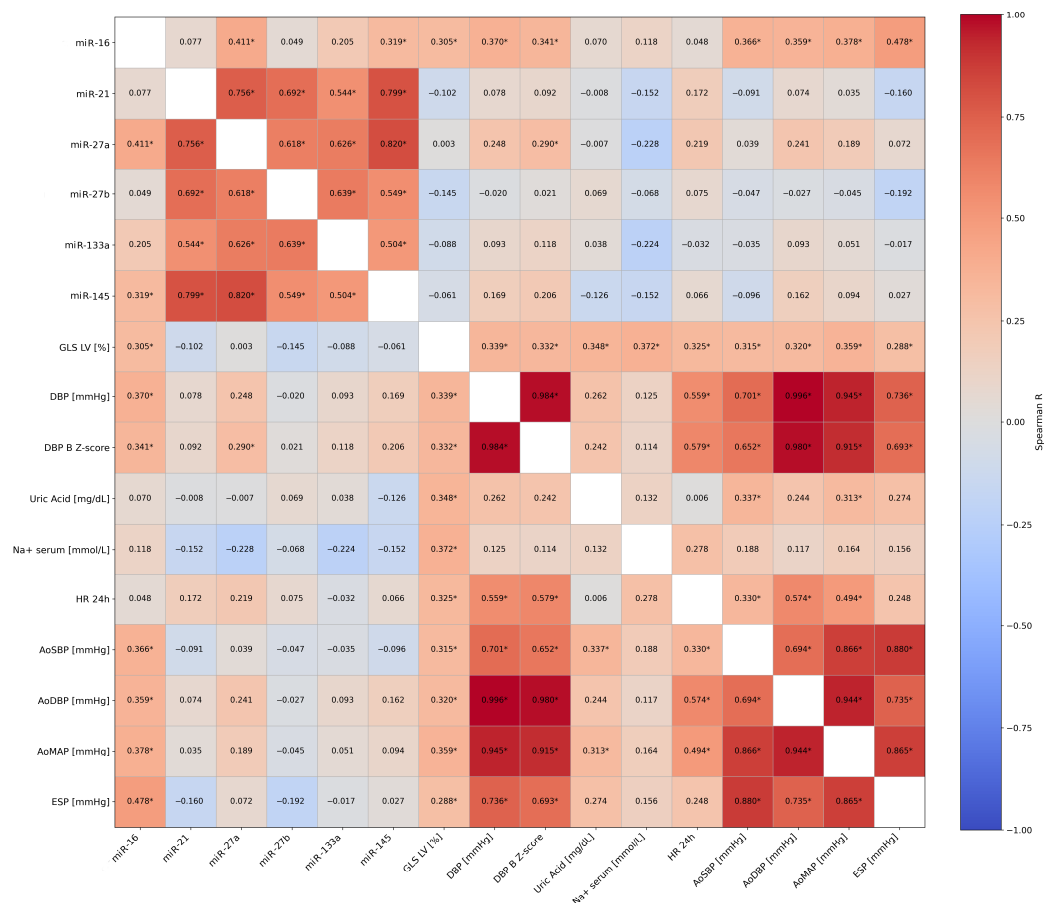
B—unstandardized regression coefficient; SE—standard error;  $\beta$ —standardized regression coefficient; CI—confidence interval; 24 h HR—mean 24 h heart rate in ambulatory blood pressure monitoring; RBC—red blood cell count. The intercept (constant) represents the expected GLS value when all covariates are set to zero. Model derived using a generalized linear model with stepwise variable selection.

In the overall cohort, circulating plasma miR-27b-3p levels were inversely associated with LV GLS values, suggesting that higher miR-27b-3p levels were associated with more favorable myocardial deformation indices (Figure 3).

**Figure 3.** Relationship between global LV strain and relative miR-27b-3p expression levels in all children. The figure shows the unadjusted Spearman correlation. Blue circles represent individual study participants included in the analysis. Solid lines are linear regression lines and dotted lines represent 95% confidence intervals. LV—left ventricle.

No comparable correlations were identified for the other microRNAs assessed.

Additionally, LV GLS exhibited significant positive correlations with peripheral DBP, DBP Z-score, 24 h HR, and all office central pressures (AoSBP, AoDBP, and AoMAP). Furthermore, LV GLS was positively correlated with end-systolic pressure (ESP) and, among biochemical variables, with serum uric acid and sodium concentrations (Figure 4).



**Figure 4.** Unadjusted Spearman correlation matrix of the blood pressure, biochemical and echocardiographic parameters (including global left ventricle strain) and microRNA in the group of hypertensive children. Adjusted associations were subsequently evaluated using multivariable regression analysis. GLS LV—left ventricular global longitudinal strain; DBP—diastolic blood pressure; DBP Z-score—Z-score of diastolic blood pressure; HR 24 h—24 h mean heart rate; AoSBP—central (aortic) systolic blood pressure; AoDBP—central (aortic) diastolic blood pressure; AoMAP—central (aortic) mean arterial pressure; ESP—estimated end-systolic pressure. \* indicates statistically significant correlations ( $p < 0.05$ ).

No significant associations were found between age and any of the microRNAs analyzed (Spearman's  $|R| \leq 0.10$ , all  $p \geq 0.49$ ), nor for LV GLS ( $R = -0.039$ ,  $p = 0.788$ ).

A multivariable generalized multiple regression (GRM) analysis was performed on a group of patients with primary hypertension. The statistically significant model ( $R^2 = 0.355$ ;  $F = 7.88$ ;  $p = 0.00027$ ) identified 24 h heart rate (24 h HR), red blood cell (RBC) count, and miR-16-5p expression level (beta = 0.331, 95CI: 0.084–0.578,  $p = 0.010$ ) as independent determinants of LV GLS (Table 4). The achieved statistical power ranged from 0.657 to 0.849, depending on the predictor.

Partial Spearman correlation analysis adjusting for age, sex, BMI Z-score, and SBP Z-score showed attenuation of the association ( $r = 0.24$ ,  $p = 0.089$ ). Further details of unadjusted and adjusted analyses are provided in Supplementary Table S2.

### 3. Discussion

Our findings showed miR-16-5p was independently positively associated with LV GLS in our cohort of hypertensive children, which persisted in multivariable regression analysis adjusting for relevant clinical covariates, pointing to a potential link with subclinical myocardial deformation. In between-group comparisons, miR-16-5p approached but did

not reach conventional statistical significance. Previous studies have described miR-16-5p as a contributing factor linked to adverse cardiovascular remodeling and complications in various disease states, particularly in adult populations [15,25].

Furthermore, miR-27b-3p differed significantly between hypertensive and control groups and was negatively correlated with LV GLS in the overall cohort. This may suggest a potential protective role for this microRNA in the context of pediatric hypertension ([26] and our yet unpublished data).

Overall, our outcomes suggest that microRNAs may be components of integrated molecular signatures, in addition to isolated biomarkers. The emerging concept of theranomiRNA proposes that selected microRNAs could provide diagnostic, prognostic, and therapeutic value simultaneously in cardiovascular disease [27]. In pediatric hypertension, microRNA panels, when combined with high-level analytical approaches such as machine learning, may in the future improve risk stratification and identify early myocardial vulnerability and drug targets [28]. Given our results, miR-16-5p and miR-27b-3p should be considered in such models.

However, due to the modest effect sizes and limited sample size in this study, these observations require prudent interpretation. Validation in larger, multicenter cohorts and investigation of expanded microRNA profiles are essential prior to considering clinical implementation.

To the best of our knowledge, this is the first study in which an association between circulating miR-27b-3p expression and LV GLS in children with PH has been demonstrated. Although one previous study has reported elevated serum miR-27b levels in hypertensive adults with established left ventricular hypertrophy compared to healthy controls [16], our findings demonstrate higher circulating plasma miR-27b-3p expression in healthy children and an inverse association with myocardial deformation, as assessed by LV GLS. Interestingly, recent experimental and clinical study in chronic pressure overload states suggests that sustained upregulation of miR-27b in cardiac tissue may alleviate—by targeting the Mff/MAVS axis—myocardial injuries, which are characteristic of advanced remodeling [29]. Moreover, another recent research has demonstrated that miR-27b can counteract several harmful effects of TNF- $\alpha$ , such as endothelial dysfunction and mitochondrial redox imbalance; it has also been shown to reduce mitochondrial apoptosis and downregulate FOXO1 expression, which can limit the effects of the Akt/FOXO1 signaling pathway and reduce oxidative stress-driven cellular injury [26]. These ambiguous observations highlight the context- and stage-dependent functions of miR-27b in cardiovascular regulation, supporting the idea that its biological role shifts from protective in the early stages of disease to potentially detrimental in established hypertensive heart disease. This apparent discrepancy may also reflect age-dependent regulation and developmental differences in microRNA expression profiles between pediatric and adult populations. In children with early-stage primary hypertension and largely preserved cardiac structure, miR-27b may play a predominantly adaptive or compensatory role in maintaining myocardial mechanics and preserving the left ventricle's systolic function. However, it cannot be unequivocally determined whether elevated miR-27b levels represent a compensatory adaptive response or developmental regulation. Further studies are needed to clarify the underlying mechanisms.

These findings further highlight the need for standardized and unified protocols for microRNA assessment. Differences in biological matrices and methodologies, such as the use of plasma in our study versus cardiomyocytes or serum in the above-cited reports, may substantially influence measured expression levels and their clinical interpretation. Therefore, they should be analyzed within a precise biological and clinical context.

Like miR-27b, the biological role of miR-16-5p appears to be context-dependent [30,31]. Some results of experiments on animal models indicate that reducing miR-16 could have a protective effect on the heart during conditions involving restricted blood supply and stress [32]. In mentioned study using rats to model heart damage, stopping the action of miR-16 has been found to reduce the death of heart muscle cells and lessen the heart's damage during ischemic state [32]. When applied to clinical practice, studies have shown that circulating miR-16 is linked to a higher chance of a future myocardial infarction and negative cardiovascular outcomes in adults, suggesting that it could be a useful tool for assessing cardiovascular risk [33].

From a mechanistic perspective, miR-16 has been implicated in pathways related to cardiomyocyte injury, including the regulation of endoplasmic reticulum stress, apoptotic signaling and cellular survival cascades [34]. These pathways may contribute to impaired myocardial mechanics and vulnerability to ischemic damage.

In our cohort, we found a positive correlation between circulating miR-16-5p levels and LV GLS values and miR-16-5p expression was independent determinants of LV GLS in multivariate model. This suggests that higher microR-16-5p expression is associated with less negative (i.e., impaired) longitudinal myocardial deformation and potentially reflects early alterations in left ventricular contractile mechanics in juvenile patients with PH.

LV GLS is an STE measure that quantifies the left ventricle's longitudinal systolic shortening and serves as a sensitive marker of subclinical ventricular dysfunction, often detecting abnormalities before changes in conventional indices, such as ejection fraction [35]. Meta-analysis by Tadic et al. demonstrates that STE can detect subclinical systolic myocardial dysfunction in hypertensive individuals, even when conventional parameters, such as left ventricular ejection fraction (LVEF), remain within the normal range [36]. Another recent meta-analysis and pediatric cohort studies have consistently demonstrated that LV GLS is less negative in hypertensive young people compared with normotensive controls [10]. This supports the concept of early, subclinical impairment of the heart's longitudinal systolic mechanics in hypertensive children. Importantly, pediatric evidence suggests that abnormalities in myocardial strain can be detected before overt left ventricular hypertrophy develops, and that changes occur even when systolic function, as assessed by LVEF and fractional shortening (FS), is fully preserved, highlighting GLS as a sensitive marker of early hypertensive cardiac involvement [10].

Consistent with these observations, our study found significantly less negative LV GLS values in children with primary hypertension than in healthy peers, suggesting that myocardial strain analysis may provide additional value beyond conventional echocardiographic measures. Collectively, these findings underscore that LV GLS is more sensitive than LVEF for detecting subclinical myocardial dysfunction in hypertensive heart disease, reflecting early impairment of longitudinal myocardial fiber mechanics that precedes a measurable decline in LVEF. From a clinical perspective, centers with access to strain imaging may consider incorporating LV GLS into the recommended diagnostic work-up to identify subclinical myocardial involvement, even in the absence of established HMOD. This could potentially enable closer monitoring and earlier initiation or intensification of pharmacological therapy in selected patients.

In addition to impaired systolic mechanics, our hypertensive pediatric cohort demonstrated significantly lower early diastolic myocardial velocities ( $E'$ ) at both the septal and lateral mitral annulus compared with controls, as well as a prolonged isovolumetric relaxation time (IVRT), indicating early diastolic dysfunction. These tissue Doppler imaging (TDI) findings are consistent with previous reports in children with primary arterial hypertension [37]. These observations support the presence of subclinical diastolic impairment in the setting of elevated blood pressure. Furthermore, meta-analytic data in pediatric

hypertension demonstrate that abnormalities in diastolic function parameters are common in hypertensive children compared with normotensive controls, underscoring the substantial burden of diastolic dysfunction even in the absence of overt systolic failure [38]. The combination of reduced  $E'$  velocities and prolonged IVRT observed in our cohort reinforces the concept that childhood hypertension is associated with early alterations in myocardial relaxation and filling dynamics. Early detection of these subclinical systolic and diastolic abnormalities using STE and TDI may provide valuable prognostic information and guide earlier therapeutic interventions to prevent progression to overt ventricular dysfunction.

Additionally, LV GLS was positively associated with blood pressure-related parameters, including DBP, AoSBP, AoDBP, and AoMAP, as well as with serum uric acid and sodium concentrations. In a recent study involving a pediatric cohort with chronic kidney disease (CKD), the association between reduced LV GLS and DBP appeared more consistent than with SBP [35]. Consistent with these observations, in our study, LV GLS was more closely associated with DBP than SBP, as significant correlations were observed with peripheral office DBP but not with peripheral office SBP. All central blood pressure parameters, including AoSBP, AoDBP and AoMAP, showed positive correlations with LV GLS in our study, which may indicate a greater role for central pressure than peripheral pressure, and for DBP than SBP, in the early changes that occur in the heart. It is noteworthy that central blood pressure parameters were not assessed in the aforementioned study. Therefore, our findings suggest that, in addition to assessing myocardial strain, evaluating central hemodynamic parameters might provide further insight into strain–blood pressure relationships during the early stages of hypertensive cardiac involvement.

Taken together, a mechanistic framework can link pediatric PH to early longitudinal myocardial dysfunction through pathways involving neurohumoral factors and oxidative stress. Pediatric PH is characterized by increased sympathetic activity and hemodynamic overdrive, which may contribute to myocardial cellular stress, including oxidative and endoplasmic reticulum stress [39,40]. Studies suggest that miR-16-5p is involved in stress response and remodeling pathways, particularly in oxidative stress signaling and the regulation of cardiomyocyte survival [34].

In this context, we can propose the following hypothesis: hypertension leads to increased adrenergic drive and oxidative stress, which, in turn, upregulate miR-16-5p, resulting in preferential impairment of longitudinal fibers and, subsequently, less negative GLS (Figure 1). While our study's cross-sectional design prevents us from inferring causality, this framework might support the biological plausibility of using miR-16-5p as a biomarker for early myocardial deformation abnormalities in pediatric PH. Further studies with a larger cohort are needed to confirm this mechanistic framework in this context.

### *Limitations and Strengths*

There are some acknowledged limitations to our study that must be addressed. Firstly, the study has a narrow focus on a small set of six preselected microRNAs and is unable to perform global normalization of microRNA expression. Another limitation of the study is the absence of a formal pubertal staging assessment, as hormonal and developmental changes during puberty may influence circulating microRNA profiles and cardiac functional parameters. Although strict pre-analytical procedures were applied, hemolysis was assessed only by visual inspection and not by molecular markers (e.g., the miR-451a/miR-23a-3p ratio), which should be considered another limitation of the study. Furthermore, all patients were from a single center, which had limited ethnic and age diversity. This makes it difficult to generalize the results. Another limitation of the study was the lack of formal assessment of LV GLS reproducibility. All measurements were performed by a single experienced investigator using the same echocardiographic device, platform and software;

however, intra-observer variability were not formally evaluated. Lastly, the relatively small sample size of the hypertensive cohort ( $n = 50$ ) may have limited the statistical power of adjusted correlation analyses. Although miR-16-5p remained independently associated with LV GLS in the multivariable regression model, the partial correlation analysis showed attenuation of the association. Finally, our study has several methodological limitations related to the analytical framework of circulating miRNA quantification. Because relative real-time PCR quantification was applied and normalization relied on exogenous spike-in controls rather than validated endogenous reference miRNAs, the reported fold changes should be interpreted as relative estimates of expression differences rather than absolute quantitative measurements. Consequently, the findings should be considered exploratory and hypothesis-generating, requiring confirmation in independent cohorts using optimized normalization strategies. However, in plasma-based circulating miRNA studies, universally stable endogenous reference miRNAs have not been consistently established yet - due to the low abundance and high biological variability of circulating miRNAs. However, the relatively homogeneous composition of our study cohort (untreated pharmacologically children with primary hypertension) is also a strength in a pioneering study that has never been conducted before, as it reduces biological and clinical heterogeneity and enhances internal validity. This is especially important when considering the abundance of microRNA binding sites, their multidirectional effects, and the difficulties in interpreting the results. Furthermore, our study has other key strengths. It is the first to compare microRNA expression in children and its association with LV GLS. We used a commercially available and reproducible methods to measure plasma microRNA expression, performed according to the manufacturer's instructions. All reactions were conducted in technical triplicates, and a no-template control (NTC) was included on each plate. All analyses were conducted by a single investigator to reduce bias. Independent bodies reviewed the study protocol, and a preliminary study was conducted to validate the procedures. Additionally, we thoroughly assessed the patients' vascular phenotype, blood pressure parameters and performed a thorough echocardiographic examination, evaluating numerous indices.

## 4. Materials and Methods

### 4.1. Study Design and Study Group

The study was designed as a prospective, cross-sectional investigation conducted according to a predefined protocol prospectively reviewed and approved by the Bioethics Committee of the Medical University of Warsaw (KB-154/2020; 14 September 2020) and externally peer-reviewed within the National Science Centre, Poland funding scheme (2021/41/N/NZ5/04194). Patients were recruited from a single Pediatric Nephrology Center from November 2021 to June 2023. The study group (SG) comprised untreated patients with PH, while the control group (CG) consisted of children without hypertension who were the same age and sex as the study participants and met the same exclusion criteria.

The inclusion criteria were as follows: a newly diagnosed case of untreated arterial hypertension, in accordance with the recommendations of the European Society of Hypertension (ESH) [41], verified using ambulatory blood pressure monitoring (ABPM) [42]. Additional eligibility criteria included a body height of at least 120 cm and written informed consent.

Patients were excluded if they had: secondary hypertension (as assessed according to the protocol described [43]), presented with significant cardiac (including heart failure), renal or hepatic disease, active inflammatory conditions, or acute infections

## 4.2. Laboratory Procedures

### 4.2.1. General Sample Handling

All blood samples collected during the study were taken on fasting (at least 10 h without a meal) between 7–11 a.m. Blood samples were collected into 6 mL EDTA Vacutainer tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). They were centrifuged at  $3000 \times g$  for 15 min at 4 °C within 10 min of blood collection, and the supernatant was centrifuged again under the same conditions to remove any trace of blood cells. In addition, hemolysis was visually inspected. Samples found to be hemolyzed were excluded from further analysis. After centrifugation, the obtained plasma was carefully transferred into two RNase- and DNase-free cryovials, each containing 1 mL of plasma, and immediately frozen at  $-80$  °C for further analysis. Plasma samples were handled only with extended, sterile, double-filtered pipette tips free of RNase, DNase, PCR inhibitors, endotoxin, and DNA (Eppendorf ep Dualfilter T.I.P.S., Hamburg, Germany) to protect against degradation of microRNAs. There was no additional freeze–thaw cycle; all samples were used only once.

For miRNA extraction, plasma samples were thawed on ice, gently vortexed, and centrifuged to remove any remaining debris. A fixed volume of 400 µL of plasma (middle part) was used for each miRNA isolation, ensuring standardization across all study participants. At the final stage of the extraction procedure, total RNA was eluted using 100 µL of RNase/DNase-free water (molecular grade). This elution volume was consistent for all samples processed. All isolation procedures were performed using mirVana PARIS kits (Thermo Fisher Scientific, Waltham, MA, USA). RNA quantity, quality, and purity were assessed using a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) (samples with an A260/280 ratio between 1.8 and 2.1 were considered acceptable for further analysis). Reverse transcription was conducted with TaqMan kits, followed by real-time PCR using TaqMan Advanced microRNA Assays (Thermo Fisher Scientific, Waltham, MA, USA) and the LightCycler 480 II (Roche Diagnostics GmbH, Mannheim, Germany). Absence of inhibition was confirmed by linear Ct shift across dilutions (Supplementary Table S3). To reduce technical variation, reactions were carried out in triplicate, and mean cycle threshold values were used for analysis (intra-assay CV 1.0–1.9%). Replicates with SD > 0.5 Ct were excluded. Inter-assay reproducibility was assessed using identical plasma samples analyzed on independent plates (CV 2.0–3.8%). Serial dilution experiments using pooled cDNA were performed to assess assay linearity, working range, and potential inhibition ( $R^2$  values ranging from approximately 0.96 to 0.99, slopes close to the expected value for efficient amplification (~3.3) and amplification efficiencies ranged from approximately 85% to 106%). microRNA expression was calculated using the  $2^{-\Delta\Delta CT}$  method [44]. Expression values were log10-transformed before analysis to improve normality.

### 4.2.2. Spike-In and Exogenous Normalization:

Given the absence of a validated housekeeping microRNA for normalization of microRNA content, we used *Caenorhabditis elegans* miR-39 (cel-miR-39) and miR-54 (cel-miR-54) as spike-in controls—exogenous normalizers [45]. In addition, a randomly selected miR-21-5p sample (reference sample) was included as an internal reference across all qPCR runs to ensure inter-run comparability. Its expression did not differ significantly between groups ( $p = 0.486$ ;  $r = 0.07$ ), confirming its stability and suitability as a reference gene.

After the addition of 2X Denaturing Solution to the plasma sample, the cel-miR-39 was immediately added and thoroughly mixed. As a control for the reverse transcription step, cel-miR-54 was incorporated into the RT Reaction Mix during the poly(A) tailing reaction in place of an equal volume of RNase-free water, ensuring identical concentrations across all samples.

All microRNA sequences and accession numbers (<https://www.mirbase.org/>; accessed on 26 January 2026) are listed in Supplementary Table S4 for reference and further analysis. The primers and probes sequences are part of the pre-formulated assay and are not publicly disclosed by the manufacturer (Thermo Fisher Scientific, Waltham, MA, USA).

Our previous manuscripts provide a detailed description of the methodology used to determine the parameters in Sections 4.3 and 4.4 [46,47]. The kits used (according to the manufacturer's instructions) for RNA isolation, reverse transcription, and qPCR, including catalog numbers and manufacturers, are listed in Supplementary Table S5. Reporting of real-time PCR experiments followed commonly recommended quality control principles described in the MIQE guidelines (Supplementary Table S6) [48].

#### 4.3. Basic Clinical and Biochemical Parameters

The following clinical variables were recorded among study participants: age, sex, duration of arterial hypertension, gestational age at birth, and birth weight. Current height, weight, and body mass index (BMI) were measured and Z-scores calculated [48]. Biochemical indices were determined using routine laboratory techniques (dry chemistry; VITROS 5600, Ortho Clinical Diagnostics, Raritan, NJ, USA) and included serum creatinine, sodium, potassium, and uric acid levels. The albumin-to-creatinine ratio was measured in a first morning urine sample. For children under 15 years old, the estimated glomerular filtration rate (eGFR) was calculated using the Schwartz equation, and for those 16 years and older, it was calculated using the CKD-EPI equation [49,50].

#### 4.4. BP Measurements and Vascular Examinations

The following parameters were measured: SBP—Systolic Blood Pressure; DBP—Diastolic Blood Pressure and Z-score [51]; MAP—Mean Arterial Pressure by Omron HBP-1320 (OMRON HEALTHCARE, Co., Ltd., Kyoto, Japan); 24 h: cSBP—Central Systolic Blood Pressure; cDBP—Central Diastolic Blood Pressure; PP—Pulse Pressure; 24 h-PWV—24-Hour Pulse Wave Velocity; TVR—Total Vascular Resistance; 24 h-AIx—24-Hour Augmentation Index; CO—Cardiac Output; SV—Stroke Volume; RM—Reflection Magnitude by Mobil-O-Graph (I.E.M. Industrielle Entwicklung Medizintechnik GmbH, Stolberg, Germany); AoSBP—Aortic Systolic Blood Pressure; AoDBP—Aortic Diastolic Blood Pressure; AoMAP—Aortic Mean Arterial Pressure; AIx75HR—Augmentation Index at a Heart Rate of 75/min; Buckberg-SERV—subendocardial viability ratio; ESP—end systolic pressure by the Sphygmocor device (AtCor Medical Pty Ltd., Sydney, Australia); cIMT—common carotid artery intima-media by the Aloka Prosound Alpha 6 (Hitachi Aloka Medical, Mitaka, Japan). Both the cfPWV and the cIMT were presented as numerical values and Z-scores [23,24].

#### 4.5. Echocardiography Measurements

Echocardiographic assessments were conducted with an EPIQ CVx 5.0 ultrasound machine (Philips Medical System, Andover, MA, USA) equipped with an X5-1 matrix transducer. The frequencies of the sector array used in the study ranged from 2.1 to 4.2 MHz. Two-dimensional guided M-mode, pulsed wave Doppler, tissue Doppler (TDI), and speckle tracking echocardiograms were performed in parasternal short-axis and apical views.

Global left-ventricular deformation parameters were obtained using a semiautomated method with the device mentioned above. Apical 4-, 2-, and 3-chamber views were acquired. Endocardial contours were generated automatically, after which the operator could refine the mitral annulus and apical reference points and adjust the reconstructed 3-dimensional endocardial surface when necessary. Papillary muscles were considered part of the LV cavity. The final value (LV GLS) was the average of the measurements from all three apical projections.

Left-ventricular mass (LVM) was calculated from M-mode echocardiographic measurements, including interventricular septal thickness in diastole (IVSd), left-ventricular end-diastolic diameter (LVEDd), and posterior wall thickness in diastole (LVPWd), using the Devereux equation [52]. In accordance with the European Society of Hypertension recommendations, left-ventricular mass index (LVMI) was obtained by dividing LVM (g) by height raised to the power of 2.7 ( $\text{Height}^{2.7}$ , [m]) [41]. LVMI Z-scores were subsequently derived using the reference equations proposed by Foster et al. [53].

#### 4.6. Statistical Methods

The study was originally designed to evaluate circulating microRNA expression, and the sample size was calculated a priori based on expected differences in microRNA levels between groups, ensuring adequate statistical power for the primary microRNA-related outcomes (statistical power 0.80,  $p = 0.05$ ). The present GLS analysis represents a predefined secondary analysis within this cohort.

The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous data are presented as mean  $\pm$  standard deviation (SD) and interquartile range (IQR), whereas categorical variables are expressed as percentages. Depending on data distribution, comparisons were performed using Student's *t*-test, Mann–Whitney U test, Spearman's rank correlation, or Pearson correlation. The  $\chi^2$  test was applied to compare categorical variables between groups. Multivariate analysis was conducted for patients in the study group using a multivariable generalized multiple regression (GRM) model and step-wise regression. Age, sex, BMI Z-score, SBP Z-score, 24 h HR, RBC count, and miR-16-5p expression level were included as clinically relevant covariates. Variables that showed significant correlations with LV GLS were included in the model, while variables with intercorrelations exceeding  $|r| = 0.6$  were excluded to avoid collinearity. *p*-values  $< 0.05$  were considered statistically significant. Given the sample size ( $n = 50$ ), the number of predictors was restricted to reduce the risk of overfitting. Additionally, a post hoc power analysis was performed for the multivariable regression model in the hypertensive study group. Partial Spearman correlation analysis was performed to assess the association between microRNA expression and LV GLS after adjustment for age, sex, BMI Z-score, and SBP Z-score. Statistical analysis was performed using Dell Statistica 13.0 PL software (TIBCO Software Inc., Palo Alto, CA, USA).

## 5. Conclusions

MiR-16-5p was independently associated with impaired left ventricular global longitudinal strain in children with primary hypertension. This finding may support a link between molecular stress responses and early subclinical myocardial dysfunction in this patient group. MiR-27b-3p differed significantly between the hypertensive and control groups. It also correlated negatively with LV GLS in the overall cohort. This suggests that distinct microRNAs may reflect opposite aspects of hypertension-related cardiac remodeling. Both microRNAs should be considered in future theranomiRNA studies as possible biomarkers and drug targets.

LV GLS was significantly reduced in pediatric primary hypertension and may serve as a sensitive marker of early myocardial involvement whenever it is available in clinical practice. However, given the cross-sectional design and modest sample size, these findings should be interpreted cautiously and validated in larger prospective studies.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms27062806/s1>.

**Author Contributions:** Conceptualization. M.S. and P.S.; methodology. M.S., P.S., R.P. and C.E.; software. M.S., K.S. and R.P.; validation. P.S. and R.P.; formal analysis. M.S., P.S. and C.E.; investigation. M.S., K.O., R.P., K.S. and P.S.; resources. M.S. and P.S.; data curation. M.S.; writing—original draft preparation. M.S. and P.S.; writing—review and editing. M.S., R.P. and P.S.; visualization. M.S.; supervision. R.P. and P.S.; project administration. M.S.; funding acquisition. M.S. and P.S. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** Prior to the start of the study, approval was obtained from the Bioethics Committee of Medical University of Warsaw (KB 154/2020; 14 September 2020). All procedures adhered to the highest ethical standards of the institutional research committee and were conducted in accordance with the tenets of the Declaration of Helsinki and its subsequent amendments.

**Informed Consent Statement:** Before the study began, patients and their representatives provided informed written and verbal consent.

**Data Availability Statement:** Due to the preliminary nature of the study and the need for further investigation, the data presented in this study are available on request from the corresponding author.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

|               |                                              |
|---------------|----------------------------------------------|
| PH            | Primary Hypertension                         |
| LV            | Left Ventricle                               |
| GLS           | Global Longitudinal Strain                   |
| STE           | Speckle-tracking Echocardiography            |
| TDI           | Tissue Doppler Imaging                       |
| BMI           | Body mass index                              |
| SBP           | Systolic Blood Pressure                      |
| DBP           | Diastolic Blood Pressure                     |
| MAP           | Mean Arterial Pressure                       |
| cSBP          | Central Systolic Blood Pressure              |
| cDBP          | Central Diastolic Blood Pressure             |
| PP            | Pulse Pressure                               |
| 24 h-PWV      | 24 h Pulse Wave Velocity                     |
| TVR           | Total Vascular Resistance                    |
| 24 h-AIx      | 24 h Augmentation Index                      |
| CO            | Cardiac Output                               |
| SV            | Stroke Volume                                |
| RM            | Reflection Magnitude                         |
| AoSBP         | Aortic Systolic Blood Pressure               |
| AoDBP         | Aortic Diastolic Blood Pressure              |
| AoMAP         | Aortic Mean Arterial Pressure                |
| AiX75HR       | Augmentation Index at a Heart Rate of 75/min |
| Buckberg-SERV | Subendocardial viability ratio               |
| ESP           | End systolic pressure                        |

## References

- O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front. Endocrinol.* **2018**, *9*, 402. [\[CrossRef\]](#)
- Huntzinger, E.; Izaurralde, E. Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* **2011**, *12*, 99–110. [\[CrossRef\]](#) [\[PubMed\]](#)
- He, L.; Liu, Y.; Widlansky, M.E.; Kriegel, A.J.; Qiu, Q.; Liang, M. microRNA and Hypertension. *Hypertension* **2025**, *82*, 181–184. [\[CrossRef\]](#) [\[PubMed\]](#)
- Kostiniuk, D.; Marttila, S.; Raitoharju, E. Circulatory miRNAs in essential hypertension. *Atherosclerosis* **2025**, *401*, 119069. [\[CrossRef\]](#) [\[PubMed\]](#)
- Song, P.; Zhang, Y.; Yu, J.; Zha, M.; Zhu, Y.; Rahimi, K.; Rudan, I. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr.* **2019**, *173*, 1154–1163. [\[CrossRef\]](#)
- Wang, C.; Yuan, Y.; Zheng, M.; Pan, A.; Wang, M.; Zhao, M.; Li, Y.; Yao, S.; Chen, S.; Wu, S.; et al. Association of Age of Onset of Hypertension With Cardiovascular Diseases and Mortality. *J. Am. Coll. Cardiol.* **2020**, *75*, 2921–2930. [\[CrossRef\]](#)
- Williams, B.; Mancia, G.; Spiering, W.; Agabiti Rosei, E.; Azizi, M.; Burnier, M.; Clement, D.L.; Coca, A.; de Simone, G.; Dominiczak, A.; et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. *Eur. Heart J.* **2018**, *39*, 3021–3104. [\[CrossRef\]](#)
- Falkner, B.; Gidding, S.S.; Baker-Smith, C.M.; Brady, T.M.; Flynn, J.T.; Malle, L.M.; South, A.M.; Tran, A.H.; Urbina, E.M. Council on Lifelong Congenital Heart Disease and Heart Health in the Young; et al. Pediatric Primary Hypertension: An Underrecognized Condition: A Scientific Statement From the American Heart Association. *Hypertension* **2023**, *80*, e101–e111. [\[CrossRef\]](#)
- Sinha, M.D.; Azukaitis, K.; Sladowska-Kozłowska, J.; Bårdsen, T.; Merkevicus, K.; Sletten, I.S.K.; Obrycki, Ł.; Pac, M.; Fernández-Aranda, F.; Bjelakovic, B.; et al. Prevalence of left ventricular hypertrophy in children and young people with primary hypertension: Meta-analysis and meta-regression. *Front. Cardiovasc. Med.* **2022**, *9*, 993513. [\[CrossRef\]](#)
- Faggiano, A.; Gherbesi, E.; Sala, C.; Carugo, S.; Grassi, G.; Cuspidi, C.; Tadic, M. Elevated Blood Pressure and Cardiac Mechanics in Children and Adolescents: A Systematic Review and Meta-Analysis. *Am. J. Hypertens.* **2025**, *38*, 370–379. [\[CrossRef\]](#)
- Lopes, E.C.P.; Paim, L.R.; Carvalho-Romano, L.F.R.S.; Marques, E.R.; Minin, E.O.Z.; Vegian, C.F.L.; Pio-Magalhães, J.A.; Velloso, L.A.; Coelho-Filho, O.R.; Sposito, A.C.; et al. Relationship Between Circulating MicroRNAs and Left Ventricular Hypertrophy in Hypertensive Patients. *Front. Cardiovasc. Med.* **2022**, *9*, 798954. [\[CrossRef\]](#) [\[PubMed\]](#)
- Fei, S.-H.; Liu, Z.-F.; Xie, H.-N.; Tong, J.-N.; Fang, Z.-M.; Chen, Y.; Yao, Y.-S. A meta-analysis of the diagnostic value of microRNA for hypertensive left ventricular hypertrophy. *Front. Cardiovasc. Med.* **2022**, *9*, 994826. [\[CrossRef\]](#) [\[PubMed\]](#)
- Stepniewski, J.; Jeż, M.; Dulak, J. Generation of miR-15a/16-1 cluster-deficient human induced pluripotent stem cell line (DMBi001-A-2) using CRISPR/Cas9 gene editing. *Stem Cell Res.* **2023**, *68*, 103046. [\[CrossRef\]](#) [\[PubMed\]](#)
- Gu, Q.; Zhao, G.; Wang, Y.; Xu, B.; Yue, J. Silencing miR-16 Expression Promotes Angiotensin II Stimulated Vascular Smooth Muscle Cell Growth. *Cell Dev. Biol.* **2017**, *6*, 181. [\[CrossRef\]](#)
- He, L.; Huang, C. MiR-19b and miR-16 cooperatively signal to target the regulator ADRA1A in Hypertensive heart disease. *Biomed. Pharmacother.* **2017**, *91*, 1178–1183. [\[CrossRef\]](#)
- Wang, Y.; Chen, S.; Gao, Y.; Zhang, S. Serum MicroRNA-27b as a Screening Biomarker for Left Ventricular Hypertrophy. *Tex. Heart Inst. J.* **2017**, *44*, 385–389. [\[CrossRef\]](#)
- Li, G.; Shao, Y.; Guo, H.C.; Zhi, Y.; Qiao, B.; Ma, K.; Du, J.; Lai, Y.Q.; Li, Y. MicroRNA-27b-3p down-regulates FGF1 and aggravates pathological cardiac remodelling. *Cardiovasc. Res.* **2022**, *118*, 2139–2151. [\[CrossRef\]](#)
- Thum, T.; Gross, C.; Fiedler, J.; Fischer, T.; Kissler, S.; Bussen, M.; Galuppo, P.; Just, S.; Rottbauer, W.; Frantz, S.; et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **2008**, *456*, 980–984. [\[CrossRef\]](#)
- Tian, C.; Hu, G.; Gao, L.; Hackfort, B.T.; Zucker, I.H. Extracellular vesicular MicroRNA-27a\* contributes to cardiac hypertrophy in chronic heart failure. *J. Mol. Cell Cardiol.* **2020**, *143*, 120–131. [\[CrossRef\]](#)
- Carè, A.; Catalucci, D.; Felicetti, F.; Bonci, D.; Addario, A.; Gallo, P.; Bang, M.-L.; Segnalini, P.; Gu, Y.; Dalton, N.D.; et al. MicroRNA-133 controls cardiac hypertrophy. *Nat. Med.* **2007**, *13*, 613–618. [\[CrossRef\]](#)
- Cordes, K.R.; Sheehy, N.T.; White, M.P.; Berry, E.C.; Morton, S.U.; Muth, A.N.; Lee, T.-H.; Miano, J.M.; Ivey, K.N.; Srivastava, D. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **2009**, *460*, 705–710. [\[CrossRef\]](#) [\[PubMed\]](#)
- Sessa, F. MicroRNAs in cardiovascular disease. In *Advances in Clinical Chemistry*; Elsevier: Amsterdam, The Netherlands, 2025.
- Reusz, G.S.; Cseprenkal, O.; Temmar, M.; Kis, E.; Cherif, A.B.; Thaleb, A.; Fekete, A.; Szabó, A.J.; Benetos, A.; Salvi, P. Reference values of pulse wave velocity in healthy children and teenagers. *Hypertension* **2010**, *56*, 217–224. [\[CrossRef\]](#) [\[PubMed\]](#)
- Doyon, A.; Kracht, D.; Bayazit, A.K.; Deveci, M.; Duzova, A.; Krmar, R.T.; Litwin, M.; Niemirska, A.; Oguz, B.; Schmidt, B.M.W. Carotid artery intima-media thickness and distensibility in children and adolescents: Reference values and role of body dimensions. *Hypertension* **2013**, *62*, 550–556. [\[CrossRef\]](#) [\[PubMed\]](#)
- Yildiz, M.; Ozkan, U.; Budak, M. The Heart's Small Molecules: The Importance of MicroRNAs in Cardiovascular Health. *J. Clin. Med.* **2025**, *14*, 7454. [\[CrossRef\]](#)

26. D'ONofrio, N.; Prattichizzo, F.; Martino, E.; Anastasio, C.; Mele, L.; La Grotta, R.; Sardu, C.; Ceriello, A.; Marfella, R.; Paolisso, G.; et al. MiR-27b attenuates mitochondrial oxidative stress and inflammation in endothelial cells. *Redox Biol.* **2023**, *62*, 102681. [\[CrossRef\]](#)
27. Sessa, F.; Pomara, C.; Schembari, F.; Esposito, M.; Capasso, E.; Pesaresi, M.; Osuna, E.; Ulas, E.; Zammit, C.; Salerno, M. MiRNA Dysregulation in Brain Injury: An In Silico Study to Clarify the Role of a MiRNA Set. *Curr. Neuropharmacol.* **2025**, *23*, 209–231. [\[CrossRef\]](#)
28. Sessa, F.; Salerno, M.; Esposito, M.; Cocimano, G.; Pisanelli, D.; Malik, A.; Khan, A.A.; Pomara, C. New Insight into Mechanisms of Cardiovascular Diseases: An Integrative Analysis Approach to Identify TheranoMiRNAs. *Int. J. Mol. Sci.* **2023**, *24*, 6781. [\[CrossRef\]](#)
29. Wang, X.; Huang, L.; Xu, J.; Li, M.; Zhang, H.; Yao, Y.; Liu, Y.; Lin, X.; Shang, X. MicroRNA-27b alleviates septic cardiomyopathy by targeting the Mff/MAVS axis. *Front. Cell Infect. Microbiol.* **2025**, *15*, 1588461. [\[CrossRef\]](#)
30. Stojkovic, S.; Wojta, J. Circulatory microRNAs in acute coronary syndrome: An update. *Pol. Heart J. (Kardiol. Pol.)* **2024**, *82*, 1192–1199.
31. Yang, J.; Yang, X.S.; Fan, S.W.; Zhao, X.Y.; Li, C.; Zhao, Z.Y.; Pei, H.J.; Qiu, L.; Zhuang, X.; Yang, C.H. Prognostic value of microRNAs in heart failure: A meta-analysis. *Medicine* **2021**, *100*, e27744. [\[CrossRef\]](#)
32. Liu, J.; Sun, F.; Wang, Y.; Yang, W.; Xiao, H.; Zhang, Y.; Lu, R.; Zhu, H.; Zhuang, Y.; Pan, Z.; et al. Suppression of microRNA-16 protects against acute myocardial infarction by reversing beta2-adrenergic receptor down-regulation in rats. *Oncotarget* **2017**, *8*, 20122–20132. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Badacz, R.; Kleczyński, P.; Legutko, J.; Żmudka, K.; Gacoń, J.; Przewłocki, T.; Kabłak-Ziembicka, A. Expression of miR-1-3p, miR-16-5p and miR-122-5p as Possible Risk Factors of Secondary Cardiovascular Events. *Biomedicines* **2021**, *9*, 1055. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Toro, R.; Pérez-Serra, A.; Mangas, A.; Campuzano, O.; Sarquella-Brugada, G.; Quezada-Feijoo, M.; Ramos, M.; Alcalá, M.; Carrera, E.; García-Padilla, C.; et al. miR-16-5p Suppression Protects Human Cardiomyocytes against Endoplasmic Reticulum and Oxidative Stress-Induced Injury. *Int. J. Mol. Sci.* **2022**, *23*, 1036. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Kula, A.J.; Xu, Y.; Hill, G.D.; Furth, S.L.; Warady, B.A.; Ng, D.K.; Seegmiller, J.; Mitsnefes, M.; CKiD Study Investigators. Hypertension and Left Ventricular Strain in Pediatric Chronic Kidney Disease. *Hypertension* **2024**, *81*, 2181–2188. [\[CrossRef\]](#)
36. Tadic, M.; Sala, C.; Carugo, S.; Mancía, G.; Grassi, G.; Cuspidi, C. Left ventricular global longitudinal strain in secondary hypertension: A meta-analysis of echocardiographic studies. *Eur. J. Intern. Med.* **2022**, *96*, 81–89. [\[CrossRef\]](#)
37. Zamojska, J.; Niewiadomska-Jarosik, K.; Wosiak, A.; Lipiec, P.; Stańczyk, J. Myocardial dysfunction measured by tissue Doppler echocardiography in children with primary arterial hypertension. *Kardiol. Pol.* **2015**, *73*, 194–200. [\[CrossRef\]](#)
38. Rus, R.R.; Pac, M.; Obrycki, Ł.; Sągśak, E.; Azukaitis, K.; Sinha, M.D.; Jankauskiene, A.; Litwin, M. Systolic and diastolic left ventricular function in children with primary hypertension: A systematic review and meta-analysis. *J. Hypertens.* **2023**, *41*, 51–62. [\[CrossRef\]](#)
39. Litwin, M. Pathophysiology of primary hypertension in children and adolescents. *Pediatr. Nephrol.* **2024**, *39*, 1725–1737. [\[CrossRef\]](#)
40. Li, Y.; Haseler, E.; Chowienczyk, P.; Sinha, M.D. Haemodynamics of Hypertension in Children. *Curr. Hypertens. Rep.* **2020**, *22*, 60. [\[CrossRef\]](#)
41. Lurbe, E.; Agabiti-Rosei, E.; Cruickshank, J.K.; Dominiczak, A.; Erdine, S.; Hirth, A.; Invitti, C.; Litwin, M.; Mancía, G.; Pall, D.; et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J. Hypertens.* **2016**, *34*, 1887–1920. [\[CrossRef\]](#)
42. Flynn, J.T.; Urbina, E.M.; Brady, T.M.; Baker-Smith, C.; Daniels, S.R.; Hayman, L.L.; Mitsnefes, M.; Tran, A.; Zachariah, J.P.; Atherosclerosis, H.O.B.O.T.; et al. Ambulatory Blood Pressure Monitoring in Children and Adolescents: 2022 Update: A Scientific Statement From the American Heart Association. *Hypertension* **2022**, *79*, e114–e124. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Tykarski, A.; Filipiak, K.J.; Januszewicz, A.; Litwin, M.; Narkiewicz, K.; Prejbisz, A.; Ostalska-Nowicka, D.; Widecka, K.; Kostka-Jeziorny, K. 2019 Guidelines for the Management of Hypertension. *Arter. Hypertens.* **2019**, *23*, 41–87. [\[CrossRef\]](#)
44. De Rosa, S.; Eposito, F.; Carella, C.; Strangio, A.; Ammirati, G.; Sabatino, J.; Abbate, F.G.; Iaconetti, C.; Liguori, V.; Pergola, V.; et al. Transcoronary concentration gradients of circulating microRNAs in heart failure. *Eur. J. Heart Fail.* **2018**, *20*, 1000–1010. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Mitchell, P.S.; Parkin, R.K.; Kroh, E.M.; Fritz, B.R.; Wyman, S.K.; Pogosova-Agadjanyan, E.L.; Peterson, A.; Noteboom, J.; O'Briant, K.C.; Allen, A.; et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 10513–10518. [\[CrossRef\]](#)
46. Szyszka, M.; Skrzypczyk, P.; Ofiara, A.; Wabik, A.M.; Pietrzak, R.; Werner, B.; Pańczyk-Tomaszewska, M. Circadian Blood Pressure Profile in Pediatric Patients with Primary Hypertension. *J. Clin. Med.* **2022**, *11*, 5325. [\[CrossRef\]](#)
47. Skrzypczyk, P.; Przychodzień, J.; Mizerska-Wasiak, M.; Kuźma-Mroczkowska, E.; Stelmaszczyk-Emmel, A.; Górska, E.; Pańczyk-Tomaszewska, M. Asymmetric dimethylarginine is not a marker of arterial damage in children with glomerular kidney diseases. *Cent. Eur. J. Immunol.* **2019**, *44*, 370–379. [\[CrossRef\]](#)

48. Kulaga, Z.; Litwin, M.; Tkaczyk, M.; Rózdżyńska, A.; Barwicka, K.; Grajda, A.; Świąder, A.; Gurzkowska, B.; Napieralska, E.; Pan, H. The height-, weight-, and BMI-for-age of Polish school-aged children and adolescents relative to international and local growth references. *BMC Public Health* **2010**, *10*, 109. [\[CrossRef\]](#)
49. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int.* **2024**, *105*, S117–S314. [\[CrossRef\]](#)
50. Schwartz, G.J.; Muñoz, A.; Schneider, M.F.; Mak, R.H.; Kaskel, F.; A Warady, B.; Furth, S.L. New equations to estimate GFR in children with CKD. *J. Am. Soc. Nephrol.* **2009**, *20*, 629–637. [\[CrossRef\]](#)
51. Kułaga, Z.; Litwin, M.; Grajda, A.; Kułaga, K.; Gurzkowska, B.; Gózdź, M.; Pan, H. Oscillometric blood pressure percentiles for Polish normal-weight school-aged children and adolescents. *J. Hypertens.* **2012**, *30*, 1942–1954. [\[CrossRef\]](#)
52. Marwick, T.H.; Gillebert, T.C.; Aurigemma, G.; Chirinos, J.; Derumeaux, G.; Galderisi, M.; Gottdiener, J.; Haluska, B.; Ofili, E.; Segers, P.; et al. Recommendations on the Use of Echocardiography in Adult Hypertension: A Report from the European Association of Cardiovascular Imaging (EACVI) and the American Society of Echocardiography (ASE). *J. Am. Soc. Echocardiogr.* **2015**, *28*, 727–754. [\[CrossRef\]](#)
53. Foster, B.J.; Mackie, A.S.; Mitsnefes, M.; Ali, H.; Mamber, S.; Colan, S.D. A novel method of expressing left ventricular mass relative to body size in children. *Circulation* **2008**, *117*, 2769–2775. [\[CrossRef\]](#)

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**7.4. Publikacja IV: „MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review”**

Review

# MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review

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## Abstract

Hypertension affects approximately 1.4 billion people worldwide. It is worth noting that it is also found in approximately 4% of children, with about half of pediatric patients having primary hypertension. Despite its widespread prevalence, the pathogenesis of primary hypertension remains poorly understood. Among the many hypotheses, research into epigenetic factors, primarily microRNA, has gained momentum in recent years. The role of microRNA is to bind to mRNA and thus influence protein translation. It is postulated that the main sites where microRNA influences blood pressure are the vascular wall, vascular smooth muscle cells, and the kidney. Many microRNAs have been identified whose importance in primary hypertension development has been demonstrated in experimental and human studies. This article discusses in detail the significance of microRNA-16, -21, -27a, -27b, -133a, and -145. It presents the current state of knowledge, as well as the challenges, limitations, and uncertainties regarding the use of these fascinating molecules as biomarkers and in potential therapy. MicroRNAs are an extremely promising area of research into the pathogenesis of primary hypertension. However, we still do not know whether they will prove to be a promising biomarker of primary hypertension.

**Keywords:** microRNA; epigenetic factors; primary hypertension; hypertension-mediated organ damage; biomarker; children

## 1. Introduction

### 1.1. The Definition, Epidemiology and Etiology of Hypertension in the Developmental Period

The European Society of Hypertension (ESH) and the HyperChildNET group use age-specific criteria to define pediatric arterial hypertension. In children aged 0–15 years, blood pressure is assessed using percentile charts; in adolescents aged 16–17 years, however, adult thresholds are applied [1,2]. Arterial hypertension is diagnosed when systolic and/or diastolic blood pressure is at or above the 95th percentile based on at least three separate measurements, or at or above 140/90 mmHg in adolescents. Both the European and American (American Academy of Pediatrics) guidelines define pediatric hypertension using age-, sex- and height-specific percentiles, which distinguishes it fundamentally from adult hypertension [3]. While these approaches are largely consistent, the American guidelines apply lower thresholds ( $\geq 130/80$  mmHg) from the age of 13 years onwards and are based on reference values derived from children of normal weight. In contrast, European reference data are based on earlier population cohorts that did not explicitly exclude overweight and obese individuals.



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According to estimates by the World Health Organization, AH occurs in 1.4 billion people aged 30–79. An analysis of our local and national data from 2018 to 2022 indicates that the number of patients with AH in Poland is stable and amounts to about 11 million—this disease affects about 35% of adult Poles [4]. However, population-based studies, such as the WOBASZ II survey, suggest a higher prevalence of approximately 42.7%, reflecting the burden of both diagnosed and undiagnosed hypertension [5].

In the literature, the percentage of patients with hypertension in the developmental period varies greatly and ranges from one to several percent, depending on the study and measurement methodology. A 2019 meta-analysis of 47 publications and 186,630 participants, published in JAMA Pediatrics, reports a 4% prevalence of AH in the developmental age group. The most common in developmental age is isolated systolic hypertension (1.5% of the population, 37.5% of children with AH). In the cited meta-analysis, there were no differences in AH incidence between boys and girls, nor were there differences by age. Still, it was shown that AH was most common in children with obesity, in whom it occurred with a frequency of 15.27%, followed by overweight children (4.99%), and least often in children with normal body weight (1.90%) [6].

Data from other epidemiological studies indicate an increasing incidence of hypertension with the age of the children studied. The disease is infrequent in infancy and early childhood, but during puberty its prevalence rises substantially, mostly among adolescent boys. Polish data indicate that AH affects 9% of people aged 18, including as many as 16% of males, which is the same as the rate of AH in the third decade of life [7]. Traditionally, it was believed that secondary hypertension dominates in the developmental age, unlike adults. However, large epidemiological data indicate that primary hypertension (pHTN) may occur in up to half of pediatric patients with AH and can be diagnosed in children in the first decade of life, especially those burdened with risk factors such as obesity and a positive family history [8,9]. In a recently published analysis of 2008 patients of one tertiary Polish center, out of 1260 patients with confirmed hypertension, pHTN was found in 49.3% of children, and pHTN secondary in the remaining 50.7% [10]. There is not the slightest doubt that pHTN is by far the most common etiology of AH in adolescence. The likelihood of pHTN is greater among males, those aged  $\geq 12.5$  years, overweight and obese children, and children with hyperuricemia [10]. In 15–20% of adolescents with pHTN, metabolic syndrome is diagnosed [11]. Although pediatric pHTN shares several pathophysiological mechanisms with adult hypertension, including genetic predisposition and neurohormonal dysregulation, lifestyle-related factors such as obesity, poor dietary habits, and physical inactivity appear to play an even more prominent role in the pediatric population.

In summary, it should be stated that taking into account the above epidemiological data, hypertension, and especially pHTN, is a significant health issue in the population of children and adolescents.

### *1.2. The Underlying Factors Contributing to the Development of Primary Hypertension*

The pathogenesis of pHTN in both adults and children is multifactorial and unclear. Mean arterial pressure (MAP) is the product of cardiac output, which in turn is determined by stroke volume and heart rate, and peripheral (arterial) resistance. Each of these elements is strictly regulated by physiological mechanisms that ensure the normal, laminar flow of blood ejected from the left ventricle during contraction to all tissues and organs [12]. The classic two theories of hypertension development are Guyton's hypothesis, assuming that pHTN is the result of increased blood volume (and thus cardiac output) due to an increase in sodium renal reuptake (the so-called increased renal threshold for sodium), and the Folkow hypothesis, indicating an increase in arterial wall tension (peripheral

resistance) as the cause of hypertension. Rather, current concepts assume the involvement of multiple factors that dynamically increase each of these elements, thereby raising blood pressure (the so-called modified Page mosaic). According to the current knowledge, the main mechanisms involved in the pathogenesis of primary hypertension in children and adolescents were described in Table 1.

**Table 1.** Selected mechanisms involved in the pathogenesis of primary hypertension in children and adolescents and the potential role of microRNAs.

| Mechanism                               | Description/Pathophysiology                                                                                                                                                                                         | Possible MicroRNA Involved                                                               |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Increased sodium sensitivity            | Excessive renal tubular sodium reabsorption [13] (not specific for children)                                                                                                                                        | MicroRNA-19 [14] and microRNA-RNA-485 [15]                                               |
| Sympathetic nervous system overactivity | Sympathetic overdrive often associated with obesity and metabolic syndrome [16] (not specific for children but may be particularly important in the pediatric population)                                           | MicroRNA-146a/b [17], microRNA-155 [17] and microRNA-181a [18]                           |
| RAAS overactivation                     | Increased activity of the renin–angiotensin–aldosterone system both systemically and locally, leading to blood pressure elevation and, e.g., left ventricular hypertrophy [19,20] (not specific for children)       | Numerous micro-RNAs (e.g., microRNA-16 [21], microRNA-21 [21,22], microRNA-133a [21,23]) |
| Altered body composition                | Increased fat mass with relative deficiency of lean body mass and skeletal muscle (sarcopenia) [24] (not specific for children but may be particularly important in the pediatric population)                       | Numerous micro-RNAs (e.g., microRNA-21, microRNA-27a) [25]                               |
| Early vascular aging                    | Accelerated biological aging with early vascular changes, including increased arterial stiffness and wall thickening (not specific for children but may be particularly important in the pediatric population) [26] | Numerous micro-RNAs (e.g., microRNA-103, microRNA-107, microRNA-128) [27]                |
| Immune system activation                | Chronic low-grade inflammation and immune system overactivation leading to blood pressure elevation and formation of hypertension-mediated organ damage [28,29] (not specific for children)                         | MicroRNA-126 [30], microRNA-130a [30], microRNA-146 [30] and microRNA-199a [31]          |

### 1.3. Epigenetic Factors in the Pathophysiology of Primary Hypertension

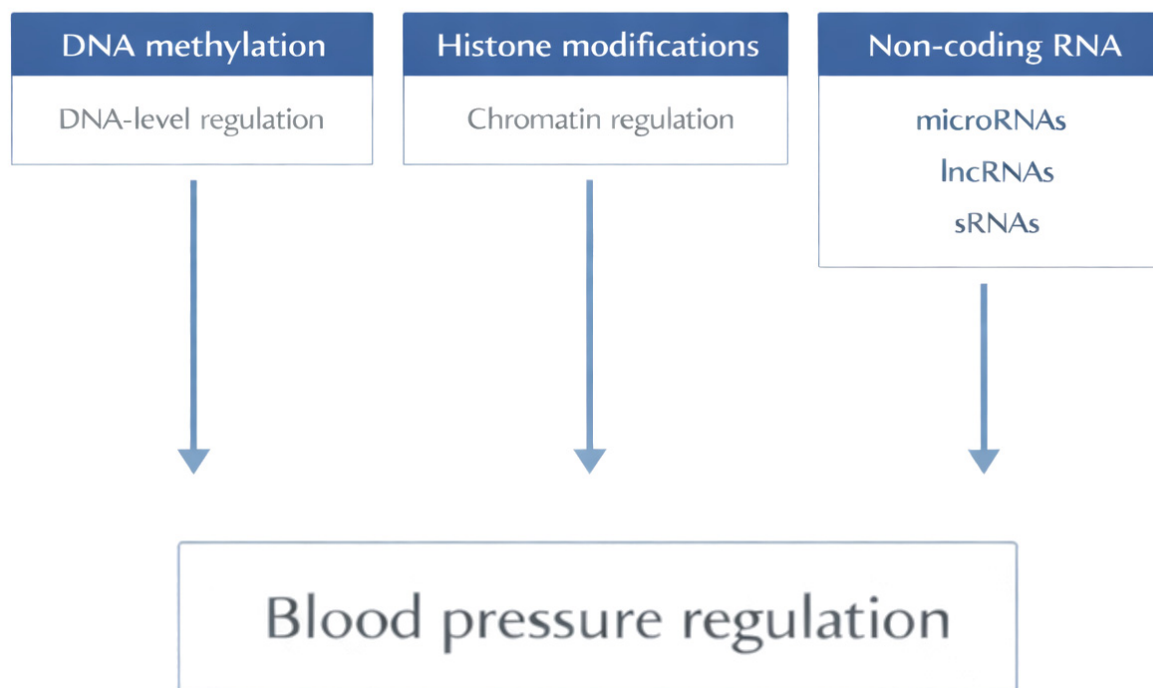
Epigenetic mechanisms regulate human homeostasis by influencing the expression of various genes. These mechanisms respond swiftly to environmental and health-related changes, regulating gene expression or cellular processes independently of DNA sequence changes.

There are three main proposed epigenetic mechanisms influencing blood pressure regulation (Figure 1). They are described briefly below.

DNA methylation is the first epigenetic mechanism discovered. DNA chains are methylated mainly at cytosine (5-methylcytosine is formed) in cytosine-guanine dinucleotides (CpG). Human DNA is methylated non-symmetrically, with some areas heavily methylated. These DNA regions are transcriptionally inactive (heterochromatin). Conversely, some other DNA fragments are variably methylated depending on various exogenous and endogenous factors, such as diet and physical exercise. Generally, when promoter regions are heavily methylated, the gene is silent and not transcribed [32–34]. Recently, another methylation mark, N6-methyladenine (6mA), was discovered [33]. The significance of various factors, e.g., high-salt diet, and the level of methylation was unmasked in a

mouse model [32]. Also, some human studies indicate a role for DNA methylation in the development of arterial hypertension [35,36]. Interestingly, in the aforementioned Indian study, methylation status differed between hypertensives with controlled and uncontrolled hypertension, and folate concentration seemed to influence methylation levels [35].

## Epigenetic mechanisms influencing blood pressure regulation



**Figure 1.** Epigenetic mechanisms influencing blood pressure regulation.

Histones are small, positively charged proteins that pack negatively charged DNA into nucleosomes, finally forming chromatin. Histone modifications influence gene activation or silencing. There are many forms of histone modifications—mainly amino acid modifications, e.g., lysine acetylation, lysine or arginine methylation, or serine or threonine phosphorylation, or larger alterations, i.e., histone protein sumoylation and ubiquitylation [37,38]. Human studies have shown a link between hypertension and the activity of histone-modifying enzymes, such as histone deacetylases (HDACs). Furthermore, in an animal model, treatment with HDAC inhibitors lowered blood pressure [37]. Lysine demethylation of histones at the TWIST1 promoter in macrophages was found to induce instability of the atherosclerotic plaque in another animal model [39].

The discovery of non-coding RNAs has opened an entirely new field of knowledge regarding the post-transcriptional regulation of gene expression. Depending on their length, non-coding RNAs are classified as short (<200 nt) and long (>200 nt). We currently know of more than 10 types of non-coding RNAs. These molecules are crucial for regulation, including basic housekeeping functions (ribosomal RNA—rRNA, transfer RNA—tRNA) and intricate gene regulation (long non-coding RNA—lncRNA and microRNA). They are implicated in numerous diseases, including cardiovascular diseases [40,41]. lncRNAs are transcripts > 200 nt, including molecules called Xist and HOTAIR, which regulate gene expression, chromatin remodeling, and epigenetic machinery. Animal models have shown that lncRNAs are involved in multiple processes involved in blood pressure regulation, including vascular smooth muscle cell (VSMC) proliferation and nitric oxide production [42]. It has recently been demonstrated that the lncRNA MALAT1 plays a role in the develop-

ment of salt-induced hypertension, which opens up possibilities for targeting this RNA as a potential approach to the prevention and treatment of hypertension [43].

The three principal epigenetic mechanisms—DNA methylation, histone modifications, and non-coding RNAs—contribute to the regulation of gene expression without altering the DNA sequence. These processes collectively influence pathways involved in blood pressure control. DNA—deoxyribonucleic acid; RNA—ribonucleic acid; lncRNAs—long non-coding RNAs; sRNAs—small RNAs.

#### 1.4. The Biology of MicroRNAs

MicroRNAs (miRNAs) are small, single-stranded, 20–25 nucleotide-long, non-coding RNAs found in humans, animals, plants, and even some viruses that are involved in post-transcriptional regulation of gene expression, primarily by silencing target messenger RNAs (mRNAs). The human genome encodes approximately 2600 pre-microRNAs (miRbase v.22, accessed 18 March 2026). Discovered initially in *C. elegans* by Victor Ambros and Gary Ruvkun (who won the 2024 Nobel Prize for this), these molecules are highly conserved across plants and animals [44,45].

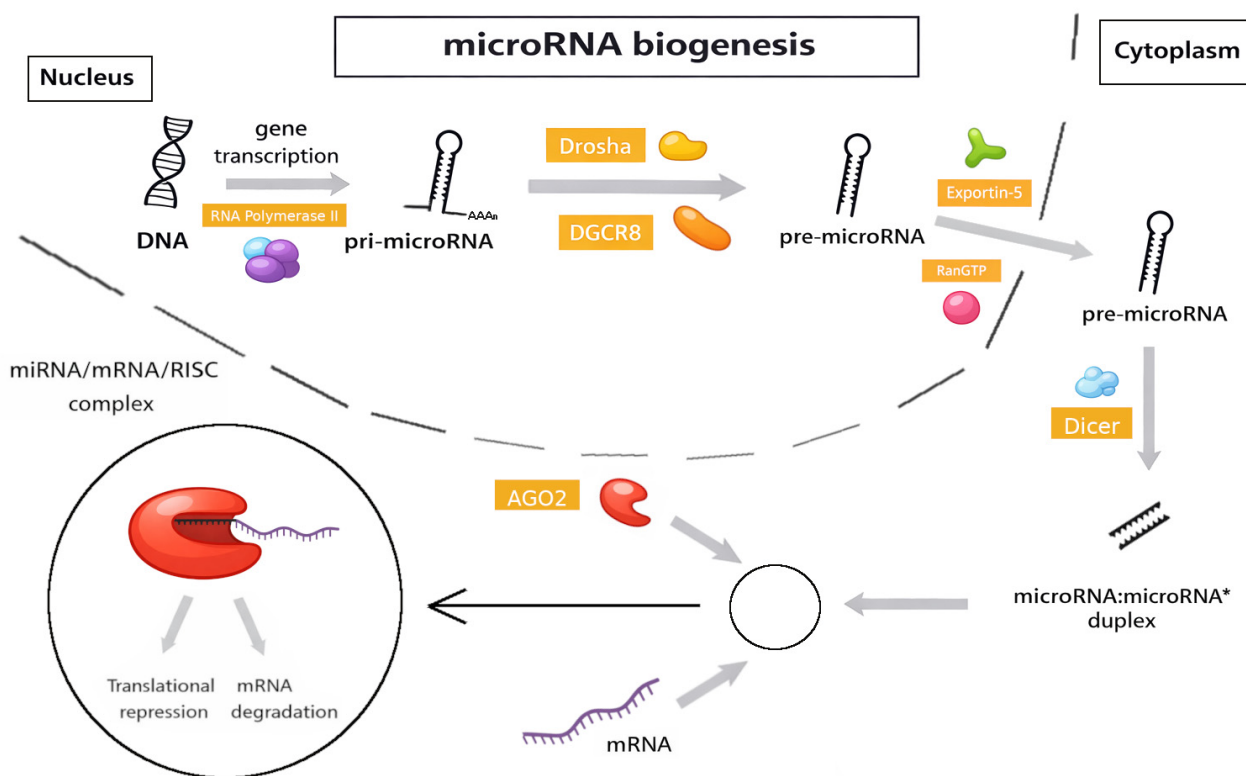
miRNAs are key regulators of numerous cellular processes, including cell proliferation and development, cell differentiation, and signaling. MiRNAs bind to the 3′ untranslated region (3′UTR) of target mRNAs, thus leading to inhibition of mRNA translation or mRNA degradation. miRNA genes are transcribed using RNA polymerase II into primary miRNA (pri-miRNA). Pri-mRNAs consist of approximately one thousand nucleotides with a local stem-loop structure made of a stem, a terminal loop, and single-stranded RNA segments at both the 5′ and 3′ ends. The nuclear microprocessor complex, composed of Drosha protein and two DGCR8 proteins, processes pri-miRNA into pre-miRNA. Pre-miRNA is a molecule consisting of 65 nucleotides shaped in a hairpin and is subsequently transferred from the nucleus to the cytoplasm by exportin 5 and Ran-GTP. There, it is cleaved by a Dicer endonuclease, thus forming a mature, single-stranded miRNA. Of note, other non-canonical mechanisms and pathways of synthesis of final mature miRNA have been identified [46,47].

This mature particle is loaded into the RNA-Induced Silencing Complex (RISC). Mature miRNAs use the Argonaute (AGO) protein within the RISC to guide binding to target mRNAs, usually through partial complementarity of the 5′-miRNA end and the 3′ UTR of the target mRNA. This interaction results in mRNA silencing via destabilization (degradation) by the endonuclease AGO activity or translational repression [46,47].

The entire process is briefly illustrated in Figure 2.

A fascinating yet challenging issue is that a single miRNA particle can target multiple mRNAs, modulating complex gene networks. Conversely, the mRNA of a single gene can be affected by numerous miRNAs [46]. Moreover, some microRNAs affect gene expression through mechanisms such as transcriptional regulation. Single-nucleotide polymorphisms (SNPs) within the 3′UTR regions of various genes may represent a key mechanism underlying individual variability and the influence of microRNAs on various diseases, as they alter genes' ability to bind microRNAs and thereby either inhibit or enhance this mechanism of gene expression [48].

MicroRNAs are crucial in development, cell growth, and apoptosis. Dereglulation of microRNA expression is linked to many diseases, primarily different malignancies, where they function as tumor suppressors or oncogenes, as well as autoimmune diseases (e.g., rheumatoid arthritis) and different viral infections. MicroRNA particles were detected in various compartments of the human body, including bodily fluids (e.g., plasma, cerebrospinal fluid, urine), often protected within vesicles (exosomes) or associated with proteins such as AGO2, and function as intercellular messengers [47,49–51].



**Figure 2.** Simplified overview of canonical microRNA biogenesis.

MicroRNA biogenesis begins in the nucleus, where transcription from DNA by RNA polymerase II generates a primary microRNA (pri-microRNA) with a characteristic hairpin structure. This transcript is processed by the microprocessor complex, composed of Drosha and DGCR8 (DiGeorge syndrome critical region 8), to produce precursor microRNA (pre-microRNA). Pre-microRNA is then exported to the cytoplasm in an Exportin-5/RanGTP-dependent manner. In the cytoplasm, the RNase III enzyme Dicer cleaves the pre-microRNA to generate a microRNA: microRNA\* duplex, of which typically only one strand becomes functionally active. The mature microRNA is incorporated into the RNA-induced silencing complex (RISC), where it associates with Argonaute (AGO) proteins. Within the RISC, microRNA guides target recognition through partial complementarity, primarily involving the seed region (nucleotides 2–8), typically located in the 3' untranslated region (3' UTR) of target mRNAs. This interaction results in translational repression or mRNA degradation, thereby regulating gene expression at the post-transcriptional level [46,47].

## 2. The Role of MicroRNAs in the Pathogenesis of Primary Hypertension and Development of Hypertension-Mediated Organ Damage

Since the discovery of microRNAs, thousands of full-text manuscripts have been published on the relationship between these molecules and arterial hypertension. Studies have identified changes in microRNA expression levels in tissues, extracellular vesicles, and circulation, examined different possible pathophysiological pathways (e.g., nitric oxide synthase or renin–angiotensin–aldosterone system), and analyzed the connections between miRNAs and sex, and ethnic differences, hypertension-mediated organ damage, and virtually all possible aspects of hypertension [52,53].

Several of the most promising microRNAs that might serve as potential biomarkers for primary hypertension or be associated with its pathogenesis were presented in Table 2 [52]. It should be emphasized that, despite the numerous limitations in the use of microRNAs described later in this article, these molecules are quite stable in plasma or serum compared

to other RNAs, remaining bound either to extracellular vesicles (EVs) or to proteins, primarily AGO2. MicroRNA can remain unchanged for several days at room temperature and can even survive numerous freeze–thaw cycles [53].

The origin of serum/plasma microRNA is not fully understood, though a study published in 2020 showed that plasma and serum microRNA expression patterns reflect those in liver, spleen, fatty tissue, and pericardium [54].

**Table 2.** MicroRNAs affecting blood pressure and arterial hypertension, according to [35,36], with our own modifications.

| MicroRNA                                                                                                | Proposed Target (mRNA)                                                                                                            | Mechanism of Action                                                                                                                                     | Cardiovascular Effect                                                                                                             |
|---------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| MicroRNA evaluated in the ATHENA study.                                                                 |                                                                                                                                   |                                                                                                                                                         |                                                                                                                                   |
| microRNA-16                                                                                             | cyclins ( <i>CCND1</i> , <i>CCND3</i> , <i>CCNE1</i> ), <i>VEGFR2</i> , <i>FGFR1</i> , <i>ADRA1A</i> , <i>VEGFA</i> , <i>BCL2</i> | Regulation of cell cycle and apoptosis, including endothelial and vascular smooth muscle cells and through sympathetic nervous system                   | Rather the anti-proliferative effect on pulmonary hypertension. The effect on systemic hypertension may be bidirectional [55,56]. |
| microRNA-21                                                                                             | Mitochondrial cytochrome b, signaling pathways including <i>SMADs</i> , <i>FGFs/FGFRs</i>                                         | Regulation of fibroblast function, cardiac cell growth, and vascular smooth muscle cell proliferation                                                   | Increase in blood pressure, development of HMOD [57,58].                                                                          |
| microRNA-27a                                                                                            | <i>HMGR</i> , <i>TGFR2</i>                                                                                                        | Regulation of cardiomyocyte and vascular smooth muscle cell function                                                                                    | Protection against hypertension and negative aortic alterations [59,60].                                                          |
| microRNA-27b                                                                                            | <i>FBW7</i> /Snail pathway, <i>SMAD-2/-3</i> pathway, <i>HMGR</i>                                                                 | a “regulatory hub” in lipid metabolism,                                                                                                                 | Contradictory data on the impact on heart fibrosis, and the impact on the risk of pHTN development [61,62].                       |
| microRNA-133a                                                                                           | <i>NOTCH</i> signaling, <i>SOX4</i> , <i>SOX9</i>                                                                                 | Protection against heart fibrosis and hypertrophy                                                                                                       | Downregulated in left ventricular hypertrophy and primary hypertension [63,64].                                                   |
| microRNA-145                                                                                            | <i>ACE</i> , <i>MRTF</i> , <i>SGRAP1</i> , <i>SGRAP2</i> , <i>SLC7A1</i> , <i>KLF4</i> , <i>KLF5</i> , <i>Mycocardin</i>          | Overexpression in damaged arteries (atherosclerotic plaque or aortic aneurysm), expression in vascular smooth muscle cells during embryonic development | Contradictory data may promote arterial damage or act as a compensatory factor<br>Elevation of blood pressure [64,65].            |
| Other important microRNAs                                                                               |                                                                                                                                   |                                                                                                                                                         |                                                                                                                                   |
| microRNA-29a-3p/-29b-3p                                                                                 | <i>Lysophospholipase 1</i> , <i>VEGF-A</i> , <i>PDGFB</i> , <i>PTEN</i> , <i>TGFB2</i> , <i>SMAD3</i>                             | Nitric oxide production                                                                                                                                 | Downregulation leads to an elevation in blood pressure [66].                                                                      |
| microRNA-30a/-30b/-30c/-30d/-30e                                                                        | <i>SMAD1</i> , <i>RUNX2</i> , <i>vimentin</i>                                                                                     | regulation of angiogenesis                                                                                                                              | microRNA-30a and -30e elevated in hypertension, -30d—lowered in hypertension [53].                                                |
| microRNA-34a                                                                                            | <i>SIRT1</i> , <i>NOTCH1</i> , <i>BCL-2</i> , <i>SMAD4</i> , <i>TGF-β1</i>                                                        | Induction of senescent cell phenotype through downregulation of <i>SIRT1</i> in endothelial and vascular smooth muscle cells                            | Elevated in human and animal models of hypertension and in atherosclerotic plaques [67].                                          |
| microRNA-92a                                                                                            | <i>FBXW7</i> , <i>CDH1</i> , <i>NF2</i> , <i>PTEN</i> , <i>p21</i> , <i>MKK4</i>                                                  | Induction of endothelial and vascular smooth muscle cell injury, reduction in nitric oxide production                                                   | Elevated in people with hypertension [25].                                                                                        |
| microRNA-126                                                                                            | <i>SPRED1</i> , <i>PIK3R2</i> , <i>VCAM1</i> , <i>EGFL7</i> , <i>VEGF-A</i>                                                       | Influences endothelial function (e.g., inhibition of expression of <i>VCAM1</i> )                                                                       | Conflicting results—associations found both with elevated and lowered blood pressure [68].                                        |
| microRNA-192-5p                                                                                         | <i>Na-K-ATPase β1</i> , <i>ZEB-1</i> , <i>ZEB-2</i>                                                                               | Increases sodium urinary excretion                                                                                                                      | Downregulation increases blood pressure [52].                                                                                     |
| microRNA-181a                                                                                           | <i>Renin</i> , <i>SIRT1</i> , <i>SMAD7</i> , <i>PDIA6</i>                                                                         | Renin–angiotensin–aldosterone system                                                                                                                    | Downregulation increases blood pressure [52].                                                                                     |
| microRNA-195-5p                                                                                         | <i>NKCC2A</i> , <i>CCND1</i> , <i>CDK6</i> , <i>CDK2</i>                                                                          | Increases sodium, potassium, and chloride tubular reabsorption in the thick ascending limb of the loop of Henle                                         | Downregulation increases blood pressure [69].                                                                                     |
| let-7 microRNA family (let-7a, let-7b, let-7c, let-7d, let-7e, let-7f, let-7g, let-7i, mir-98, mir-202) | <i>CCND1</i> , <i>K-RAS</i> , <i>H-RAS</i> , <i>N-RAS</i> , <i>HMGA2</i>                                                          | Regulation of a pro-inflammatory NF-κB pathway in endothelial cells                                                                                     | Elevated in people with hypertension in selected studies [25,70]                                                                  |

The question remains open as to how microRNAs influence blood pressure and can induce the progression from normotension to primary hypertension.

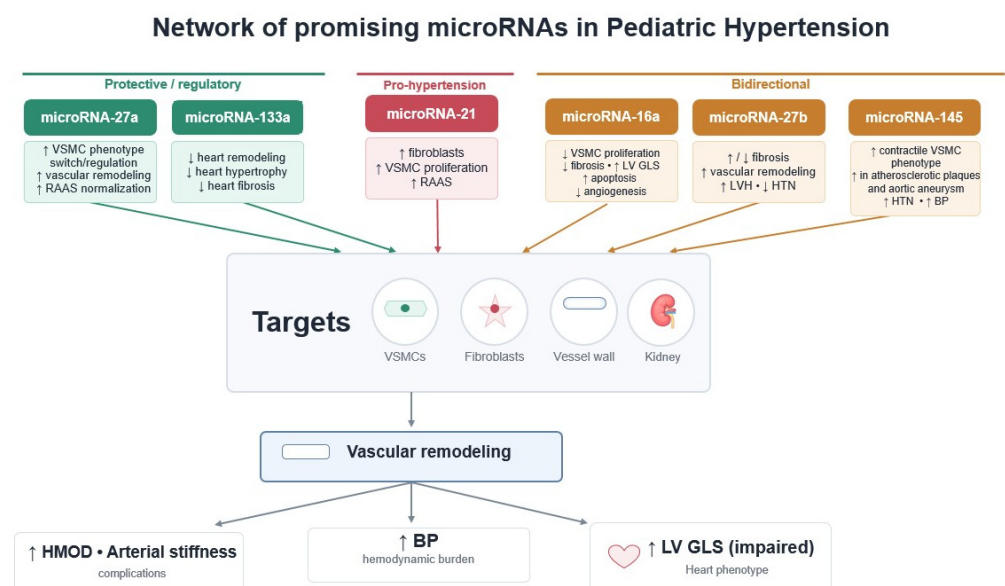
The first proposed mechanism (according to Folkov’s concept) is an effect on smooth muscle tone, thereby increasing peripheral resistance. This may be a mechanism of direct

effect on VSMCs or via angiotensin II or nitric oxide. This seems to be the mechanism of action of microRNA-143, microRNA-145, microRNA-29a-3b, and microRNA-29b-3b.

The second proposed mechanism is the effect on the kidney and increased sodium retention in the renal tubule (the hypertension mechanism proposed by Guyton), and again through direct action, e.g., on the NKCC2a co-transporter (sensitive to loop diuretics) in the nephron (Henle's) loop, and indirectly through various elements of the renin–angiotensin–aldosterone system. MicroRNA-133a, microRNA-181a, and microRNA-195-p appear to act on the kidney.

In this way, microRNAs fit into the classic concepts of the development of pHTN in humans. However, it is now believed that Guyton and Folkow's concepts are extreme models that more closely correspond to monogenic forms of hypertension (Liddle's syndrome on the one hand and Bilginturan's syndrome on the other) than to the complexity of primary hypertension's pathogenesis. pHTN can more readily be explained using Page's modified mosaic model [12]. And in this context, microRNAs should be considered as one of the players—possibly key ones—but ones that interact dynamically with other systems (including the sympathetic nervous system and the RAA system) mentioned in Section 3.

In 2020, we launched the ATHENA (ArTerial HypErTension PATHogenesis—microRNA as a missing link in the disease development in children) project. The study aimed to evaluate the expression levels of microRNA particles in children with primary hypertension and their correlation with clinical and biochemical data, blood pressure, and hypertension-mediated organ damage (HMOD) [64]. We selected 6 molecules as promising candidates potentially involved in the regulation of blood pressure. The selection was based on data from adult studies and preceded by an in-depth analysis of the available literature. Since then, many studies have been published confirming the importance of these molecules in the development of primary hypertension and organ damage. The complex and interconnected mechanisms of action of these microRNAs are summarized in Figure 3.



**Figure 3.** Network of selected, promising circulating microRNAs involved in pediatric hypertension, demonstrating heterogeneous and context-dependent effects on vascular smooth muscle cells, fibroblasts, vessel wall remodeling, and the renal regulatory system. These interactions contribute to vascular remodeling, hypertension-mediated organ damage (HMOD), increased blood pressure (BP), and impaired left ventricular global longitudinal strain (LV GLS). VSMCs—vascular smooth muscle cells; RAAS—renin–angiotensin–aldosterone system; LVH—left ventricular hypertrophy; HTN—hypertension.

### 2.1. MicroRNA-16

MicroRNA-16 is a potent suppressor of cancer growth and a key regulator in the cell cycle and is frequently downregulated in neoplasms such as chronic lymphocytic leukemia. MicroRNA-16 is located on chromosome 13q14. It inhibits cell proliferation by targeting cyclins (CCND1, CCND3, CCNE1) and CDK6, and modulates immune responses, angiogenesis, and neuronal function. MicroRNA-16 is a well-documented tumor suppressor. Reduced expression (downregulation) is observed in various malignancies, including chronic lymphocytic leukemia, prostate cancer, and ovarian cancer, often acting to stop the G1/S phase transition. Its main mechanisms of action include regulating the cell cycle by inhibiting the expression of genes that promote cell division, such as Cyclin D1 (CCND1) and CDK6. MicroRNA-16 also plays a role in inflammatory responses by improving insulin sensitivity and influencing the translation of programmed cell death ligand 1 (PD-L1), thereby affecting macrophage polarization [55,71].

This molecule seems to be involved in the development and progression of pulmonary arterial hypertension [72]. What is more, experimental data suggest that microRNA-16 may be involved in blood pressure regulation by modulating sympathetic signaling. In a mouse model, it was demonstrated that microRNA-16 directly targets the ADRA1A gene, which encodes the  $\alpha$ 1A-adrenergic receptor. Inhibiting microRNA-16 increased ADRA1A expression and was associated with reductions in systolic and diastolic blood pressure, as well as attenuating myocardial apoptosis and fibrosis [73]. This indicates a potential role for microRNA-16 in hypertensive cardiac remodeling.

Additionally, microRNA-16 has recently been linked to subclinical myocardial deformation in children. Notably, our recently published data demonstrated an association between increased circulating microRNA-16-5p and impaired left ventricular global longitudinal strain (LV GLS) in children with pHTN [56]. This suggests that microRNA-16-5p may be a potential biomarker of early subclinical myocardial dysfunction. Furthermore, previous experimental studies have demonstrated the involvement of microRNA-16 in the renin–angiotensin II system [74]. In the aforementioned study, angiotensin II was found to downregulate the expression of microRNA-16, and silencing it was found to enhance the proliferation of vascular smooth muscle cells, suggesting a protective role in vascular remodeling. This apparent discrepancy may reflect the tissue- and context-specific effects of microRNA-16, as well as its potential role as a compensatory biomarker rather than a direct mediator of pathological processes. In particular, the activation of the renin–angiotensin–aldosterone system (RAAS) in hypertension may initially lead to the downregulation of microRNA-16 at the tissue level. The observed increase in circulating microRNA-16, meanwhile, could represent a systemic compensatory response aimed at counteracting vascular remodeling and hypertrophic signaling. However, further investigation is required to confirm this.

Additionally, some reports indicate altered expression of microRNA-16 in patients with essential hypertension or hypertension-associated chronic kidney disease [53,75,76]. However, the direction and magnitude of these changes are still inconsistent across studies. Overall, the current evidence base supports the potential involvement of microRNA-16 in pathways relevant to arterial hypertension, particularly in the regulation of the sympathetic nervous system and as mentioned above, cardiovascular remodeling. However, further well-designed clinical studies are required to clarify its significance in human disease.

### 2.2. MicroRNA-21

MicroRNA-21 acts as a potent oncogene (“oncomiR”) by regulating gene expression and suppressing apoptosis. Although this molecule is frequently overexpressed in numerous cancer types, it is also becoming an increasingly significant area of interest in the study

of cardiovascular and inflammatory diseases. Known as an “oncomiR,” microRNA-21 promotes tumor growth, invasion, and metastasis by targeting tumor suppressor genes, including *PTEN* and *PDCD4*. MicroRNA-21 is upregulated in most cancers, including breast, lung, and glioblastoma, and inhibits apoptosis [57].

In the cardiovascular system, microRNA-21 regulates fibroblast function, cardiac cell growth, and vascular smooth muscle cell proliferation. It was found that microRNA-21 controls numerous signaling pathways, including mTOR, NF- $\kappa$ B, and TGF- $\beta$ 1. These pathways are related to cell (also VSMC) proliferation, injury repair, and fibrosis.

Some studies have shown that microRNA-21 may play a cardioprotective role. On the other hand, other papers revealed that this particle expression level is high in patients with myocardial infarction and may act as a promoter of fibrosis and heart dysfunction. One cannot exclude the dual role of this microRNA in the pathogenesis of cardiovascular diseases, depending on the cell, body fluid, and clinical circumstances [77].

Accumulating current evidence indicates that microRNA-21 is closely involved in the pathogenesis of pHTN, particularly through its contribution to vascular dysfunction and cardiovascular remodeling [78]. MicroRNA-21 influences the TGF- $\beta$ 1/SMAD7 and RAAS pathways, affects the immune response, and, through these mechanisms, may be a key player in pHTN pathogenesis.

Notably, it has been demonstrated that microRNA-21 regulates key processes such as fibrosis, hypertrophy, inflammation, and cell survival [79]. Moreover, the aforementioned processes play a central role in the development of vascular stiffness and HMOD, including arterial and heart damage [63,80]. MicroRNA-21 negatively affects VSMC biology. Proatherogenic stimuli induce microRNA-21 expression in VSMCs and their differentiation into lipid-accumulating cells [81]. Not to mention, microRNA-21 overexpression was found in atherosclerotic plaques [67] and correlated with common carotid artery intima-media thickness (cIMT) [58].

A recent study showed higher levels of microRNA-21 expression in individuals with resistant hypertension compared to normotensives and newly diagnosed hypertensives. In this study, the expression of microRNA-21 correlated positively with blood pressure and aldosterone concentration [82]. Another study has shown that circulating levels of microRNA-21 are correlated with aldosterone, blood pressure, and HMOD, such as albuminuria [83]. These findings further support that microRNA-21 expression reflects the activation of the RAAS, hemodynamic load, and early HMOD in pHTN. Interestingly, a meta-analysis including 12 articles, involving 546 cases and 436 controls, on the relationship between microRNA-21 and arterial hypertension was published. The pooled results revealed that the expression levels of microRNA-21 were significantly elevated in hypertensives compared with normotensives [84]. Unfortunately, there are still some conflicting data. A prospective 5-year Japanese study failed to demonstrate that microRNA-21 predicts the development of pHTN [59].

Considering the above, elevated circulating levels of microRNA-21 have been associated with disease severity and adverse cardiovascular outcomes, supporting its potential as a biomarker reflecting ongoing vascular injury and remodeling. Therefore, microRNA-21 may serve not only as a mechanistic regulator but also as a clinically relevant marker for monitoring disease progression and early cardiovascular involvement, perhaps in pediatric patients with pHTN as well.

In summary, microRNA-21 appears to be closely associated with pHTN and may be a promising biomarker. This may be the most important microRNA molecule in this disease entity. Prospective and interventional studies are undoubtedly needed.

### 2.3. MicroRNA-27a and MicroRNA-27b

MicroRNA-27a and microRNA-27b belong to a larger microRNA cluster (microRNA-23a~27a~24-2) located on human chromosome 19 and chromosome 9 (C9orf3 gene, 9q22.1), respectively. This cluster is a crucial post-transcriptional regulator of gene expression, involved, e.g., in cancer biology [85]. MicroRNA-27a seems to act alongside microRNA-21, as a potent oncogene (“oncomiR”) or, inversely, may suppress tumor growth, depending on the specific tissue and cancer type. It promotes proliferation and invasion in cancers such as gastric, breast, ovarian, and laryngeal carcinoma. On the other hand, it can inhibit growth and metastasis in renal cell carcinoma, esophageal squamous cell carcinoma, and colon cancer. In addition, microRNA-27a is a critical regulator of cholesterol biosynthesis by targeting the *HMGCR* gene. It also negatively regulates adipocyte (fat cell) differentiation by suppressing PPAR $\gamma$ . In addition, microRNA-27a is essential for maintaining bone homeostasis. It functions as a negative regulator of osteoclast differentiation; its loss can lead to severe osteoporosis [86,87].

Like microRNA-27a, microRNA-27b plays an essential role in regulating lipid homeostasis and is often referred to as a “regulatory hub” in lipid metabolism. The expression level of microRNA-27b is commonly altered in various forms of dyslipidemia [61].

MicroRNA-27a and microRNA-27b have also numerous potential roles in the cardiovascular system. MicroRNA-27a regulates cardiac function [88] and cardiomyocyte apoptosis [89], and also affects vascular smooth muscle cell proliferation and migration [90]. An experimental Chinese study showed that exercise training in spontaneously hypertensive rats increased microRNA-27a levels in the aortas, restoring disturbed renin–angiotensin–aldosterone system biology and, at least in part, explaining the protective effect of exercise on aortic function and structure [60]. Suzuki et al. reported that the expression level of circulating microRNA-27a may predict the occurrence of hypertension (“a negative biomarker”). Serum microRNA-27a was significantly lower in newly hypertensive subjects than in normotensive subjects [59].

As for microRNA-27b, it can promote myocardial fibrosis by modulating the FBW7/Snail pathway [62]. Conversely, another study revealed that microRNA-27b ameliorated atrial fibrosis and atrial fibrillation by inactivating the Smad-2/3 pathway via targeting *ALK5*. The latter results suggest an anti-fibrotic potential of this molecule in the heart. It was even proposed as a target for the therapies of heart failure [91]. An interesting human study showed that serum microRNA-27b expression levels were elevated in subjects with AH and left ventricular hypertrophy (LVH) compared to hypertensive patients without LVH and in normotensives. The authors concluded that the expression level of microRNA-27b is a potential biomarker for left ventricular hypertrophy [92].

DROSHA is a class 2 ribonuclease III enzyme, which, together with DGCR8, is involved in an initial step in microRNA processing. DROSHA participates in many other pathways of RNA maturation and degradation. DROSHA plays a role in vascular smooth muscle cell proliferation and differentiation, thus potentially influencing arterial tonus and blood pressure [93]. The SNP rs10719, located within microRNA-27b, is associated with the risk of pHTN. Chinese authors reported that rs10719 prevents the interplay between microRNA-27b and DROSHA, which may underlie the association between this SNP and the risk of pHTN [94].

### 2.4. MicroRNA 133a

MicroRNA-133a is encoded by two genes, miR-133a-1 and miR-133a-2, which are placed on chromosomes 18 and 20. It is a muscle-enriched (“myomiR”) that regulates cardiac and skeletal muscle development and also inhibits the growth of various cancers. The expression of microRNA-133a is very high in the myocardium and is crucial

for heart development and function, where it suppresses cardiomyocyte apoptosis and regulates gene expression [95]. It is downregulated during heart hypertrophy and acts as an anti-apoptotic factor in damaged cardiomyocytes, making it a potential therapeutic target. Also, a MicroRNA-133a/b complex, by influencing the Notch signaling pathway, promotes atherosclerotic plaque formation and adverse alterations (e.g., fibrosis) in the myocardium [63].

The study by Akerman revealed elevated plasma expression levels of microR-133a in individuals with hypertension compared to those with normal blood pressure [96]. Also, Koval et al. investigated plasma microRNA-133a levels in patients with primary hypertension. In their study, plasma microRNA-133a levels were lower in patients with AH than in healthy individuals. The authors have also reported significantly diminished microRNA-133a expression levels in the plasma of subjects with LVH compared with patients with normal heart structure. The authors identified no additional determinants (e.g., comorbidities) of plasma microRNA-133a expression levels. The authors concluded that reducing plasma levels of miR-133a is essential in the development of pHTN and in the pathological remodeling of the heart [97]. In another study by the same group analyzing hypertensive patients, the expression of microRNA-133a was decreased in patients with left ventricular diastolic dysfunction. It was negatively correlated with left ventricular mass index [98].

Kontaraki et al. unmasked similar results. They also reported that patients with arterial hypertension had diminished microRNA-133a expression ( $5.06 \pm 0.50$  vs.  $13.20 \pm 2.15$ ,  $p < 0.001$ ) than healthy controls. In hypertensive patients, a significant negative correlation was observed between miR-133a expression levels and the indexed left ventricular mass ( $r = -0.431$ ,  $p < 0.001$ ) [99].

Furthermore, in our previous study, changes in microRNA-133a expression after exercise were positively correlated with LVMI [64]. However, this association was not observed in the full cohort. Our currently unpublished preliminary data also suggest that children with pHTN have lower baseline and post-exercise levels of microRNA-133a, along with a blunted exercise-induced response, compared to healthy controls. The results also showed a positive correlation between microRNA-133a expression and BMI Z-scores, as well as measures of adiposity (waist and hip circumference). This indicates a potential, albeit currently limited, role for miR-133a as a biomarker of myocardial hypertrophy in pHTN.

### 2.5. MicroRNA-145

The primary role of microRNA-145 is to suppress tumor growth by regulating the expression of genes such as *c-MYC*. It acts as a molecular brake, often reducing the expression of proteins that promote cancer progression, such as MUC1, or stemness, such as OCT4 [100,101].

Beyond cancer, microRNA-145 is the most abundant microRNA in vascular smooth muscle cells (VSMCs), essential for maintaining their stability and playing an important role in VSMC differentiation. The expression of microRNA-145 is also found in plasma and the endothelium, highlighting its significance in the cardiovascular system. MicroRNA-145 targets crucial pathways and molecules in the cardiovascular system, including angiotensin-converting enzyme (ACE) or myocardin-related transcription factor B [102]. Additionally, microRNA-145 plays a central role in maintaining the contractile phenotype of VSMCs by regulating key transcription factors, including KLF4, myocardin, and SRF [103]. A loss of microRNA-145 expression can lead to a switch in phenotype from contractile to synthetic and proliferative, which contributes to vascular remodeling, increased arterial stiffness, and impaired regulation of vascular tone [104,105]. Thus, these mechanisms

directly link microRNA-145 dysregulation to blood pressure control and the development of hypertension.

From more clinical perspective, Santovito et al. examined material obtained during endarterectomy from patients with AH and found very high levels of microRNA-145 expression in atherosclerotic plaques [106]. Experimental data suggest that microRNA-145 might play a harmful role in the pathogenesis of both systemic and pulmonary hypertension [107].

Another experimental study investigated the microRNA-145 in spontaneously hypertensive rats (SHR). The expression of microRNA-15 in the aorta was higher in the SHR group than in healthy animals. In rat vascular endothelial cells (RVECs), in which microRNA-145 was silenced, the expression of solute carrier family 7 member 1 (SLC7A1) and phosphorylated endothelial nitric oxide synthase rose. A dual-luciferase reporter assay confirmed the hypothesis that SLC7A1 is directly targeted by microRNA-145. The authors concluded that microRNA-145 might be a crucial player in the development of arterial hypertension.

In our group, microRNA-145 showed a distinct pattern, suggesting a possible beneficial effect in children with pHTN [64]. In our study, the expression of this microRNA increased after a single exercise session and was negatively correlated with blood pressure and HMOD. The explanation of our findings may be found in an experimental study by Li et al. The authors revealed that microRNA-145 negatively regulated the production of GATA-binding protein 6 (GATA6), thereby attenuating cardiomyocyte hypertrophy in the experimental model [65].

Our results are consistent with Özkan's study. The authors found that downregulation of microRNA-145 was an independent predictor of arterial hypertension. Renalase concentration also showed a positive correlation with microRNA-145 expression [108].

### 3. Potential Therapeutic and Diagnostic Implementations

Non-coding RNAs can be a new therapeutic measure in the treatment of both rare and common diseases. The use of antisense RNA—interfering with mRNA (small interfering RNA)—allows protein production to be inhibited despite increased gene expression. SiRNA-based drugs are now approved medications for many rare entities, including primary hyperoxaluria type 1 [109]. Clinical trials are also conducted in hypertensive patients. Zilebesiran is a siRNA that targets angiotensinogen mRNA, thereby inhibiting its hepatic synthesis for up to half a year after a single injection. Once animal studies confirmed its safety and efficacy comparable to those of oral RAS blockers, human clinical trials were conducted.

The study of zilebesiran in humans had 3 parts. In the first part, hypertensive subjects randomly received either a single subcutaneous injection (doses from 10 to 800 mg) or a placebo and were observed for 24 weeks. Another part evaluated the efficacy of the 800 mg single dose of zilebesiran on blood pressure, depending on salt intake. In the final part, participants received the 800 mg zilebesiran injection in combination with irbesartan. The drug was effective in lowering blood pressure for a whole 24-week period with only mild side effects (transient injection-site reactions). No patients experienced hypotension, hyperkalemia, or deterioration in kidney function [110]. Elevated RAAS activity is crucial in certain clinical circumstances, such as hypovolemic shock. For these situations, a REVERSIR technology was developed. REVERSIR uses short, high-affinity, single-stranded oligonucleotides designed to reverse siRNA-mediated RNA interference in vivo [79] rapidly. We are deeply convinced that siRNA technology could revolutionize antihypertensive therapies, especially for non-adherent patients, thereby substantially reducing the impact of non-adherence on health care costs [111].

#### 4. Gaps in Current Knowledge

While microRNA research has advanced significantly—highlighted by the 2024 Nobel Prize in Physiology or Medicine—several critical knowledge gaps and technical bottlenecks remain. Firstly, many discovered microRNAs lack specific biological functions and the gene networks they regulate. Also, there is a general lack of data on microRNA expression at the cell level. Researchers often do not know if a microRNA and its predicted target are ever present in the same cell at the same time. In addition, many circulating microRNAs identified as “biomarkers” are not disease-specific (e.g., hypertension-specific). The same microRNA may be up- or downregulated in multiple unrelated conditions and in response to different stimuli, e.g., food or exercise. The results of the microRNA studies on the same entity are highly inconsistent, often due to small sample sizes, varying subject characteristics, and different isolation and evaluation protocols. As miRNAs are found in the nucleus, mitochondria, and endoplasmic reticulum, the precise mechanisms by which they are transported to these organelles remain unclear. It also remains an open question whether the specific functions of individual microRNAs are conserved across species, particularly in non-model organisms. Standardizing detection for short, highly homologous sequences remains difficult. Current methods are prone to bias during isolation and struggle with single-nucleotide specificity [50,112].

Finally, there are numerous bioinformatic limitations. Namely, analyzing the relationships between hundreds of candidate microRNAs and their targets is often impossible. There is a lack of integrated, automated software that efficiently extracts these functions from complex gene networks.

#### 5. Limitations of Using MicroRNA in Clinical Practice as Biomarkers or Therapeutic Targets

Using microRNAs as disease markers or therapeutic goals holds significant potential, but numerous limitations hamper their translation into everyday clinical practice. These include technical challenges in measurement, lack of standardization, poor target specificity, and issues with delivery and stability [113].

First, to reliably select biomarkers for diseases such as pHTN, one should not preselect based on a single study; rather, conduct a preliminary profiling analysis. Such an analysis should be carried out on a smaller group (a pilot study) comprising at least 10 individuals, including both those with and without hypertension. Based on the results obtained, potential microRNAs should be selected for verification by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) in two large groups (hypertensive vs. normotensive), with sample sizes estimated using statistical methods [53].

While miRNAs are rather stable in biofluids (serum, plasma, urine), several obstacles prevent their routine use as biomarkers. First, pre-analytical variability represents a major source of bias. Circulating miRNA levels are highly sensitive to sample collection, processing, and storage conditions. There is no consensus on protocols for sample type (serum, plasma, etc.), sample collection, handling, storage, and isolation. These issues lead to significant variability and discrepancies across published studies. Further limitations include the lack of standardization in RNA isolation and quantification methods. A variety of extraction kits, protocols and input volumes can produce inconsistent results. Different quantification methods (qRT-PCR, RNA sequencing, microarrays) often yield opposite results. In addition, there is a lack of universally accepted normalizers (internal controls) for measuring circulating microRNA levels. Although endogenous reference miRNAs are frequently utilized in studies of tissue and cells, their stability in plasma or serum remains uncertain. Consequently, many studies rely on exogenous spike-in controls to monitor extraction and reverse transcription efficiency. However, these controls do not account

for biological variability between samples. Alternative approaches have been proposed, such as global mean normalization or normalization to plasma volume, but none have been universally accepted. It should be noted that global mean normalization usually necessitates the measurement of a sufficiently large number of miRNAs to ensure stable estimation of the average expression level, which could restrict its applicability in studies focusing on a small panel of selected miRNAs.

Notably, the analytical validation of RT-qPCR assays is frequently inadequately documented. Key parameters such as amplification efficiency, dynamic range, and intra- and inter-assay variability are not consistently evaluated, nor is the presence of PCR inhibitors. This may limit the interpretability of quantitative results. The validity of relative quantification methods, such as the  $2^{\Delta\Delta C_q}$  approach, depends on the assumption that amplification efficiencies are comparable between targets and reference controls, a factor that is not always verified.

Furthermore, the biological variability and heterogeneity of circulating microRNAs complicate final analysis and conclusions. These molecules originate from multiple tissues and are present in various forms, such as protein-bound fractions and extracellular vesicles, potentially reflecting distinct biological processes. Consequently, differences observed between studies may be influenced by disease-related mechanisms and methodological inconsistencies.

Finally, many reported microRNA biomarkers are not disease-specific and are frequently detected in multiple, different, or overlapping entities. For instance, as already mentioned, microRNA-21 is upregulated in numerous cancers (colorectal, lung, breast, prostate, etc.), and in cardiovascular diseases, which limits its value as a disease-specific biomarker. Additional factors, such as hemolysis, may falsely elevate plasma or serum microRNA levels, as many microRNAs are abundant within cells. Patient-related factors such as age, sex, ethnicity, diet, comorbidities, and medications can significantly affect circulating microRNA expression levels [114,115].

Given that in adults, hypertension is rarely the only condition affecting a patient and often occurs in conjunction with metabolic syndrome, atherosclerosis, and, not infrequently, type 2 diabetes and chronic kidney disease, it is difficult to unequivocally demonstrate a link between a specific microRNA and elevated blood pressure alone. Therefore, it seems crucial to study a population as free of comorbidities as possible. In this regard, the ATHENA study, which reliably assesses the expression of selected microRNAs in children and adolescents with primary hypertension—with secondary forms, CKD, and DM excluded—and includes a detailed evaluation of clinical data and HMOD, offers hope for identifying genuine associations between microRNAs and primary hypertension [64].

Although siRNAs (RNA-based therapeutics that induce sequence-specific silencing of target mRNA) are slowly entering clinical practice, it is not as easy with drugs that affect microRNA. Developing microRNA-targeted medications faces numerous challenges. Because a single miRNA particle targets numerous mRNAs, therapeutic modulation of a single miRNA-based drug may cause unintended, broad-spectrum gene silencing in non-target tissues, leading to unforeseen sequelae. Delivering microRNAs to specific tissues (e.g., kidneys, heart, or cancer cells) remains a major bottleneck. Unmodified microRNAs are rapidly degraded by nucleases in the bloodstream and are cleared by the kidneys. In addition, some microRNA-based treatments have been found to trigger an immune response, leading to the activation of inflammatory cytokines. Finally, many miRNA inhibitors or mimics show really moderate efficacy in early clinical trials, with none, for example, having progressed to Phase III by now [116,117].

## 6. Conclusions

Recent years have brought significant progress in research into the pathogenesis of primary hypertension on the one hand, and the role of small non-coding RNAs, including microRNAs, in numerous human pathologies on the other. While the contribution of individual genes does not appear significant, post-transcriptional changes in microRNAs may open a new avenue of research into primary hypertension. Although the current evidence on microRNAs in pediatric primary hypertension is inconsistent, this variability likely reflects their context- and tissue-specific roles rather than a lack of biological relevance. In this context, microRNAs can be considered not only as promising biomarkers, but also as dynamic regulators of the vascular, renal, and cardiac pathways involved in blood pressure control. Our review highlights their dual role as both potential biomarkers and context-dependent regulators, which may explain the inconsistencies observed across studies.

Thus, microRNAs can be both valuable biomarkers and therapeutic targets. The most promising molecules have already been selected. However, the pleiotropic and context-dependent effects of microRNAs—where they may exert beneficial actions in one tissue while being detrimental in another or have different roles across disease stages—make identifying the appropriate therapeutic target in the right place and at the right time a key challenge for future research.

In our opinion, studying children is particularly valuable, as there are fewer environmental factors to consider that have accumulated during their lifetime, making it easier to derive conclusions. This makes it easier to identify microRNA involvement in the development of diseases such as primary hypertension. Future research should focus on standardized methodologies, larger, well-characterized pediatric cohorts, and longitudinal studies, to better define the clinical utility and translational potential of microRNAs in primary hypertension.

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## References

1. Lurbe, E.; Mancia, G.; Calpe, J.; Drożdż, D.; Erdine, S.; Fernandez-Aranda, F.; Hadjipanayis, A.; Hoyer, P.F.; Jankauskiene, A.; Jiménez-Murcia, S.; et al. Joint statement for assessing and managing high blood pressure in children and adolescents: Chapter 1. How to correctly measure blood pressure in children and adolescents. *Front. Pediatr.* **2023**, *11*, 1140357. [[CrossRef](#)]
2. Lurbe, E.; Agabiti-Rosei, E.; Cruickshank, J.K.; Dominiczak, A.; Erdine, S.; Hirth, A.; Invitti, C.; Litwin, M.; Mancia, G.; Pall, D.; et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J. Hypertens.* **2016**, *34*, 1887–1920. [[CrossRef](#)]

3. Flynn, J.T.; Kaelber, D.C.; Baker-Smith, C.M.; Blowey, D.; Carroll, A.E.; Daniels, S.R.; de Ferranti, S.D.; Dionne, J.M.; Falkner, B.; Flinn, S.K.; et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. *Pediatrics* **2017**, *140*, e20171904. [\[CrossRef\]](#)
4. Ceglowska, U.; Burzyńska, M.; Prejbisz, A.; Stępińska, J.; Gellert, R.; Pinkas, J.; Jankowski, P. Incidence and prevalence of registered hypertension in Poland. *Pol. Arch. Intern. Med.* **2024**, *134*, 16746. [\[CrossRef\]](#)
5. Drygas, W.; Niklas, A.A.; Piwońska, A.; Piotrowski, W.; Flotyńska, A.; Kwaśniewska, M.; Nadrowski, P.; Puch-Walczak, A.; Szafranec, K.; Bielecki, W.; et al. Multi-centre National Population Health Examination Survey (WOBASZ II study): Assumptions, methods, and implementation. *Kardiol. Pol.* **2016**, *74*, 681–690. [\[CrossRef\]](#)
6. Song, P.; Zhang, Y.; Yu, J.; Zha, M.; Zhu, Y.; Rahimi, K.; Rudan, I. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr.* **2019**, *173*, 1154–1163. [\[CrossRef\]](#)
7. Symonides, B.; Jedrusik, P.; Artyszuk, L.; Gryboś, A.; Dziliński, P.; Gaciong, Z. Different diagnostic criteria significantly affect the rates of hypertension in 18-year-old high school students. *Arch. Med. Sci.* **2010**, *6*, 689–694. [\[CrossRef\]](#)
8. Gupta-Malhotra, M.; Banker, A.; Shete, S.; Hashmi, S.S.; Tyson, J.E.; Barratt, M.S.; Hecht, J.T.; Milewicz, D.M.; Boerwinkle, E. Essential hypertension vs. secondary hypertension among children. *Am. J. Hypertens.* **2015**, *28*, 73–80. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Wyszynska, T.; Cichocka, E.; Wieteska-Klimczak, A.; Jobs, K.; Januszewicz, P. A single pediatric center experience with 1025 children with hypertension. *Acta Paediatr.* **1992**, *81*, 244–246. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Obrycki, Ł.; Skoczyński, K.; Sikorski, M.; Koziej, J.; Mitoraj, K.; Pilip, J.; Pac, M.; Feber, J.; Litwin, M. Current etiology of hypertension in European children—Factors associated with primary hypertension. *Pediatr. Nephrol.* **2025**, *40*, 3233–3244. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Litwin, M.; Sladowska, J.; Antoniewicz, J.; Niemirska, A.; Wierzbicka, A.; Daszkowska, J.; Wawer, Z.T.; Janas, R.; Grenda, R. Metabolic abnormalities, insulin resistance, and metabolic syndrome in children with primary hypertension. *Am. J. Hypertens.* **2007**, *20*, 875–882. [\[CrossRef\]](#)
12. Litwin, M. Pathophysiology of primary hypertension in children and adolescents. *Pediatr. Nephrol.* **2024**, *39*, 1725–1737. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Hanevold, C.D. Salt sensitivity of blood pressure in childhood and adolescence. *Pediatr. Nephrol.* **2022**, *37*, 993–1005. [\[CrossRef\]](#)
14. Farrell, C.E.; Liu, X.; Yagan, N.O.; Suda, A.C.; Cerqueira, D.M.; Bodnar, A.J.; Kashlan, O.B.; Subramanya, A.R.; Ho, J.; Butterworth, M.B. MicroRNA-19 is regulated by aldosterone in a sex-specific manner to alter kidney sodium transport. *Am. J. Physiol. Cell Physiol.* **2024**, *326*, C282–C293. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Gildea, J.J.; Xu, P.; Schiermeyer, K.A.; Yue, W.; Carey, R.M.; Jose, P.A.; Felder, R.A. Inverse Salt Sensitivity of Blood Pressure Is Associated with an Increased Renin-Angiotensin System Activity. *Biomedicines* **2022**, *10*, 2811. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Niemirska, A.; Litwin, M.; Feber, J.; Jurkiewicz, E. Blood pressure rhythmicity and visceral fat in children with hypertension. *Hypertension* **2013**, *62*, 782–788. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Mitrokhin, V.M.; Kamkin, A.G.; Babkina, I.I.; Savinkova, I.G.; Shileiko, S.A.; Ovchinnikov, R.S.; Mladenov, M.I. Heart-Brain Axis in Viral Myocarditis: Shared Cytokine Pathways, Blood-Brain Barrier Crosstalk, and Neuroinflammatory Consequences. *Int. J. Mol. Sci.* **2026**, *27*, 2603. [\[CrossRef\]](#)
18. Biancardi, V.C.; Sharma, N.M. Connecting sympathetic and renin-angiotensin system overdrive in neurogenic hypertension through miRNA-181a. *Hypertens. Res.* **2020**, *43*, 1309–1310. [\[CrossRef\]](#)
19. Litwin, M.; Michałkiewicz, J.; Trojanek, J.; Niemirska, A.; Wierzbicka, A.; Szalecki, M. Altered genes profile of renin-angiotensin system, immune system, and adipokines receptors in leukocytes of children with primary hypertension. *Hypertension* **2013**, *61*, 431–436. [\[CrossRef\]](#)
20. South, A.M.; Arguelles, L.; Finer, G.; Langman, C.B. Race, obesity, and the renin-angiotensin-aldosterone system: Treatment response in children with primary hypertension. *Pediatr. Nephrol.* **2017**, *32*, 1585–1594. [\[CrossRef\]](#)
21. Adamcova, M.; Kawano, I.; Simko, F. The Impact of microRNAs in Renin-Angiotensin-System-Induced Cardiac Remodelling. *Int. J. Mol. Sci.* **2021**, *22*, 4762. [\[CrossRef\]](#)
22. Rajabi, S.; Saberi, S.; Najafipour, H.; Askaripour, M.; Rajizadeh, M.A.; Shahraki, S.; Kazemina, S. Interaction of estradiol and renin-angiotensin system with microRNAs-21 and -29 in renal fibrosis: Focus on TGF- $\beta$ /smad signaling pathway. *Mol. Biol. Rep.* **2024**, *51*, 137. [\[CrossRef\]](#)
23. Sharma, N.M.; Nandi, S.S.; Zheng, H.; Mishra, P.K.; Patel, K.P. A novel role for miR-133a in centrally mediated activation of the renin-angiotensin system in congestive heart failure. *Am. J. Physiol. Heart Circ. Physiol.* **2017**, *312*, H968–H979. [\[CrossRef\]](#)
24. Pludowski, P.; Litwin, M.; Sladowska, J.; Antoniewicz, J.; Niemirska, A.; Wierzbicka, A.; Lorenc, R.S. Bone mass and body composition in children and adolescents with primary hypertension: Preliminary data. *Hypertension* **2008**, *51*, 77–83. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Improtta Caria, A.C.; Nonaka, C.K.V.; Pereira, C.S.; Soares, M.B.P.; Macambira, S.G.; Souza, B.S.F. Exercise Training-Induced Changes in MicroRNAs: Beneficial Regulatory Effects in Hypertension, Type 2 Diabetes, and Obesity. *Int. J. Mol. Sci.* **2018**, *19*, 3608. [\[CrossRef\]](#)

26. Obrycki, Ł.; Sarnecki, J.; Pac, M.; Dereziński, T.; Lewandowska, W.; Feber, J.; Litwin, M. Accelerated vascular age in adolescents with primary hypertension. *J. Hypertens.* **2023**, *41*, 171–179. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Ya, J.; Bayraktutan, U. Vascular Ageing: Mechanisms, Risk Factors, and Treatment Strategies. *Int. J. Mol. Sci.* **2023**, *24*, 1538. [\[CrossRef\]](#)
28. Dziedzic-Jankowska, K.; Kołodziej, M.; Skrzypczyk, P. Association of Subclinical Inflammation Markers with Primary Hypertension in Children-A Systematic Review with Meta-Analysis. *J. Clin. Med.* **2025**, *14*, 2319. [\[CrossRef\]](#)
29. Dziedzic-Jankowska, K.; Pietrzak, R.; Szyszka, M.; Bujanowicz, A.; Stelmaszczyk-Emmel, A.; Werner, B.; Skrzypczyk, P. Monocyte-to-Neutrophil Ratio as an Immunological Marker of Left Ventricular Hypertrophy in Children with Primary Hypertension. *J. Clin. Med.* **2025**, *14*, 3896. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Guerrero, F.; Carmona, A.; Jiménez, M.J.; Ariza, F.; Obrero, T.; Berdud, I.; Carrillo-Carrión, C.; Rodríguez, M.; Soriano, S.; Muñoz-Castañeda, J.R.; et al. New biomarkers of inflammation associated with haemodialysis. *Clin. Kidney J.* **2025**, *18*, sfaf223. [\[CrossRef\]](#)
31. Guo, S.; Li, J.; Pang, S.; Li, J.; Tian, X. Exosome miR-199a-5p modulated vascular remodeling and inflammatory infiltration of Takayasu's arteritis. *Arthritis Res. Ther.* **2025**, *27*, 11. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Huang, D.; Shang, W.; Xu, M.; Wan, Q.; Zhang, J.; Tang, X.; Shen, Y.; Wang, Y.; Yu, Y. Genome-Wide Methylation Analysis Reveals a KCNK3-Prominent Causal Cascade on Hypertension. *Circ. Res.* **2024**, *135*, e76–e93. [\[CrossRef\]](#)
33. Chaudhary, M. Novel methylation mark and essential hypertension. *J. Genet. Eng. Biotechnol.* **2022**, *20*, 11. [\[CrossRef\]](#)
34. Moore, L.D.; Le, T.; Fan, G. DNA methylation and its basic function. *Neuropsychopharmacology* **2013**, *38*, 23–38. [\[CrossRef\]](#)
35. Kaushik, A.; Bhattacharjee, D.; Chaudhary, V.; Dahal, S.; Devi, N.K.; Mitra, R.P.; Dhamija, R.K.; Krishan, K.; Pandey, R.; Saraswathy, K.N. Hypertension and global DNA methylation: A population-based study in rural, Punjab, India. *Sci. Rep.* **2024**, *14*, 25826. [\[CrossRef\]](#)
36. Zhao, L.; Jia, Y.N.; Liu, Q.S.J.; Liu, Z.Q.; Lin, H.S.; Shui, X.Y.; Guo, L.Q.; Hou, S.K. Association between Mitochondrial DNA Methylation and Hypertension Risk: A Cross-sectional Study in Chinese Northern Population. *Biomed. Environ. Sci.* **2023**, *36*, 972–978. [\[CrossRef\]](#)
37. Ray, A.; Stelloh, C.; Liu, Y.; Meyer, A.; Geurts, A.M.; Cowley, A.W., Jr.; Greene, A.S.; Liang, M.; Rao, S. Histone Modifications and Their Contributions to Hypertension. *Hypertension* **2024**, *81*, 229–239. [\[CrossRef\]](#) [\[PubMed\]](#)
38. Stoll, S.; Wang, C.; Qiu, H. DNA Methylation and Histone Modification in Hypertension. *Int. J. Mol. Sci.* **2018**, *19*, 1174. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Sun, Y.; Yao, J.; Wang, C.; Jin, Y.; Wan, X.; Meng, Q.; Wu, J.; Liu, Q.; Sun, H. Epigenetic modification of TWIST1 in macrophages promotes hypertension-induced atherosclerotic plaque instability. *Int. Immunopharmacol.* **2024**, *127*, 111313. [\[CrossRef\]](#)
40. Riffo-Campos, A.L.; Perez-Hernandez, J.; Martinez-Arroyo, O.; Ortega, A.; Flores-Chova, A.; Redon, J.; Cortes, R. Biofluid Specificity of Long Non-Coding RNA Profile in Hypertension: Relevance of Exosomal Fraction. *Int. J. Mol. Sci.* **2022**, *23*, 5199. [\[CrossRef\]](#)
41. Leimena, C.; Qiu, H. Non-Coding RNA in the Pathogenesis, Progression and Treatment of Hypertension. *Int. J. Mol. Sci.* **2018**, *19*, 927. [\[CrossRef\]](#)
42. Jiang, X.; Ning, Q. Long noncoding RNAs as novel players in the pathogenesis of hypertension. *Hypertens. Res.* **2020**, *43*, 597–608. [\[CrossRef\]](#)
43. Khan, M.M.; Kirabo, A. Long Noncoding RNA MALAT1: Salt-Sensitive Hypertension. *Int. J. Mol. Sci.* **2024**, *25*, 5507. [\[CrossRef\]](#)
44. Ambros, V.; Bartel, B.; Bartel, D.P.; Burge, C.B.; Carrington, J.C.; Chen, X.; Dreyfuss, G.; Eddy, S.R.; Griffiths-Jones, S.; Marshall, M.; et al. A uniform system for microRNA annotation. *RNA* **2003**, *9*, 277–279. [\[CrossRef\]](#)
45. Kozomara, A.; Birgaoanu, M.; Griffiths-Jones, S. miRBase: From microRNA sequences to function. *Nucleic Acids Res.* **2019**, *47*, D155–D162. [\[CrossRef\]](#)
46. Ha, M.; Kim, V.N. Regulation of microRNA biogenesis. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 509–524. [\[CrossRef\]](#)
47. Ranganathan, K.; Sivasankar, V. MicroRNAs—Biology and clinical applications. *J. Oral Maxillofac. Pathol.* **2014**, *18*, 229–234. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Arora, P.; Wu, C.; Khan, A.M.; Bloch, D.B.; Davis-Dusenbery, B.N.; Ghorbani, A.; Spagnolli, E.; Martinez, A.; Ryan, A.; Tainsh, L.T.; et al. Atrial natriuretic peptide is negatively regulated by microRNA-425. *J. Clin. Investig.* **2013**, *123*, 3378–3382. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Matsuyama, H.; Suzuki, H.I. Systems and Synthetic microRNA Biology: From Biogenesis to Disease Pathogenesis. *Int. J. Mol. Sci.* **2020**, *21*, 132. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Pogribny, I.P. MicroRNAs as biomarkers for clinical studies. *Exp. Biol. Med.* **2018**, *243*, 283–290. [\[CrossRef\]](#)
51. O'Brien, J.; Hayder, H.; Zayed, Y.; Peng, C. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. *Front. Endocrinol.* **2018**, *9*, 402. [\[CrossRef\]](#) [\[PubMed\]](#)
52. He, L.; Liu, Y.; Widlansky, M.E.; Kriegel, A.J.; Qiu, Q.; Liang, M. microRNA and Hypertension. *Hypertension* **2025**, *82*, 181–184. [\[CrossRef\]](#)

53. Kostiniuk, D.; Marttila, S.; Raitoharju, E. Circulatory miRNAs in essential hypertension. *Atherosclerosis* **2025**, *401*, 119069. [\[CrossRef\]](#)
54. Cui, C.; Cui, Q. The relationship of human tissue microRNAs with those from body fluids. *Sci. Rep.* **2020**, *10*, 5644. [\[CrossRef\]](#)
55. Yan, L.; Liang, M.; Hou, X.; Zhang, Y.; Zhang, H.; Guo, Z.; Jinyu, J.; Feng, Z.; Mei, Z. The role of microRNA-16 in the pathogenesis of autoimmune diseases: A comprehensive review. *Biomed. Pharmacother.* **2019**, *112*, 108583. [\[CrossRef\]](#)
56. Szyszka, M.; Pietrzak, R.; Obszajczyk, K.; Skubisz, K.; Eyileten, C.; Skrzypczyk, P. Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension. *Int. J. Mol. Sci.* **2026**, *27*, 2806. [\[CrossRef\]](#)
57. Rhim, J.; Baek, W.; Seo, Y.; Kim, J.H. From Molecular Mechanisms to Therapeutics: Understanding MicroRNA-21 in Cancer. *Cells* **2022**, *11*, 2791. [\[CrossRef\]](#)
58. Yildirim, E.; Ermis, E.; Allahverdiyev, S.; Ucar, H.; Yavuzer, S.; Cengiz, M. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. *Medicine* **2019**, *98*, e17297. [\[CrossRef\]](#)
59. Suzuki, K.; Yamada, H.; Fujii, R.; Munetsuna, E.; Yamazaki, M.; Ando, Y.; Ohashi, K.; Ishikawa, H.; Mizuno, G.; Tsuboi, Y.; et al. Circulating microRNA-27a and -133a are negatively associated with incident hypertension: A five-year longitudinal population-based study. *Biomarkers* **2022**, *27*, 496–502. [\[CrossRef\]](#) [\[PubMed\]](#)
60. Gu, Q.; Wang, B.; Zhang, X.F.; Ma, Y.P.; Liu, J.D.; Wang, X.Z. Contribution of renin-angiotensin system to exercise-induced attenuation of aortic remodeling and improvement of endothelial function in spontaneously hypertensive rats. *Cardiovasc. Pathol.* **2014**, *23*, 298–305. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Vickers, K.C.; Shoucri, B.M.; Levin, M.G.; Wu, H.; Pearson, D.S.; Osei-Hwedieh, D.; Collins, F.S.; Remaley, A.T.; Sethupathy, P. MicroRNA-27b is a regulatory hub in lipid metabolism and is altered in dyslipidemia. *Hepatology* **2013**, *57*, 533–542. [\[CrossRef\]](#)
62. Fu, Q.; Lu, Z.; Fu, X.; Ma, S.; Lu, X. MicroRNA 27b promotes cardiac fibrosis by targeting the FBW7/Snail pathway. *Aging* **2019**, *11*, 11865–11879. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Anwar, A.; Khan, M.J. Pathogenic and clinical significance of inflammation-associated MicroRNAs in cardiovascular diseases. *Mol. Biol. Rep.* **2025**, *52*, 940. [\[CrossRef\]](#)
64. Szyszka, M.; Skrzypczyk, P. MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension-A Preliminary Study. *J. Clin. Med.* **2024**, *13*, 6929. [\[CrossRef\]](#)
65. Li, R.; Yan, G.; Zhang, Q.; Jiang, Y.; Sun, H.; Hu, Y.; Sun, J.; Xu, B. miR-145 inhibits isoproterenol-induced cardiomyocyte hypertrophy by targeting the expression and localization of GATA6. *FEBS Lett.* **2013**, *587*, 1754–1761. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Kriegel, A.J.; Liu, Y.; Fang, Y.; Ding, X.; Liang, M. The miR-29 family: Genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol. Genom.* **2012**, *44*, 237–244. [\[CrossRef\]](#)
67. Raitoharju, E.; Lyytikäinen, L.P.; Levula, M.; Oksala, N.; Mennander, A.; Tarkka, M.; Klopp, N.; Illig, T.; Kähönen, M.; Karhunen, P.J.; et al. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. *Atherosclerosis* **2011**, *219*, 211–217. [\[CrossRef\]](#)
68. Harris, T.A.; Yamakuchi, M.; Ferlito, M.; Mendell, J.T.; Lowenstein, C.J. MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 1516–1521. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Hao, S.; Zhao, H.; Hao, D.H.; Ferreri, N.R. MicroRNA-195a-5p Regulates Blood Pressure by Inhibiting NKCC2A. *Hypertension* **2023**, *80*, 426–439. [\[CrossRef\]](#)
70. Kontaraki, J.E.; Marketou, M.E.; Zacharis, E.A.; Parthenakis, F.I.; Vardas, P.E. Differential expression of vascular smooth muscle-modulating microRNAs in human peripheral blood mononuclear cells: Novel targets in essential hypertension. *J. Hum. Hypertens.* **2014**, *28*, 510–516. [\[CrossRef\]](#)
71. Li, N.; Yang, L.; Sun, Y.; Wu, X. MicroRNA-16 inhibits migration and invasion via regulation of the Wnt/ $\beta$ -catenin signaling pathway in ovarian cancer. *Oncol. Lett.* **2019**, *17*, 2631–2638. [\[CrossRef\]](#)
72. Liu, J.J.; Tang, M.M.; Zhu, M.L.; Xie, C.X.; Kang, P.F.; Ling, X.; Zhang, H.; Wang, X.J.; Tang, B. Docosahexaenoic acid inhibits  $\text{Ca}^{2+}$  influx and downregulates CaSR by upregulating microRNA-16 in pulmonary artery smooth muscle cells. *J. Biochem. Mol. Toxicol.* **2020**, *34*, e22573. [\[CrossRef\]](#)
73. He, L.; Huang, C. MiR-19b and miR-16 cooperatively signaling target the regulator ADRA1A in Hypertensive heart disease. *Biomed. Pharmacother.* **2017**, *91*, 1178–1183. [\[CrossRef\]](#)
74. Gu, Q.; Zhao, G.; Wang, Y.; Xu, B.; Yue, J. Silencing miR-16 Expression Promotes Angiotensin II Stimulated Vascular Smooth Muscle Cell Growth. *Cell Dev. Biol.* **2017**, *6*, 181. [\[CrossRef\]](#)
75. Cheng, B.; Yang, R.; Xu, H.; Wang, L.; Jiang, N.; Song, T.; Dong, C. Peripheral Blood miRNA Expression in Patients with Essential Hypertension in the Han Chinese Population in Hefei, China. *Biochem. Genet.* **2025**, *63*, 3170–3186. [\[CrossRef\]](#)
76. Nandakumar, P.; Tin, A.; Grove, M.L.; Ma, J.; Boerwinkle, E.; Coresh, J.; Chakravarti, A. MicroRNAs in the miR-17 and miR-15 families are downregulated in chronic kidney disease with hypertension. *PLoS ONE* **2017**, *12*, e0176734. [\[CrossRef\]](#)
77. Jafari-Nozad, A.M.; Rostami, N.; Esmaeili, M.; Vahdati, H.; Hosseini, S.; Farkhondeh, T.; Samarghandian, S. A Review of the Dual Role of MicroRNA-21 in Cardiovascular Diseases: Risk Factor or a Potential Therapeutic Target. *Curr. Mol. Pharmacol.* **2024**, *17*, e18761429287057. [\[CrossRef\]](#)

78. Iside, C.; Picone, F.; Di Pietro, P.; Abate, A.C.; Prete, V.; Damato, A.; Venturini, E.; Akeel, S.; Petralia, S.; Vecchione, C.; et al. MicroRNA Signatures in Cardiometabolic Disorders as a Next-Generation Diagnostic Approach: Current Insight. *Int. J. Mol. Sci.* **2025**, *26*, 10769. [\[CrossRef\]](#) [\[PubMed\]](#)
79. He, F.; Guan, W. The role of miR-21 as a biomarker and therapeutic target in cardiovascular disease. *Clin. Chim. Acta* **2025**, *574*, 120304. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Parthenakis, F.; Marketou, M.; Kontaraki, J.; Patrianakos, A.; Nakou, H.; Touloupaki, M.; Vernardos, M.; Kochiadakis, G.; Chlouverakis, G.; Vardas, P. Low Levels of MicroRNA-21 Are a Marker of Reduced Arterial Stiffness in Well-Controlled Hypertension. *J. Clin. Hypertens.* **2017**, *19*, 235–240. [\[CrossRef\]](#) [\[PubMed\]](#)
81. Zhu, J.; Liu, B.; Wang, Z.; Wang, D.; Ni, H.; Zhang, L.; Wang, Y. Exosomes from nicotine-stimulated macrophages accelerate atherosclerosis through miR-21-3p/PTEN-mediated VSMC migration and proliferation. *Theranostics* **2019**, *9*, 6901–6919. [\[CrossRef\]](#)
82. Kara, S.P.; Ozkan, G.; Yilmaz, A.; Bayrakçı, N.; Güzel, S.; Geyik, E. MicroRNA 21 and microRNA 155 levels in resistant hypertension, and their relationships with aldosterone. *Ren. Fail.* **2021**, *43*, 676–683. [\[CrossRef\]](#)
83. Błaszczyk, R.; Biskupski, M.; Wiśniowski, Ł.; Kondracki, B.; Siwiec, J.; Mlak, R.; Głowniak, A. Non-coding RNA biomarkers in resistant hypertension: A scoping review. *Front. Mol. Biosci.* **2026**, *13*, 1786399. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Xin, C.; He, W.; Sun, X.; Li, M.; Wang, H. The Association of Micro-RNA 21 and Hypertension: A Meta-Analysis. *Glob. Heart* **2024**, *19*, 41. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Ding, L.; Ni, J.; Yang, F.; Huang, L.; Deng, H.; Wu, Y.; Ding, X.; Tang, J. Promising therapeutic role of miR-27b in tumor. *Tumour Biol.* **2017**, *39*, 1010428317691657. [\[CrossRef\]](#)
86. Bi, W.; Li, J.; Xiong, M.; Pan, B.; Zhang, Z.; Nasifu, L.; He, B.; Wang, P. The diagnostic and prognostic role of miR-27a in cancer. *Pathol. Res. Pract.* **2023**, *247*, 154544. [\[CrossRef\]](#)
87. Zhang, J.; Cao, Z.; Yang, G.; You, L.; Zhang, T.; Zhao, Y. MicroRNA-27a (miR-27a) in Solid Tumors: A Review Based on Mechanisms and Clinical Observations. *Front. Oncol.* **2019**, *9*, 893. [\[CrossRef\]](#)
88. Qin, X.D.; Liu, L. Loss of microRNA-27a induces cardiac dysfunction through activating FoxO1. *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 5941–5948. [\[CrossRef\]](#)
89. Yeh, C.H.; Chen, T.P.; Wang, Y.C.; Lin, Y.M.; Fang, S.W. MicroRNA-27a regulates cardiomyocytic apoptosis during cardioplegia-induced cardiac arrest by targeting interleukin 10-related pathways. *Shock* **2012**, *38*, 607–614. [\[CrossRef\]](#) [\[PubMed\]](#)
90. Xu, M.M.; Deng, H.Y.; Li, H.H. MicroRNA-27a regulates angiotensin II-induced vascular smooth muscle cell proliferation and migration by targeting  $\alpha$ -smooth muscle-actin in vitro. *Biochem. Biophys. Res. Commun.* **2019**, *509*, 973–977. [\[CrossRef\]](#)
91. Wang, Y.; Cai, H.; Li, H.; Gao, Z.; Song, K. Atrial overexpression of microRNA-27b attenuates angiotensin II-induced atrial fibrosis and fibrillation by targeting ALK5. *Hum. Cell* **2018**, *31*, 251–260. [\[CrossRef\]](#)
92. Wang, Y.; Chen, S.; Gao, Y.; Zhang, S. Serum MicroRNA-27b as a Screening Biomarker for Left Ventricular Hypertrophy. *Tex. Heart Inst. J.* **2017**, *44*, 385–389. [\[CrossRef\]](#)
93. Fan, P.; Chen, Z.; Tian, P.; Liu, W.; Jiao, Y.; Xue, Y.; Bhattacharya, A.; Wu, J.; Lu, M.; Guo, Y.; et al. miRNA biogenesis enzyme Drosha is required for vascular smooth muscle cell survival. *PLoS ONE* **2013**, *8*, e60888. [\[CrossRef\]](#)
94. Zhang, Y.; Cao, A.L.; Dong, C. rs10719 Polymorphism Located within DROSHA 3'-Untranslated Region is Responsible for Development of Primary Hypertension by Disrupting Binding with microRNA-27b. *Med. Sci. Monit.* **2017**, *23*, 911–918. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Liu, N.; Bezprozvannaya, S.; Williams, A.H.; Qi, X.; Richardson, J.A.; Bassel-Duby, R.; Olson, E.N. microRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. *Genes Dev.* **2008**, *22*, 3242–3254. [\[CrossRef\]](#)
96. Akerman, A.W.; Blanding, W.M.; Stroud, R.E.; Nadeau, E.K.; Mukherjee, R.; Ruddy, J.M.; Zile, M.R.; Ikonomidis, J.S.; Jones, J.A. Elevated Wall Tension Leads to Reduced miR-133a in the Thoracic Aorta by Exosome Release. *J. Am. Heart Assoc.* **2019**, *8*, e010332. [\[CrossRef\]](#) [\[PubMed\]](#)
97. Koval, S.; Snihurska, I.; Yushko, K.; Lytvynova, O.; Berezin, A. Plasma microRNA-133a level in patients with essential arterial hypertension. *Georgian Med. News* **2019**, 52–59.
98. Koval, S.M.; Snihurska, I.O.; Yushko, K.O.; Mysnychenko, O.V.; Penkova, M.Y.; Lytvynova, O.M.; Berezin, A.E.; Lytvynov, V.S. Circulating microRNA-133a in Patients with Arterial Hypertension, Hypertensive Heart Disease, and Left Ventricular Diastolic Dysfunction. *Front. Cardiovasc. Med.* **2020**, *7*, 104. [\[CrossRef\]](#) [\[PubMed\]](#)
99. Kontaraki, J.E.; Marketou, M.E.; Parthenakis, F.I.; Maragkoudakis, S.; Zacharis, E.A.; Petousis, S.; Kochiadakis, G.E.; Vardas, P.E. Hypertrophic and antihypertrophic microRNA levels in peripheral blood mononuclear cells and their relationship to left ventricular hypertrophy in patients with essential hypertension. *J. Am. Soc. Hypertens.* **2015**, *9*, 802–810. [\[CrossRef\]](#)
100. Chen, Z.; Qin, Y. Role of miRNA-145-5p in cancer (Review). *Oncol. Rep.* **2025**, *53*, 39. [\[CrossRef\]](#)
101. Xu, W.; Hua, Y.; Deng, F.; Wang, D.; Wu, Y.; Zhang, W.; Tang, J. MiR-145 in cancer therapy resistance and sensitivity: A comprehensive review. *Cancer Sci.* **2020**, *111*, 3122–3131. [\[CrossRef\]](#)

102. Sawant, D.; Lilly, B. MicroRNA-145 targets in cancer and the cardiovascular system: Evidence for common signaling pathways. *Vasc. Biol.* **2020**, *2*, R115–R128. [\[CrossRef\]](#)
103. Cordes, K.R.; Sheehy, N.T.; White, M.P.; Berry, E.C.; Morton, S.U.; Muth, A.N.; Lee, T.H.; Miano, J.M.; Ivey, K.N.; Srivastava, D. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **2009**, *460*, 705–710. [\[CrossRef\]](#)
104. Cheng, Y.; Liu, X.; Yang, J.; Lin, Y.; Xu, D.Z.; Lu, Q.; Deitch, E.A.; Huo, Y.; Delphin, E.S.; Zhang, C. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ. Res.* **2009**, *105*, 158–166. [\[CrossRef\]](#)
105. Wang, J.; Liew, O.W.; Richards, A.M.; Chen, Y.T. Overview of MicroRNAs in Cardiac Hypertrophy, Fibrosis, and Apoptosis. *Int. J. Mol. Sci.* **2016**, *17*, 749. [\[CrossRef\]](#)
106. Santovito, D.; Mandolini, C.; Marcantonio, P.; De Nardis, V.; Bucci, M.; Paganelli, C.; Magnacca, F.; Uchino, S.; Mastroiacovo, D.; Desideri, G.; et al. Overexpression of microRNA-145 in atherosclerotic plaques from hypertensive patients. *Expert Opin. Ther. Targets* **2013**, *17*, 217–223. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Wang, Y.; Jin, L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp. Ther. Med.* **2018**, *15*, 548–552. [\[CrossRef\]](#)
108. Özkan, G.; Ulusoy, Ş.; Geyik, E.; Erdem, Y. Down-regulation of miRNA 145 and up-regulation of miRNA 4516 may be associated with primary hypertension. *J. Clin. Hypertens.* **2019**, *21*, 1724–1731. [\[CrossRef\]](#) [\[PubMed\]](#)
109. Groothoff, J.W.; Metry, E.; Deesker, L.; Garrelfs, S.; Acquaviva, C.; Almardini, R.; Beck, B.B.; Boyer, O.; Cerkauskiene, R.; Ferraro, P.M.; et al. Clinical practice recommendations for primary hyperoxaluria: An expert consensus statement from ERKNet and OxalEurope. *Nat. Rev. Nephrol.* **2023**, *19*, 194–211. [\[CrossRef\]](#) [\[PubMed\]](#)
110. Desai, A.S.; Webb, D.J.; Taubel, J.; Casey, S.; Cheng, Y.; Robbie, G.J.; Foster, D.; Huang, S.A.; Rhyee, S.; Sweetser, M.T.; et al. Zilebesiran, an RNA Interference Therapeutic Agent for Hypertension. *N. Engl. J. Med.* **2023**, *389*, 228–238. [\[CrossRef\]](#)
111. Gardezi, S.K.M.; Aitken, W.W.; Jilani, M.H. The Impact of Non-Adherence to Antihypertensive Drug Therapy. *Healthcare* **2023**, *11*, 2979. [\[CrossRef\]](#)
112. Ekins, S.; Shigeta, R.; Bunin, B.A. Bottlenecks caused by software gaps in miRNA and RNAi research. *Pharm. Res.* **2012**, *29*, 1717–1721. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Takizawa, S.; Matsuzaki, J.; Ochiya, T. Circulating microRNAs: Challenges with their use as liquid biopsy biomarkers. *Cancer Biomark.* **2022**, *35*, 1–9. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Momin, M.Y.; Gaddam, R.R.; Kravitz, M.; Gupta, A.; Vikram, A. The Challenges and Opportunities in the Development of MicroRNA Therapeutics: A Multidisciplinary Viewpoint. *Cells* **2021**, *10*, 3097. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Condrat, C.E.; Thompson, D.C.; Barbu, M.G.; Bugnar, O.L.; Boboc, A.; Cretoiu, D.; Suciu, N.; Cretoiu, S.M.; Voinea, S.C. miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells* **2020**, *9*, 276. [\[CrossRef\]](#)
116. Rhee, S.W.; Stimers, J.R.; Wang, W.; Pang, L. Vascular smooth muscle-specific knockdown of the noncardiac form of the L-type calcium channel by microRNA-based short hairpin RNA as a potential antihypertensive therapy. *J. Pharmacol. Exp. Ther.* **2009**, *329*, 775–782. [\[CrossRef\]](#)
117. Ju, J. Challenges and opportunities in microRNA-based cancer therapeutics. *Cell Rep. Med.* **2025**, *6*, 102057. [\[CrossRef\]](#)

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## 8. Omówienie

Przedstawiony cykl publikacji dotyczy znaczenia wybranych krążących mikroRNA w patogenezie nadciśnienia tętniczego pierwotnego u dzieci i młodzieży oraz ich związku z wartościami ciśnienia tętniczego, wczesnymi zmianami sercowo-naczyniowymi i odpowiedzią organizmu na wysiłek fizyczny. Badania przeprowadzono w grupie dzieci i młodzieży z nieleczonym farmakologicznie nadciśnieniem tętniczym pierwotnym oraz w grupie zdrowych rówieśników.

W pierwszej publikacji przedstawiono wyniki pilotażowego badania obejmującego 22 dzieci z nadciśnieniem tętniczym pierwotnym. Głównym celem tej części projektu była weryfikacja zastosowanego protokołu badawczego oraz wstępna ocena zależności pomiędzy zmianami ekspresji wybranych mikroRNA po wysiłku fizycznym a parametrami ciśnienia tętniczego, przerostem lewej komory oraz wykładnikami uszkodzenia naczyń. Wykazano dodatnią korelację pomiędzy wzrostem ekspresji mikroRNA-133a po wysiłku a wartościami indeksu masy lewej komory ( $r = 0,491$ ;  $p = 0,020$ ). Ponadto wzrost ekspresji mikroRNA-145 był związany z niższymi wartościami ciśnienia tętniczego, mniejszą grubością kompleksu intima-media tętnicy szyjnej wspólnej oraz niższymi wartościami prędkości fali tętna (odpowiednio:  $r = -0,428$ ;  $p = 0,047$ ;  $r = -0,449$ ;  $p = 0,036$ ;  $r = -0,430$ ;  $p = 0,046$ ;  $r = -0,557$ ;  $p = 0,007$ ;  $r = -0,455$ ;  $p = 0,033$ ). Uzyskane wyniki sugerowały potencjalny udział tych cząsteczek w procesach prowadzących do rozwoju nadciśnieniowego uszkodzenia narządowego i stanowiły podstawę do przeprowadzenia dalszych badań w większej grupie pacjentów.

W drugiej publikacji przedstawiono główne wyniki badania ATHENA dotyczące podstawowej ekspresji wybranych krążących mikroRNA oraz zmian ich ekspresji po pojedynczej sesji standaryzowanego wysiłku aerobowego. Do badania włączono 56 dzieci z nadciśnieniem tętniczym pierwotnym oraz 59 zdrowych rówieśników. Chłopcy stanowili 75% grupy badanej, co jest zgodne z obserwowaną w populacji pediatrycznej przewagą płci męskiej wśród pacjentów z nadciśnieniem tętniczym pierwotnym, szczególnie w okresie dojrzewania. Na uwagę zasługuje również wysoki odsetek pacjentów z otyłością, którzy stanowili 41% badanej grupy. Może to częściowo wynikać z prowadzenia rekrutacji w okresie bezpośrednio po pandemii COVID-19, kiedy zaobserwowano istotny wzrost częstości otyłości wśród dzieci i młodzieży. W pracy wykazano istotnie niższą ekspresję mikroRNA-27b oraz mikroRNA-133a u pacjentów z nadciśnieniem tętniczym w porównaniu do zdrowych rówieśników. Ponadto stwierdzono, że pojedyncza sesja wysiłku aerobowego prowadzi do istotnego obniżenia

wartości ciśnienia tętniczego jedynie u dzieci z NTP oraz wpływa na ekspresję wybranych mikroRNA – różnie u dzieci z nadciśnieniem i u dzieci zdrowych. W grupie dzieci zdrowych po wysiłku obserwowano wzrost ekspresji mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145, natomiast u pacjentów z nadciśnieniem istotny wzrost dotyczył jedynie mikroRNA-145. Analizując zaś wartości ciśnienia, po pojedynczej, standaryzowanej sesji wysiłku aerobowego u pacjentów z nadciśnieniem tętniczym stwierdzono istotne obniżenie gabinetowego ciśnienia tętniczego (SBP: 136,8 vs 133,1 mm Hg; DBP: 82,1 vs 78,6 mm Hg), 24-godzinnego ciśnienia tętniczego (SBP: 131,2 vs 129,1 mm Hg; DBP: 73,6 vs 71,6 mm Hg) oraz 24-godzinnego ciśnienia centralnego (cSBP: 114,5 vs 112,2 mm Hg; cDBP: 76,0 vs 74,2 mm Hg), natomiast w grupie kontrolnej nie obserwowano hipotensyjnego efektu wysiłku, a odnotowano nawet niewielki, ale istotny statystycznie, wzrost 24-godzinowego skurczowego ciśnienia tętniczego. Zaobserwowano również liczne istotne statystycznie ( $p < 0,05$ ) dodatnie korelacje ekspresji mikroRNA-16, -21, -27a i -145 z parametrami ciśnienia tętniczego mogące wskazywać na ich udział w mechanizmach związanych z rozwojem NTP. Ekspresja mikroRNA-16 korelowała z wartościami Z-score skurczowego i rozkurczowego ciśnienia tętniczego (odpowiednio  $r = 0,268$  i  $r = 0,279$ ), a także z wartościami centralnego ciśnienia skurczowego, rozkurczowego i średniego (odpowiednio  $r = 0,295$ ,  $r = 0,296$  i  $r = 0,303$ ). Ekspresja mikroRNA-21 korelowała dodatnio z 24-godzinnymi wartościami SBP Z-score, DBP Z-score i MAP Z-score (odpowiednio  $r = 0,337$ ,  $r = 0,409$  i  $r = 0,373$ ). Najsilniejsze zależności z parametrami ABPM wykazano jednak dla mikroRNA-145, którego ekspresja korelowała z 24-godzinnymi wartościami SBP Z-score, DBP Z-score i MAP Z-score (odpowiednio  $r = 0,414$ ,  $r = 0,511$  i  $r = 0,478$ ). Ponadto analiza wieloczynnikowa wykazała, że wyjściowa ekspresja mikroRNA-16 była jedynym niezależnym czynnikiem związanym z wyższymi wartościami 24-godzinnego rozkurczowego ciśnienia tętniczego (DBP Z-score  $\geq 2,1$ ). Analiza ROC wykazała umiarkowaną wartość predykcyjną wyjściowej ekspresji mikroRNA-16 w identyfikacji pacjentów z DBP Z-score  $\geq 2,1$  (AUC = 0,770;  $p = 0,003$ ), przy czułości 79% i swoistości 64%. Dodatkowo analiza ROC również wykazała, że mikroRNA-27b charakteryzowało się najlepszą zdolnością różnicowania dzieci z nadciśnieniem tętniczym od zdrowych rówieśników, co może wskazywać na jego potencjalną przydatność jako biomarkera choroby. Nie stwierdzono natomiast istotnych zależności pomiędzy analizowanymi mikroRNA a klasycznymi wykładnikami nadciśnieniowego uszkodzenia narządowego.

W trzeciej publikacji oceniono zależności pomiędzy ekspresją wybranych mikroRNA a parametrami deformacji mięśnia sercowego ocenianymi metodą echokardiografii śledzenia markerów akustycznych (ang. *speckle-tracking echocardiography* – STE). Do analizy

włączono 50 dzieci z nadciśnieniem tętniczym pierwotnym oraz 57 zdrowych rówieśników. Wykazano, że dzieci z nadciśnieniem cechują się istotnie większymi (mniej ujemnymi) wartościami globalnego odkształcenia podłużnego lewej komory (GLS:  $-19.28\% \pm 3.02$  vs.  $-20.99\% \pm 3.11$ ), pomimo zachowanej frakcji wyrzutowej, co może świadczyć o obecności wczesnej subklinicznej dysfunkcji skurczowej mięśnia sercowego. Ponadto stwierdzono obniżone wartości prędkości wczesnorozkurczowego ruchu pierścienia mitralnego (E') oraz wydłużenie czasu relaksacji izowolumetrycznej (IVRT) u dzieci z NTP, sugerujące współistnienie wczesnych zaburzeń funkcji rozkurczowej w tej grupie pacjentów. Wykazano również istotne zależności pomiędzy ekspresją mikroRNA-16 ( $r=0,305$ ,  $p=0,031$ ) i mikroRNA-27b ( $r=0,330$ ,  $p<0,001$ ) a wartościami GLS. W analizie wieloczynnikowej ekspresja mikroRNA-16 była niezależnym czynnikiem związanym z wyższymi wartościami GLS. Uzyskane wyniki sugerują, że ocena GLS może stanowić wartościowe uzupełnienie klasycznych parametrów echokardiograficznych w identyfikacji wczesnych, subklinicznych zaburzeń funkcji mięśnia sercowego u dzieci z nadciśnieniem tętniczym pierwotnym.

Czwarta publikacja miała charakter pracy przeglądowej i stanowiła podsumowanie aktualnej wiedzy dotyczącej roli mikroRNA w patogenezie nadciśnienia tętniczego pierwotnego u dzieci. Szczególną uwagę poświęcono cząsteczkom analizowanym w badaniach własnych: mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a oraz mikroRNA-145. Omówiono ich potencjalny udział w regulacji aktywności układu renina–angiotensyna–aldosteron, funkcji śródbłonna, przebudowie naczyń, rozwoju przerostu mięśnia sercowego oraz nadciśnieniowego uszkodzenia narządowego. Istotnym elementem pracy była również analiza ograniczeń badań nad mikroRNA, które mogą wpływać na interpretację uzyskiwanych wyników oraz utrudniać porównywanie rezultatów między poszczególnymi badaniami. Omówiono między innymi wpływ rodzaju analizowanego materiału biologicznego (osocze, surowica, tkanki lub komórki), obecności mikroRNA w różnych frakcjach biologicznych (wolno krążących, związanych z białkami lub transportowanych w pęcherzykach zewnątrzkomórkowych) oraz znaczenie zmienności przedanalizacyjnej związanej z pobieraniem, przetwarzaniem i przechowywaniem próbek.

Zwrócono również uwagę na brak standaryzacji metod izolacji i oznaczania mikroRNA, wynikający ze stosowania różnych zestawów odczynników, platform analitycznych oraz metod normalizacji wyników. Omówiono znaczenie walidacji metod laboratoryjnych, w tym oceny wydajności amplifikacji, powtarzalności oznaczeń (ang. *intra- i inter-assay variability*) oraz wpływu potencjalnych inhibitorów reakcji PCR. Przedstawiono również problem hemolizy, która może prowadzić do sztucznego zawyżania stężenia niektórych krążących mikroRNA.

Podkreślono ponadto, że większość mikroRNA wykazuje działanie plejotropowe i uczestniczy jednocześnie w regulacji wielu procesów biologicznych oraz w rozwoju różnych chorób. Ogranicza to ich swoistość jako biomarkerów nadciśnienia tętniczego i utrudnia jednoznaczną interpretację obserwowanych zmian ekspresji. Wskazano również, że ekspresja mikroRNA może zależeć od wieku, płci, pochodzenia etnicznego, współistniejących chorób, stosowanych leków oraz innych czynników środowiskowych.

W pracy omówiono także możliwe kierunki dalszych badań, podkreślając znaczenie stosowania ujednoliconych procedur laboratoryjnych, walidacji wyników w niezależnych kohortach oraz prowadzenia badań w dobrze scharakteryzowanych populacjach pacjentów, szczególnie w wieku rozwojowym.

Łącznie uzyskane wyniki wskazują, że wybrane mikroRNA mogą odgrywać istotną rolę we wczesnych etapach rozwoju nadciśnienia tętniczego pierwotnego u dzieci. Szczególne znaczenie mogą mieć mikroRNA-16, mikroRNA-27b oraz mikroRNA-133a, których ekspresja była związana z obecnością nadciśnienia tętniczego, odpowiedzią na wysiłek fizyczny lub wczesnymi wykładnikami przebudowy sercowo-naczyniowej. Brak istotnych zależności pomiędzy ekspresją badanych mikroRNA a klasycznymi wykładnikami nadciśnieniowego uszkodzenia narządowego oraz aktywnością układu renina–angiotensyna–aldosteron w głównym badaniu może wskazywać, że analizowane cząsteczki odzwierciedlają wcześniejsze etapy rozwoju choroby, poprzedzające wystąpienie utrwalonych zmian narządowych. Uzyskane wyniki uzasadniają potrzebę dalszych badań nad wykorzystaniem mikroRNA jako potencjalnych biomarkerów nadciśnienia tętniczego pierwotnego w wieku rozwojowym.

Należy także podkreślić kilka ograniczeń przedstawionego cyklu prac. Po pierwsze, badania przeprowadzono w jednym ośrodku, na grupie nie zróżnicowanych etnicznie chorych w podobnym wieku, co może ograniczać możliwość uogólniania uzyskanych wyników na całą populację dzieci z NTP. Po drugie, część analiz miała charakter przekrojowy, co uniemożliwia jednoznaczne określenie zależności przyczynowo-skutkowych między badanymi markerami a rozwojem nadciśnienia tętniczego oraz jego narządowymi powikłaniami.

Istotnym ograniczeniem badań dotyczących mikroRNA był brak długoterminowej obserwacji klinicznej pozwalającej ocenić ich wartość prognostyczną oraz możliwość przewidywania progresji choroby i rozwoju nadciśnieniowego uszkodzenia narządowego. Ponadto analiza obejmowała wybrane mikroRNA wskazane na podstawie dostępnych danych literaturowych, a nie pełne profilowanie transkryptomiczne.

W badaniach oceniających funkcję układu sercowo-naczyniowego zastosowano nowoczesne metody obrazowania i oceny hemodynamicznej; część analiz miała charakter

eksploracyjny, a liczba ocenianych parametrów była duża w stosunku do liczebności grup – obliczonej na zmiany w obrębie mikroRNA. Ponadto nie wszystkie potencjalne czynniki zakłócające, takie jak poziom aktywności fizycznej przed badaniem, nawyki żywieniowe, w tym spożycie sodu, czy status socjoekonomiczny, mogły zostać uwzględnione w analizach statystycznych.

Wreszcie, badania prowadzono w okresie następującym bezpośrednio po pandemii COVID-19, która mogła wpływać na częstość występowania otyłości, poziom aktywności fizycznej oraz inne czynniki ryzyka sercowo-naczyniowego w populacji pediatrycznej.

Pomimo wymienionych ograniczeń przedstawione prace dostarczają nowych danych dotyczących roli mikroRNA oraz wczesnych zmian sercowo-naczyniowych w patogenezie nadciśnienia tętniczego pierwotnego u dzieci i młodzieży. Wskazują również na korzystny wpływ pojedynczej sesji wysiłku fizycznego na wartości ciśnienia tętniczego, zarówno obwodowego, jak i centralnego u dzieci z NTP. Ze względu na ich charakter uzyskane wyniki należy interpretować przede wszystkim jako generujące hipotezy badawcze (ang. *hypothesis-generating*), a nie jako dowód istnienia zależności przyczynowo-skutkowych. Potwierdzenie obserwowanych zależności wymaga dalszych badań prowadzonych w większych, wieloośrodkowych kohortach oraz z wykorzystaniem długoterminowej obserwacji pacjentów. Wskazane są także badania profilowe pozwalające na wytypowanie innych obiecujących cząsteczek mikroRNA, które mogłyby posłużyć jako biomarkery w tej grupie chorych.

## 9. Wnioski

1. Dzieci z nadciśnieniem tętniczym pierwotnym cechują się odmiennym profilem ekspresji wybranych krążących mikroRNA w porównaniu ze zdrowymi rówieśnikami, co może wskazywać na udział mechanizmów epigenetycznych w patogenezie nadciśnienia tętniczego w wieku rozwojowym. Ekspresja mikroRNA-27b oraz mikroRNA-133a jest obniżona u dzieci z nadciśnieniem tętniczym pierwotnym.
2. U nieleczonych dzieci z nadciśnieniem tętniczym pierwotnym ekspresja mikroRNA-16, mikroRNA-21, mikroRNA-27a i mikroRNA-145 koreluje dodatnio z wartościami ciśnienia tętniczego, co może sugerować udział tych cząstek w patogenezie nadciśnienia tętniczego pierwotnego. Wysoka ekspresja mikroRNA-16 jest związana z wyższymi wartościami ciśnienia tętniczego rozkurczowego i może stanowić potencjalny biomarker ciężkości nadciśnienia u dzieci.
3. U dzieci z nadciśnieniem tętniczym pierwotnym brak jest korelacji pomiędzy klasycznymi parametrami uszkodzenia narządowego a ekspresją cząsteczek mikroRNA-16, mikroRNA-21, mikroRNA-27a, mikroRNA-27b, mikroRNA-133a i mikroRNA-145.
4. MikroRNA-16 może stanowić potencjalny biomarker wczesnego uszkodzenia skurczowego lewej komory serca, co może wskazywać na udział tej cząsteczki we wczesnych etapach przebudowy układu sercowo-naczyniowego u dzieci z nadciśnieniem tętniczym pierwotnym.
5. Pojedyncza sesja standaryzowanego wysiłku aerobowego prowadzi, inaczej niż u zdrowych rówieśników, do istotnego obniżenia wartości ciśnienia tętniczego u dzieci z nadciśnieniem tętniczym pierwotnym oraz wpływa na ekspresję wybranych mikroRNA, co podkreśla potencjalne znaczenie aktywności fizycznej w modulacji mechanizmów związanych z rozwojem nadciśnienia tętniczego w wieku rozwojowym.

## 10. Zgoda Komisji Bioetycznej



### Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym

Tel.: 022/ 57 - 20 -303

Fax: 022/ 57 - 20 -165

ul. Żwirki i Wigury nr 61

02-091 Warszawa

e-mail: komisja.bioetyczna@wum.edu.pl

www.komisja-bioetyczna.wum.edu.pl

KB/<sup>154</sup>...../2020

Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym  
w dniu 14 września 2020 r. po zapoznaniu się z wnioskiem:

**Dr hab. n. med. Małgorzata Pańczyk-Tomaszewska**  
**Katedra i Klinika Pediatrii i Nefrologii,**  
**ul. Żwirki i Wigury 63a, 02-091 Warszawa**

**dotyczącym:** wyrażenia opinii w sprawie badania pt.: „Patogeneza nadciśnienia tętniczego-mikroRNA jako brakujące ogniwo w rozwoju nadciśnienia tętniczego u dzieci.”

#### wyraża następującą o p i n i ę

- stwierdza, że jest ono dopuszczalne i zgodne z zasadami naukowo-etycznymi\*.
- ~~stwierdza, że jest ono niedopuszczalne i niezgodne z zasadami naukowo-etycznymi.\*~~

#### Uwagi Komisji – *verte*

Komisja działa na podstawie art.29 ustawy z dnia 5.12.1996r. o zawodzie lekarza /Dz.U.nr 28/97 poz.152 wraz z późn.zm./, zarządzenia MZiOS z dn.11.05.1999r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych /Dz.U.nr 47 poz.480/, Ustawy prawo farmaceutyczne z dnia 6 września 2001r. (Dz.U.Nr 126, poz. 1381 z późn. zm.) oraz Zarządzenie nr 56/2007 z dnia 15 października 2007r. w sprawie działania Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym /Regulamin Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym/.  
Komisja działa zgodnie z zasadami GCP .

\*niepotrzebne skreślić

strona podpisowa do uchwały Komisji Bioetycznej przy Warszawskim  
Uniwersytecie Medycznym nr KB/.....159..... z dnia 14 września 2020r.

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## **11. Finansowanie badań**

Projekt będący podstawą niniejszej rozprawy doktorskiej był realizowany dzięki finansowaniu uzyskanemu zarówno w ramach Grantu Młodego Badacza Warszawskiego Uniwersytetu Medycznego (nr 1WI/1/M/MBM/N/21), który umożliwił przeprowadzenie badania pilotażowego (Publikacja 1), jak i grantu zewnętrznego Narodowego Centrum Nauki przyznanego w konkursie PRELUDIUM 20 (nr 2021/41/N/NZ5/04194), finansującego realizację głównego projektu badawczego (Publikacje 2 i 3).

## **12. Oświadczenia współautorów publikacji**

WARSZAWA, 07.07.2026  
(miejscowość, data)

Piotr Skrzypczyk

## OŚWIADCZENIE

Jako współautor pracy pt *MicroRNA-133a and MicroRNA-145 May Be Involved in the Development of Hypertension-Mediated Organ Damage in Children with Primary Hypertension—A Preliminary Study* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, walidację uzyskanych wyników, współudział w analizie formalnej danych, realizację części badawczej projektu, zapewnienie zasobów niezbędnych do przeprowadzenia badań, przygotowanie i redakcję manuskryptu, nadzór merytoryczny nad realizacją projektu oraz współudział w pozyskaniu finansowania.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz koordynację prac związanych z przygotowaniem publikacji. Ponadto lek. Michał Szyszka uczestniczył w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Szyszki.

(podpis oświadczającego)

Ceren Eyiletlen

## OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w opracowaniu metodyki badania, przygotowaniu i walidacji zastosowanych narzędzi analitycznych, przeprowadzeniu analizy formalnej, udział w przygotowaniu materiałów graficznych przedstawiających wyniki badań, przygotowaniu i redakcji manuskryptu, sprawowaniu nadzoru merytorycznego.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Szyszki.

.....  
(podpis oświadczającego)

.....  
(miejscowość, data)

Jolanta Małyszko

## OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował: udział w interpretacji wyników, przygotowanie i redakcję manuskryptu, krytyczną analizę jego treści, koordynację procesu publikacyjnego oraz sprawowanie merytorycznego nadzoru nad przygotowaniem publikacji.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Szyszki.

.....

Warszawa, 08.06.2016  
(miejscowość, data)

Karolina Skubisz

## OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował współudział opracowaniu metodologii badań, udział w przygotowaniu i przeprowadzeniu analiz ekspresji mikroRNA metodą qPCR, opracowywanie wyników badań molekularnych, przygotowanie danych do dalszych analiz oraz udział w redakcji manuskryptu.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Szyszki.

(podpis oświadczającego)

Radosław Pietrzak

## OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował udział w realizacji części badawczej projektu, wykonywanie i opracowywanie badań echokardiograficznych wykorzystanych w analizie, interpretację wyników badań kardiologicznych oraz udział w redakcji i korekcie manuskryptu.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

(podpis oświadczającego)

.....  
(miejscowość, data)

Dr hab. n. med. Hubert Krysztofiak  
Instytut Medycyny Doświadczalnej i Klinicznej  
im. M. Mossakowskiego PAN

## OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował: udział w opracowaniu metodyki badania związanej z oceną wydolności fizycznej i protokołem wysiłkowym zastosowanym w badaniu, zapewnienie zasobów niezbędnych do realizacji projektu, konsultacje merytoryczne dotyczące interpretacji wyników oraz udział w redakcji i korekcie manuskryptu..

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

.....  
(podpis oświadczającego)

Warszawa, 07.07.2026  
(miejscowość, data)

Piotr Skrzypczyk

### OŚWIADCZENIE

Jako współautor pracy pt *ATHENA Study – The Role of Circulating Plasma MicroRNAs in Pediatric Patients with Untreated Primary Hypertension: Baseline Expression and Exercise-Induced Changes* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w opracowaniu metodyki badania, przygotowaniu i walidacji zastosowanych narzędzi analitycznych, przeprowadzeniu analizy formalnej, zapewnieniu zasobów niezbędnych do realizacji projektu, przygotowaniu i redakcji manuskryptu, sprawowaniu nadzoru merytorycznego nad projektem, koordynacji jego realizacji oraz współudział w pozyskaniu finansowania.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował opracowanie koncepcji pracy, współudział w przygotowaniu metodyki badania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, przygotowanie pierwszej wersji manuskryptu, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych oraz udział w pozyskaniu finansowania projektu. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Michała Szyszki.

  
(podpis oświadczającego)

Radosław Pietrzak

## OŚWIADCZENIE

Jako współautor pracy pt *Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował: udział w opracowaniu metodyki badania, przygotowanie narzędzi wykorzystywanych do analizy danych, walidację wyników, realizację części badawczej projektu, udział w opracowaniu i interpretacji wyników badań echokardiograficznych, udział w redakcji i korekcie manuskryptu oraz sprawowanie nadzoru merytorycznego nad realizacją projektu.

Wkład lek. Michała Szyski w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie i obsługę narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, interpretację wyników, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych, koordynację prac związanych z przygotowaniem publikacji oraz współudział w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyski.

(podpis oświadczającego)

Warszawa 05.06.2016  
(miejscowość, data)

Klaudia Obsznajczyk

## OŚWIADCZENIE

Jako współautor pracy pt *Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował udział w realizacji części badawczej projektu, wykonywanie i opracowywanie wykonanych badań echokardiograficznych wykorzystanych w analizie, konsultacje merytoryczne dotyczące interpretacji wyników echokardiograficznych oraz gromadzenie danych wykorzystanych w analizie i przygotowanie manuskryptu.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie i obsługę narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, interpretację wyników, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych, koordynację prac związanych z przygotowaniem publikacji oraz współudział w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

(podpis oświadczającego)

Warszawa, 08.06.2026  
(miejscowość, data)

Karolina Skubisz

## OŚWIADCZENIE

Jako współautor pracy pt *Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował współudział opracowaniu metodologii badań, udział w przygotowaniu i przeprowadzeniu analiz ekspresji mikroRNA metodą qPCR, opracowywanie wyników badań molekularnych, przygotowanie danych do dalszych analiz oraz udział w redakcji manuskryptu.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie i obsługę narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, interpretację wyników, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych, koordynację prac związanych z przygotowaniem publikacji oraz współudział w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

(podpis oświadczającego)

Ceren Eyiletlen

## OŚWIADCZENIE

Jako współautor pracy pt *Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował udział w opracowaniu metodyki badań molekularnych, nadzór merytoryczny nad analizami ekspresji mikroRNA metodą qPCR, analizę formalną i interpretację uzyskanych wyników, oraz współudział w przygotowaniu i redakcji manuskryptu.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie i obsługę narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, interpretację wyników, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych, koordynację prac związanych z przygotowaniem publikacji oraz współudział w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

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(podpis oświadczającego)

WARSZAWA, 07.07.2026  
(miejscowość, data)

Piotr Skrzypczyk

## OŚWIADCZENIE

Jako współautor pracy pt *Circulating miR-16 as an Early Biomarker of Subclinical Myocardial Strain Impairment in Pediatric Primary Hypertension* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, walidację wyników, współudział w analizie formalnej danych, realizację części badawczej projektu, zapewnienie zasobów niezbędnych do przeprowadzenia badań, interpretację wyników, przygotowanie i redakcję manuskryptu, sprawowanie nadzoru merytorycznego nad realizacją projektu oraz współudział w pozyskaniu finansowania.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował współtworzenie koncepcji badania, udział w opracowaniu metodyki, przygotowanie i obsługę narzędzi wykorzystywanych do analizy danych, gromadzenie i opracowanie danych badawczych, przeprowadzenie analiz statystycznych, realizację części badawczej projektu, interpretację wyników, przygotowanie pierwszej wersji manuskryptu, udział w redakcji i korekcie publikacji, opracowanie materiałów graficznych, koordynację prac związanych z przygotowaniem publikacji oraz współudział w pozyskaniu finansowania niezbędnego do realizacji projektu.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

(podpis oświadczającego)

Piotr Skrzypczyk

## OŚWIADCZENIE

Jako współautor pracy pt *MicroRNAs in Pediatric Primary Hypertension: Promising Biomarkers or Context-Dependent Regulators?—A Narrative Review* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: udział w opracowaniu metodyki badania, przygotowanie i walidacja zastosowanych narzędzi analitycznych, przeprowadzenie analizy formalnej, zapewnienie zasobów niezbędnych do realizacji projektu, przygotowanie i redakcja manuskryptu, sprawowanie nadzoru merytorycznego nad projektem oraz koordynacja jego realizacji.

Wkład lek. Michała Szyszki w powstawanie publikacji obejmował: opracowanie koncepcji pracy, współudział w przygotowaniu metodyki opracowania, opracowanie i obsługę narzędzi wykorzystywanych w analizie danych, współudział w redakcji i korekcie publikacji, opracowanie materiałów graficznych. Ponadto lek. Michał Szyszka brał aktywny udział w koordynacji prac związanych z przygotowaniem publikacji i odpowiadał za przygotowanie jej ostatecznej wersji od strony technicznej i redakcyjnej.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek Michała Szyszki.

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(podpis oświadczającego)

## 13. Piśmiennictwo

1. Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. *J Hypertens*. 2016;34(10):1887-920.
2. Olszanecka A, Chrostowska M, Litwin M, Obrycki Ł, Ostalska-Nowicka D, Niklas A, et al. Nadciśnienie tętnicze u młodych dorosłych. Stanowisko Polskiego Towarzystwa Nadciśnienia Tętniczego. *Nadciśnienie Tętnicze w Praktyce*. 2022;8(3):109-38.
3. Tykarski A, Filipiak KJ, Januszewicz A, Litwin M, Narkiewicz K, Prejbisz A, et al. Zasady postępowania w nadciśnieniu tętniczym — 2019 rok. Wytyczne Polskiego Towarzystwa Nadciśnienia Tętniczego. *Nadciśnienie Tętnicze w Praktyce*. 2019;5(1):1-86.
4. Lurbe E, Mancia G, Calpe J, Drożdż D, Erdine S, Fernandez-Aranda F, et al. Joint statement for assessing and managing high blood pressure in children and adolescents: Chapter 1. How to correctly measure blood pressure in children and adolescents. *Front Pediatr*. 2023;11:1140357.
5. Niklas A, Flotyńska A, Puch-Walczak A, Polakowska M, Topór-Mądry R, Polak M, et al. Prevalence, awareness, treatment and control of hypertension in the adult Polish population - Multi-center National Population Health Examination Surveys - WOBASZ studies. *Arch Med Sci*. 2018;14(5):951-61.
6. Song P, Zhang Y, Yu J, Zha M, Zhu Y, Rahimi K, et al. Global Prevalence of Hypertension in Children: A Systematic Review and Meta-analysis. *JAMA Pediatr*. 2019;173(12):1154-63.
7. Symonides B, Jędrusik P, Artyszuk L, Gryboś A, Dziliński P, Gaciong Z. Different diagnostic criteria significantly affect the rates of hypertension in 18-year-old high school students. *Arch Med Sci*. 2010;6(5):689-94.
8. Obrycki Ł, Skoczyński K, Sikorski M, Koziej J, Mitoraj K, Pilip J, et al. Current etiology of hypertension in European children - factors associated with primary hypertension. *Pediatr Nephrol*. 2025;40(10):3233-44.
9. Gupta-Malhotra M, Banker A, Shete S, Hashmi SS, Tyson JE, Barratt MS, et al. Essential hypertension vs. secondary hypertension among children. *Am J Hypertens*. 2015;28(1):73-80.
10. Litwin M, Feber J. Origins of Primary Hypertension in Children. *Hypertension*. 2020;76(5):1400-9.
11. Litwin M, Kułaga Z. Obesity, metabolic syndrome, and primary hypertension. *Pediatr Nephrol*. 2021;36(4):825-37.
12. Chen X, Wang Y. Tracking of blood pressure from childhood to adulthood: a systematic

review and meta-regression analysis. *Circulation*. 2008;117:3171-80.

13. Tirosh A, Afek A, Rudich A, Percik R, Gordon B, Ayalon N. Progression of normotensive adolescents to hypertensive adults: a study of 26,980 teenagers. *Hypertension*. 2010;56:203-9.
14. Litwin M. Pathophysiology of primary hypertension in children and adolescents. *Pediatr Nephrol*. 2024;39(6):1725-37.
15. Wójcik M, Koziół-Kozakowska A. Obesity, Sodium Homeostasis, and Arterial Hypertension in Children and Adolescents. *Nutrients*. 2021;13(11):4032.
16. Rossi GP, Bagordo D, Rossi FB, Pintus G, Rossitto G, Seccia TM. 'Essential' arterial hypertension: time for a paradigm change. *J Hypertens*. 2024;42(8):1298-304.
17. Vogt L, Cunha V, Dominiczak AF, Grassi G, Rajzer M, Virdis A, et al. Is It Time to Abandon the Kidney-Centered View on the Origin of Primary Hypertension? *Hypertension*. 2025;82(10):1590-8.
18. He L, Liu Y, Widlansky ME, Kriegel AJ, Qiu Q, Liang M. microRNA and Hypertension. *Hypertension*. 2025;82(2):181-4.
19. Kostiniuk D, Marttila S, Raitoharju E. Circulatory miRNAs in essential hypertension. *Atherosclerosis*. 2025;401:119069.
20. Poller W, Dimmeler S, Heymans S, Zeller T, Haas J, Karakas M, et al. Non-coding RNAs in cardiovascular diseases: diagnostic and therapeutic perspectives. *Eur Heart J*. 2018;39(29):2704-16.
21. de Gonzalo-Calvo D, Vea A, Bär C, Fiedler J, Couch LS, Brotons C, et al. Circulating non-coding RNAs in biomarker-guided cardiovascular therapy: a novel tool for personalized medicine? *Eur Heart J*. 2019;40(20):1643-50.
22. Improta Caria AC, Nonaka CKV, Pereira CS, Soares MBP, Macambira SG, Souza BSF. Exercise Training-Induced Changes in MicroRNAs: Beneficial Regulatory Effects in Hypertension, Type 2 Diabetes, and Obesity. *Int J Mol Sci*. 2018;19(11):3607.
23. Yan L, Liang M, Hou X, Zhang Y, Zhang H, Guo Z, et al. The role of microRNA-16 in the pathogenesis of autoimmune diseases: A comprehensive review. *Biomed Pharmacother*. 2019;112:108583.
24. Li N, Yang L, Sun Y, Wu X. MicroRNA-16 inhibits migration and invasion via regulation of the Wnt/ $\beta$ -catenin signaling pathway in ovarian cancer. *Oncol Lett*. 2019;17(3):2631-8.
25. Gu Q, Zhao G, Wang Y, Xu B, Yue J. Silencing miR-16 Expression Promotes Angiotensin II Stimulated Vascular Smooth Muscle Cell Growth. *Cell Dev Biol*. 2017;6(1):173.
26. He L, Huang C. MiR-19b and miR-16 cooperatively signaling target the regulator ADRA1A in Hypertensive heart disease. *Biomed Pharmacother*. 2017;91:1178-83.
27. Nandakumar P, Tin A, Grove ML, Ma J, Boerwinkle E, Coresh J, et al. MicroRNAs in the

- miR-17 and miR-15 families are downregulated in chronic kidney disease with hypertension. *PLoS One*. 2017;12(8):e0176734.
28. He F, Guan W. The role of miR-21 as a biomarker and therapeutic target in cardiovascular disease. *Clinica Chimica Acta*. 2025;574:120304.
  29. Yildirim E, Ermis E, Allahverdiyev S, Ucar H, Yavuzer S, Cengiz M. Circulating miR-21 levels in hypertensive patients with asymptomatic organ damage. *Medicine (Baltimore)*. 2019;98(39):e17297.
  30. Rhim J, Baek W, Seo Y, Kim JH. From Molecular Mechanisms to Therapeutics: Understanding MicroRNA-21 in Cancer. *Cells*. 2022;11(18):2791.
  31. Adamcova M, Kawano I, Simko F. The Impact of microRNAs in Renin-Angiotensin-System-Induced Cardiac Remodelling. *Int J Mol Sci*. 2021;22(9):4762.
  32. Parthenakis F, Marketou M, Kontaraki J, Patrianakos A, Nakou H, Touloupaki M, et al. Low Levels of MicroRNA-21 Are a Marker of Reduced Arterial Stiffness in Well-Controlled Hypertension. *J Clin Hypertens (Greenwich)*. 2017;19(3):235-40.
  33. Raitoharju E, Lyytikäinen LP, Levula M, Oksala N, Mennander A, Tarkka M, et al. miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. *Atherosclerosis*. 2011;219(1):211-7.
  34. Kara SP, Ozkan G, Yılmaz A, Bayrakçı N, Güzel S, Geyik E. MicroRNA 21 and microRNA 155 levels in resistant hypertension, and their relationships with aldosterone. *Ren Fail*. 2021;43(1):676-83.
  35. Błaszczyk R, Biskupski M, Wiśniowski Ł, Kondracki B, Siwiec J, Mlak R, et al. Non-coding RNA biomarkers in resistant hypertension: a scoping review. *Front Mol Biosci*. 2026;13:1786399.
  36. Xin C, He W, Sun X, Li M, Wang H. The Association of Micro-RNA 21 and Hypertension: A Meta-Analysis. *Glob Heart*. 2024;19(1):41.
  37. Ding L, Ni J, Yang F, Huang L, Deng H, Wu Y, et al. Promising therapeutic role of miR-27b in tumor. *Tumour Biol*. 2017;39(3):1010428317691657.
  38. Goyal R, Goyal D, Leitzke A, Gheorghe CP, Longo LD. Brain renin-angiotensin system: fetal epigenetic programming by maternal protein restriction during pregnancy. *Reprod Sci*. 2010;17(3):227-38.
  39. Yeh CH, Chen TP, Wang YC, Lin YM, Fang SW. MicroRNA-27a regulates cardiomyocytic apoptosis during cardioplegia-induced cardiac arrest by targeting interleukin 10-related pathways. *Shock*. 2012;38(6):607-14.
  40. Gu Q, Wang B, Zhang XF, Ma YP, Liu JD, Wang XZ. Contribution of renin-angiotensin system to exercise-induced attenuation of aortic remodeling and improvement of endothelial

- function in spontaneously hypertensive rats. *Cardiovasc Pathol*. 2014;23(5):298-305.
41. Suzuki K, Yamada H, Fujii R, Munetsuna E, Yamazaki M, Ando Y, et al. Circulating microRNA-27a and -133a are negatively associated with incident hypertension: a five-year longitudinal population-based study. *Biomarkers*. 2022;27(5):496-502.
  42. Keshavarz R, Aghaee-Bakhtiari SH, Pakzad P, Banach M, Sahebkar A. Evaluation of miRNA-27a/b expression in patients with familial hypercholesterolemia. *Arch Med Sci*. 2024;20(4):1314-20.
  43. Qun L, Wenda X, Weihong S, Jianyang M, Wei C, Fangzhou L, et al. miRNA-27b modulates endothelial cell angiogenesis by directly targeting Naa15 in atherogenesis. *Atherosclerosis*. 2016;254:184-92.
  44. Fu Q, Lu Z, Fu X, Ma S, Lu X. MicroRNA 27b promotes cardiac fibrosis by targeting the FBW7/Snail pathway. *Aging (Albany NY)*. 2019;11(24):11865-79.
  45. Wang Y, Cai H, Li H, Gao Z, Song K. Atrial overexpression of microRNA-27b attenuates angiotensin II-induced atrial fibrosis and fibrillation by targeting ALK5. *Hum Cell*. 2018;31(3):251-60.
  46. Anwar A, Khan MJ. Pathogenic and clinical significance of inflammation-associated MicroRNAs in cardiovascular diseases. *Mol Biol Rep*. 2025;52(1):940.
  47. Hao S, Salzo J, Zhao H, Hao M, Darzynkiewicz Z, Ferreri NR. MicroRNA-133a-Dependent Inhibition of Proximal Tubule Angiotensinogen by Renal TNF (Tumor Necrosis Factor). *Hypertension*. 2020;76(6):1744-52.
  48. Wang J, Liew OW, Richards AM, Chen YT. Overview of MicroRNAs in Cardiac Hypertrophy, Fibrosis, and Apoptosis. *Int J Mol Sci*. 2016;17(5):745.
  49. Kontaraki JE, Marketou ME, Parthenakis FI, Maragkoudakis S, Zacharis EA, Petousis S, et al. Hypertrophic and antihypertrophic microRNA levels in peripheral blood mononuclear cells and their relationship to left ventricular hypertrophy in patients with essential hypertension. *J Am Soc Hypertens*. 2015;9(10):802-10.
  50. Koval SM, Snihurska IO, Yushko KO, Mysnychenko OV, Penkova MY, Lytvynova OM, et al. Circulating microRNA-133a in Patients With Arterial Hypertension, Hypertensive Heart Disease, and Left Ventricular Diastolic Dysfunction. *Front Cardiovasc Med*. 2020;7:104.
  51. Gomes CP, Oliveira GP, Jr., Madrid B, Almeida JA, Franco OL, Pereira RW. Circulating miR-1, miR-133a, and miR-206 levels are increased after a half-marathon run. *Biomarkers*. 2014;19(7):585-9.
  52. Sawant D, Lilly B. MicroRNA-145 targets in cancer and the cardiovascular system: evidence for common signaling pathways. *Vasc Biol*. 2020;2(1):R115-R28.
  53. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, et al. miR-145 and

- miR-143 regulate smooth muscle cell fate and plasticity. *Nature*. 2009;460(7256):705-10.
54. Özkan G, Ulusoy Ş, Geyik E, Erdem Y. Down-regulation of miRNA 145 and up-regulation of miRNA 4516 may be associated with primary hypertension. *J Clin Hypertens (Greenwich)*. 2019;21(11):1724-31.
55. Wang Y, Jin L. miRNA-145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp Ther Med*. 2018;15(1):548-52.
56. Cornelissen VA, Smart NA. Exercise training for blood pressure: a systematic review and meta-analysis. *J Am Heart Assoc*. 2013;2(1):e004473.
57. Farpour-Lambert NJ, Aggoun Y, Marchand LM, Martin XE, Herrmann FR, Beghetti M. Physical activity reduces systemic blood pressure and improves early markers of atherosclerosis in pre-pubertal obese children. *J Am Coll Cardiol*. 2009;54(25):2396-406.
58. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA. American College of Sports Medicine position stand. Exercise and hypertension. *Med Sci Sports Exerc*. 2004;36(3):533-53.
59. Halliwill JR, Buck TM, Lacewell AN, Romero SA. Postexercise hypotension and sustained postexercise vasodilatation: what happens after we exercise? *Exp Physiol*. 2013;98(1):7-18.
60. Alvarez-Pitti J, Herceg-Čavrak V, Wójcik M, Radovanović D, Brzeziński M, Grabitz C, et al. Blood pressure response to exercise in children and adolescents. *Front Cardiovasc Med*. 2022;9:1004508.
61. Baggish AL, Park J, Min PK, Isaacs S, Parker BA, Thompson PD, et al. Rapid upregulation and clearance of distinct circulating microRNAs after prolonged aerobic exercise. *J Appl Physiol (1985)*. 2014;116(5):522-31.
62. Desai AS, Webb DJ, Taubel J, Casey S, Cheng Y, Robbie GJ, et al. Zilebesiran, an RNA Interference Therapeutic Agent for Hypertension. *N Engl J Med*. 2023;389(3):228-38.
63. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee IM, et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc*. 2011;43(7):1334-59.