lek. Michał Konwerski

Epikardialna tkanka tłuszczowa a ryzyko chorób układu sercowo-naczyniowego: mechanizmy patofizjologiczne i implikacje kliniczne

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

Promotor: **dr hab. n. med. Tomasz Mazurek**

Promotor pomocniczy: **dr hab. n. med. Aleksandra Gąsecka-van der Pol**

I Katedra i Klinika Kardiologii Warszawskiego Uniwersytetu Medycznego

Kierownik Kliniki: prof. dr hab. n. med. Marcin Grabowski

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych
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SPIS TREŚCI

1. Wykaz skrótów

ADAMTS- *A Disintegrin-like And Metalloprotease with Thrombospondin motifs*

CABG- *coronary artery bypass grafting,* pomostowanie aortalno- wieńcowe

CXCL2- chemokina 2

CXCL8- chemokina 8

DEGs- *differentially expressed genes,* geny o zróżnicowanej ekspresji

EAT- *epicardial adipose tissue*, nasierdziowa tkanka tłuszczowa

ESCIT- *evolutionarily conserved signaling intermediate in toll pathway*

FC- *fold change*

GPZ- gałąź przednia zstępująca

GO- gałąź okalająca

HMGB2- *high mobility group box 2*

IPA- *Ingenuity Pathway Analysis*

LDL-C- *low density lipoprotein,* cholesterol frakcji lipoprotein niskiej gęstości

Non- HDL- C- *non high density lipoprotein,* cholesterol frakcji lipoprotein niewysokiej gęstości

PCAT- *pericoronary adipose tissue*, okołowieńcowa tkanka tłuszczowa

PDGF- *platelet-derived growth factor*, płytkopochodny czynnik wzrostu

PK- prawa komora

PTW- prawa tętnica wieńcowa

TFPI- t*issue factor pathway inhibitor*, inhibitor szlaku czynnika tkankowego

2. Streszczenie w języku polskim

Wprowadzenie:

Choroby układu sercowo-naczyniowego pozostają jedną z głównych przyczyn zgonów w skali globalnej. Do klasycznych i potencjalnie modyfikowalnych czynników ryzyka sercowo-naczyniowego należą nikotynizm, otyłość, niska aktywność fizyczna, zaburzenia lipidowe i nadciśnienie tętnicze. Coraz większą wagę przykłada się jednak również do nieklasycznych czynników ryzyka sercowo-naczyniowego, takiego jak tkanka tłuszczowa otaczająca narządy wewnętrzne.

Tkanka tłuszczowa otaczająca mięsień sercowy jest podtypem tłuszczu trzewnego, którego ilość koreluje z nasileniem otyłości brzusznej i jest niezależnie związana z ryzykiem zdarzeń sercowo-naczyniowych. Podobnie jak otyłość brzuszna, okołosercowa tkanka tłuszczowa stanowi jeden z czynników ryzyka rozwoju chorób sercowo-naczyniowych o podłożu miażdżycowym.

Klasyfikacja tkanki tłuszczowej otaczającej serce różni się między publikacjami. Z punktu widzenia niniejszej pracy, najważniejsze znaczenie ma tkanka tłuszczowa nasierdziowa (ang. *epicardial adipose tissue*, EAT) i okołowieńcowa (ang. *pericoronary adipose tissue*, PCAT). EAT leży pomiędzy trzewną blaszką osierdzia a mięśniem sercowym, natomiast jako PCAT definiuje się tłuszcz otaczający naczynia wieńcowe, niezależnie od jego lokalizacji, uznając go za część tłuszczu epikardialnego.

Tkanka tłuszczowa otaczająca serce jest bardzo aktywna metabolicznie, wydziela w bezpośrednim otoczeniu mięśnia sercowego liczne cytokiny i chemokiny. W warunkach zdrowia, EAT i PCAT odgrywają rolę ochronną, biorąc m.in. udział w procesach metabolicznych, energetycznych i przeciwzapalnych. W warunkach patofizjologicznych, np. u pacjentów z cukrzycą czy zespołem metabolicznym, właściwości ochronne zanikają, a dominować zaczynają procesy promujące stan zapalny i rozwój chorób układu krążenia, w tym choroby wieńcowej. Wydaje się, że nasierdziowa tkanka tłuszczowa może w przyszłości być interesującym celem dla nowych terapii.

Cel pracy:

Celem niniejszej rozprawy doktorskiej była (i) identyfikacja zależności pomiędzy ekstremalnie intensywnym wysiłkiem fizycznym a ilością i aktywnością zapalną EAT (ii) identyfikacja potencjalnych mechanizmów patofizjologicznych na poziomie ekspresji genów, mogących stanowić punkt uchwytu dla terapii celowanych w przyszłości, (iii) podsumowanie dotychczasowych danych na temat roli nasierdziowej tkanki tłuszczowej w patofizjologii chorób sercowo-naczyniowych.

Wyniki:

W pierwszej z prezentowanych prac oceniano wpływ ekstremalnie intensywnego wysiłku fizycznego na ilość EAT oraz jej korelację z czynnikami ryzyka choroby wieńcowej. Do badania włączono 30 zdrowych ultramaratończyków amatorów i 9 ochotników prowadzących siedzący tryb życia. Pole powierzchni EAT oceniano przy użyciu rezonansu magnetycznego w 4 lokalizacjach: dookoła 3 głównych tętnic wieńcowych (gałąź przednia zstępująca, gałąź okalająca, prawa tętnica wieńcowa) oraz na powierzchni prawej komory. Dodatkowo oceniano skład ciała, lipidogram, osoczowe stężenie interleukiny 6 oraz grubość kompleksu intima-media w tętnicach szyjnych uczestników badania. Ilość EAT w grupie ultramaratończyków była istotnie mniejsza we wszystkich badanych lokalizacjach, w porównaniu z grupą kontrolną ($p < 0.001$). Zgodnie z oczekiwaniami, ultramaratończycy mieli mniejszy odsetek tłuszczu trzewnego oraz lepszy profil lipidowy niż grupa kontrolna ($p < 0.001$). Nie zaobserwowano natomiast różnic w grubości kompleksu intima- media. Nie było także istotnej statystycznie różnicy w stężeniu interleukiny 6 pomiędzy grupami, jednak w grupie biegaczy częstość występowania patologicznie wysokiego stężenia interleukiny 6 (jako punkt odcięcia przyjęto stężenie > 1 pg/ml) była 3-krotnie niższa niż w grupie kontrolnej (17% vs. 56%, $p < 0.05$). Dodatkowo, w grupie ultramaratończyków uzyskano dodatnią korelację pomiędzy polem powierzchni tkanki tłuszczowej otaczającej gałąź przednią zstępującą, gałąź okalającą oraz prawą komorę a odsetkiem całkowitego tłuszczu trzewnego oraz pomiędzy ilością tłuszczu wokół gałęzi okalającej a stężeniem frakcji LDL i nie-HDL cholesterolu ($p < 0.05$).

W kolejnej pracy porównano ekspresję genów w PCAT u pacjentów z zaawansowaną chorobą wieńcową i w grupie kontrolnej. Próbki PCAT uzyskano w czasie operacji pomostowania aortalno-wieńcowego (CABG) (n = 21, grupa badana) lub kardiochirurgicznej operacji niewieńcowej u chorych z wcześniej wykluczoną chorobą wieńcową (n = 19, grupa kontrolna). Spośród 67 528 transkryptów, 1348 zostało zidentyfikowanych jako tzw. geny o zróżnicowanej ekspresji (ang. *differentially expressed genes,* DEGs). Spośród nich, 416 (30,9%) wykazywało nadekspresję, a 932 (69,1%) zaklasyfikowano do grupy o zmniejszonej ekspresji w porównaniu z grupą kontrolą. Wśród genów wykazujących zwiększoną ekspresję znalazły się m. in. te kodujące molekuły o działaniu prozapalnym i proaterogennym, takie jak chemokiny CXCL8, CXCL2, interleukina 6, selektyna E, receptor dla lipoprotein o niskiej gęstości, metaloproteinazy z grupy ADAMTS. Wśród genów o zmniejszonej ekspresji zidentyfikowano m.in. geny kodujące białka sygnałowe, enzymy, mikroRNA czy różne typy kolagenu.

Dodatkowo wyróżniono grupę tzw. „upstream regulators" związanych z genami o zróżnicowanej ekspresji. Termin ten odnosi się do dowolnej molekuły, która może wpływać na ekspresję, transkrypcję czy fosforylację innej cząsteczki. W tej niejednorodnej grupie uwagę zwracają m.in. geny kodujące płytkopochodny czynnik wzrostu, białko grupy 2 o wysokiej mobilności (ang. *high mobility group box 2*, HMGB2) czy ESCIT (ang. *evolutionarily conserved signaling intermediate in toll pathway*), które literatura wskazuje jako potencjalne cząsteczki prozapalne i promiażdżycowe. Co więcej, użyte oprogramowanie Ingenuity Pathway Analysis (IPA) powiązało geny o zróżnicowanej ekspresji w całe sieci powiązań i zależności, tzw. ścieżki kanoniczne i sieci. Wśród aktywowanych szlaków znalazła się m.in. „ścieżka układu krzepnięcia" zawierająca molekuły znane ze swojego promiażdżycowego i prozapalnego charakteru (inhibitor szlaku czynnika tkankowego, aktywator plazminogenu, receptor dla urokinazy, trombomodulina).

Trzecią pracą jest artykuł poglądowy poświęcony podsumowaniu aktualnego stanu wiedzy na temat udziału EAT w patogenezie chorób układu sercowo-naczyniowego, w tym choroby wieńcowej, niewydolności serca i migotania przedsionków. Zwrócono także uwagę na możliwy związek pomiędzy EAT a przebiegiem COVID-19. Na koniec zaprezentowano EAT jako potencjalny cel terapeutyczny w leczeniu chorób układu sercowo-naczyniowego w przyszłości.

Wnioski:

W pierwszym badaniu wykazano, że ekstremalnie intensywny trening fizyczny może obniżać ryzyko sercowo-naczyniowe poprzez redukcję ilości i prozapalnej aktywności nasierdziowej tkanki tłuszczowej, jednak potrzebne są dalsza badania potwierdzające zależności zidentyfikowane w niniejszej pracy doktorskiej.

Drugie badanie było jednym z pierwszych, w którym wykazano zmienioną ekspresję nie tylko pojedynczych genów, ale całych sieci i ścieżek z nich stworzonych w okołowieńcowej tkance tłuszczowej, co dostarcza kolejnych dowodów na to, że badana tkanka jest aktywnym źródłem promiażdżycowych molekuł mogących przyspieszać rozwój choroby wieńcowej.

Trzecia praca wskazuje ogromne zainteresowanie tematem EAT w ostatnich latach, sugerując jej potencjał jako nowego celu terapeutycznego w prewencji i leczeniu chorób układu sercowo-naczyniowego

Podsumowując, cykl prezentowanych prac demonstruje, że okołosercowa tkanka tłuszczowa jest jednym z najważniejszych elementów biorących udział w patogenezie chorób układu sercowo-naczyniowego, jednak kolejne badania na poziomie molekularnym są kluczowe, aby zidentyfikować konkretne geny i kodowane przez nie białka, których modulacja mogłaby zmienić niekorzystny fenotyp EAT u pacjentów z chorobami sercowo-naczyniowymi. Negatywny wpływ EAT na układ krążenia może być ograniczony już teraz poprzez interwencje niefarmakologiczne, a dzięki dalszemu rozwojowi badań na poziomie ekspresji genów, istnieje szansa identyfikacji nowych celów terapeutycznych w obrębie EAT.

3. Streszczenie w języku angielskim.

Epicardial adipose tissue and the risk of cardiovascular diseases: pathophysiological mechanisms and clinical implications.

Introduction:

Cardiovascular diseases remain one of the leading causes of death globally. Classic and potentially modifiable cardiovascular risk factors include smoking, obesity, low physical activity, lipid disorders, and hypertension. However, increasing attention is also being paid to non-classical cardiovascular risk factors, such as adipose tissue surrounding internal organs.

The adipose tissue surrounding the heart is a subtype of visceral fat, the amount of which correlates with the severity of abdominal obesity and is independently associated with the risk of cardiovascular events. Like abdominal obesity, pericardial adipose tissue is a risk factor for the development of atherosclerotic cardiovascular diseases.

The classification of pericardial adipose tissue varies in the literature. For the purposes of this work, the most important is epicardial adipose tissue (EAT) and pericoronary adipose tissue (PCAT). EAT lies between the visceral layer of the pericardium and the myocardium, while PCAT is defined as fat surrounding the coronary vessels, regardless of its location, considering it as part of epicardial fat.

Pericardial adipose tissue is highly metabolically active, secreting numerous cytokines and chemokines in the vicinity of the heart muscle. Under healthy conditions, EAT and PCAT play a protective role, participating in metabolic, energetic, and anti-inflammatory processes. In pathophysiological conditions, such as in patients with diabetes or metabolic syndrome, these protective properties are replaced with processes promoting inflammation and the development of cardiovascular diseases, including coronary artery disease. Therefore, pericardial adipose tissue may be an interesting target for new cardiovascular therapies in the future.

Aim of the study:

The aim of this doctoral thesis was (i) to identify the relationship between extremely intense physical exertion and the quantity and inflammatory activity of EAT, (ii) to identify potential pathophysiological mechanisms at the level of gene expression in EAT that could be a target for future therapies, and (iii) to summarize the existing data on the role of EAT in the pathophysiology of cardiovascular diseases.

Results:

In the first study, the effect of extremely intense physical activity on the amount of EAT and the correlation between EAT and cardiovascular risk factors were evaluated. The study included 30 healthy amateur ultramarathon runners and 9 volunteers leading a sedentary lifestyle. EAT surface area was assessed using magnetic resonance imaging at 4 locations: around the 3 main coronary arteries (left anterior descending branch, circumflex branch, right coronary artery) and on the surface of the right ventricle. Additionally, body composition, lipid profile, serum interleukin-6 concentration, and intima-media thickness in the carotid arteries were evaluated. The amount of EAT in the ultramarathon runners was significantly lower at all examined locations compared to the control group ($p < 0.001$). As expected, ultramarathon runners had a lower percentage of visceral fat and a better lipid profile than the control group ($p < 0.001$). However, no differences in intima-media thickness were observed. There was also no statistically significant difference in interleukin-6 levels between the groups, but the frequency of pathologically high interleukin-6 levels (defined as concentration > 1 pg/ml) was three times lower in the ultramarathon runners group than in the control group (17% vs. 56%, $p < 0.05$). Additionally, a positive correlation was found in the ultramarathon runner group between the surface area of EAT surrounding the left anterior descending branch, circumflex branch, and right ventricle and the percentage of total visceral fat, and between the amount of EAT around the circumflex branch and the concentration of LDL and non-HDL cholesterol ($p < 0.05$).

In the second study, gene expression in PCAT was compared between patients with advanced coronary artery disease and a control group. PCAT samples were obtained during aorto-coronary bypass surgery (n = 21, study group) or non-coronary cardiac surgery in patients with previously excluded coronary artery disease (n = 19, control group). Out of 67,528 transcripts, 1348 were identified as differentially expressed genes (DEGs). Among them, 416 (30.9%) showed overexpression, and 932 (69.1%) were classified as underexpressed compared to the control group. Among the genes showing increased expression were those encoding molecules with pro-inflammatory and pro-atherogenic activity, such as CXCL8, CXCL2, interleukin-6, selectin E, low-density lipoprotein receptor, and ADAMTS metalloproteinases. Genes encoding signalling proteins, enzymes, microRNAs, and various types of collagen were identified among the underexpressed genes.

Additionally, a group of "upstream regulators" associated with differentially expressed genes was distinguished. This term refers to any molecule that can affect the expression, transcription, or phosphorylation of another molecule. In this heterogeneous group, attention was drawn to genes encoding platelet-derived growth factor, high mobility group box 2 protein

(HMGB2), and evolutionarily conserved signalling intermediate in toll pathway (ESCIT), which are considered pro-inflammatory and pro-atherosclerotic molecules. Moreover, the Ingenuity Pathway Analysis (IPA) software used linked differentially expressed genes to entire networks of connections, canonical pathways, and networks. The activated pathways included the "coagulation system pathway" containing molecules known for their pro-atherosclerotic and pro-inflammatory nature (tissue factor pathway inhibitor, plasminogen activator, urokinase receptor, thrombomodulin).

The third work is a review article summarizing the current state of knowledge regarding the involvement of EAT in the pathogenesis of cardiovascular diseases, including coronary artery disease, heart failure, and atrial fibrillation. Attention is also drawn to the possible relationship between EAT and the course of COVID-19. Finally, EAT is presented as a potential therapeutic target in the prevention and treatment of cardiovascular diseases in the future.

Conclusions:

The first study demonstrated that extremely intense physical training may reduce cardiovascular risk by reducing the amount and pro-inflammatory activity of epicardial adipose tissue. However, further research is needed to confirm the relationships identified in this doctoral thesis.

The second study was one of the first to demonstrate altered gene expression not only in individual genes but in entire networks and pathways derived from PCAT, providing further evidence that the tissue under study is an active source of pro-atherosclerotic molecules that may accelerate the development of coronary artery disease.

The third work indicates an extensive interest in EAT in the recent years, suggesting its potential as a new therapeutic target in the prevention and treatment of cardiovascular diseases.

In summary, the series of studies presented demonstrate that pericardial adipose tissue is one of the key elements involved in the pathogenesis of cardiovascular diseases. However, further research at the molecular level is crucial to identify specific genes and proteins encoded by them whose modulation could change the unfavourable phenotype of EAT in patients with cardiovascular diseases. The negative impact of EAT on the circulatory system can be limited through non-pharmacological interventions, and with further development of gene expression studies, there is a chance to identify new therapeutic targets within EAT.

4. Wstęp

Choroby układu sercowo- naczyniowego pozostają jedną z głównych przyczyn zgonów w skali globalnej [1]. Do klasycznych i potencjalnie modyfikowalnych czynników ryzyka sercowo-naczyniowego należą nikotynizm, otyłość, niska aktywność fizyczna, zaburzenia lipidowe i nadciśnienie tętnicze. Coraz większą wagę przykłada się jednak również do nieklasycznych czynników ryzyka sercowo-naczyniowego, takiego jak tkanka tłuszczowa otaczająca narządy wewnętrzne.

Tkanka tłuszczowa otaczająca mięsień sercowy jest podtypem tłuszczu trzewnego, którego ilość koreluje z nasileniem otyłości brzusznej i jest niezależnie związana z ryzykiem zdarzeń sercowo-naczyniowych [2-4]. Podobnie jak otyłość brzuszna, okołosercowa tkanka tłuszczowa stanowi jeden z czynników ryzyka rozwoju chorób sercowo-naczyniowych o podłożu miażdżycowym [5].

Klasyfikacja tkanki tłuszczowej otaczającej serce różni się między publikacjami. Z punktu widzenia niniejszej pracy, najważniejsze znaczenie ma tkanka tłuszczowa nasierdziowa (ang. *epicardial adipose tissue*, EAT) i okołowieńcowa (ang. *pericoronary adipose tissue*, PCAT). EAT leży pomiędzy trzewną blaszką osierdzia a mięśniem sercowym [2], natomiast jako PCAT definiuje się tłuszcz otaczający naczynia wieńcowe, niezależnie od jego lokalizacji, uznając go za część tłuszczu epikardialnego [6].

Istnieje wiele różnic pomiędzy EAT a tkanką tłuszczową w innych lokalizacjach na poziomie anatomicznym, histologicznym i molekularnym [7, 8]. Co ważne, pomiędzy EAT a otaczającymi strukturami, w tym mięśniem sercowym i naczyniami wieńcowymi, nie ma anatomicznej bariery (np. powięzi), dzięki czemu możliwe są ich interakcje na drodze m.in. parakrynnej [9]. W warunkach zdrowia, EAT odgrywa rolę protekcyjną, m.in. chroniąc przed hipotermią [10], zapewniając mechaniczną ochronę naczyń wieńcowych [11] i biorąc udział w licznych procesach metabolicznych mięśnia sercowego [12]. Jako źródło adiponektyny, EAT poprawia funkcję śródbłonka naczyń wieńcowych, redukuje stres oksydacyjny i nasilenie procesów zapalnych [13, 14]. W warunkach patofizjologicznych, np. u pacjentów z cukrzycą czy zespołem metabolicznym, właściwości ochronne zanikają, a dominować zaczynają procesy promujące stan zapalny i rozwój chorób układu krążenia, w tym choroby wieńcowej.

Regularna, umiarkowana aktywność fizyczna zmniejsza ryzyko chorób sercowo-naczyniowych oraz redukuje śmiertelność całkowitą [15]. W ostatnich latach rośnie liczba osób uprawiających sporty wytrzymałościowe o dużej intensywności, jednak o wpływie tak ekstremalnie intensywnej aktywności fizycznej na czynniki ryzyka sercowo- naczyniowego wiadomo zdecydowanie mniej. Z tego powodu w pierwszej z prezentowanych prac oceniono wpływ intensywnego treningu wytrzymałościowego na niektóre z czynników ryzyka rozwoju choroby wieńcowej.

Wiele badań naukowych wykazało, że stan zapalny odgrywa kluczową rolę w rozwoju miażdżycy, a także w destabilizacji już istniejących blaszek miażdżycowych [16, 17], jednak nadal nieznany jest dokładny mechanizm leżący u podłoża tego zjawiska. Jak wcześniej wspomniano, nasierdziowa tkanka tłuszczowa, w tym tkanka okołonaczyniowa, są strukturami aktywnymi metabolicznie i mogą uwalniać liczne cytokiny i chemokiny. Zależność pomiędzy nasileniem procesu zapalnego w przydance tętnic wieńcowych i okołowieńcowej tkance tłuszczowej a zaawansowaniem choroby wieńcowej i niestabilnością blaszek miażdżycowych wykazano w licznych badaniach, w tym autopsyjnych [18], tomografii komputerowej [19] czy przy użyciu metod medycyny nuklearnej [20, 21]. Wspomniano także, że pomiędzy EAT a tłuszczem w innych lokalizacjach istnieje wiele różnic [7, 8]. Istnieją doniesienia pokazujące, że różnice w ekspresji genów w obrębie tkanki tłuszczowej zależą od jej lokalizacji nie tylko w organizmie [22], ale także w obrębie samego serca [23]. Dostępne są wyniki badań wskazujące, że ekspresja genów w EAT różni się także w zależności od braku lub obecności choroby wieńcowej [8]. Coraz więcej mówi się także o okołowieńcowej tkance tłuszczowej jako potencjalnym punkcie uchwytu terapii przeciwmiażdżycowej [24]. W związku z powyższym, w drugiej z prezentowanych prac porównano ekspresję genów w okołowieńcowej tkance tłuszczowej w populacji z zaawansowaną chorobą wieńcową i bez niej.

EAT jest jednak obiektem badań nie tylko w kontekście choroby wieńcowej, ale także m.in. niewydolności serca (ze szczególnym uwzględnieniem niewydolności serca z zachowaną frakcją wyrzutową) czy migotania przedsionków, a nawet chorób potencjalnie „niekardiologicznych”, jak COVID-19. Przegląd literatury na ten temat podsumowano w trzeciej pracy.

5. Cel pracy

Celem niniejszej rozprawy doktorskiej były:

- identyfikacja zależności pomiędzy ekstremalnie intensywnym wysiłkiem fizycznym a ilością i aktywnością zapalną EAT;
- identyfikacja potencjalnych mechanizmów patofizjologicznych na poziomie ekspresji genów, mogących stanowić punkt uchwytu dla terapii celowanych w przyszłości;
- podsumowanie dotychczasowych danych na temat roli nasierdziowej tkanki tłuszczowej w patofizjologii chorób sercowo-naczyniowych.

6. Kopie opublikowanych prac

6.1. Epicardial Adipose Tissue and Cardiovascular Risk Assessment in Ultra-Marathon Runners: A Pilot Study.

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Epicardial Adipose Tissue and Cardiovascular Risk Assessment in Ultra-Marathon Runners: A Pilot Study

Michał Konwerski [1], Marek Postuła [2], Marzena Barczuk-Falęcka [3], Anna Czajkowska [4], Anna Mróz [5], Katarzyna Witek [5], Wawrzyniec Bakalarski [5], Aleksandra Gąsecka [1], Łukasz A. Małek [6,†] and Tomasz Mazurek [1,*,†]

1 1st Chair and Department of Cardiology, Medical University of Warsaw, 02-097 Warsaw, Poland; konwerski.mich@gmail.com (M.K.); aleksandra.gasecka@wum.edu.pl (A.G.)
2 Center for Preclinical Research and Technology CEPT, Department of Experimental and Clinical Pharmacology, Medical University of Warsaw, 02-097 Warsaw, Poland; m.postula@wum.edu.pl
3 Department of Pediatric Radiology, Medical University of Warsaw, 02-091 Warsaw, Poland; marzena.barczuk-falecka@wum.edu.pl
4 Faculty of Tourism and Recreation, Józef Piłsudski University of Physical Education in Warsaw, 00-968 Warsaw, Poland; anna.czajkowska@awf.edu.pl
5 Faculty of Physical Education, Józef Piłsudski University of Physical Education in Warsaw, 00-968 Warsaw, Poland; anna.mroz@awf.edu.pl (A.M.); katarzyna.witek@awf.edu.pl (K.W.); wawbak@gmail.com (W.B.)
6 Department of Epidemiology, Cardiovascular Disease Prevention and Health Promotion Institute of Cardiology, 04-628 Warsaw, Poland; lukasz.a.malek@gmail.com
* Correspondence: tmazurek@kardia.edu.pl; Tel.: +48-22-599-1958
† Senior authors.

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Abstract: Epicardial adipose tissue (EAT) volume is associated with cardiovascular disease (CVD). Data regarding the influence of extremely intensive training on CVD are scarce. We compared EAT volume among ultra-marathon runners and in the sedentary control group, and assessed the correlations between EAT and risk factors of coronary artery disease (CAD). EAT volume around three main coronary vessels and right ventricle (RV) was measured in 30 healthy amateur ultrarunners and 9 sex- and age-matched sedentary controls using cardiac magnetic resonance. In addition, body composition, lipid profile, interleukin-6 (IL-6) plasma concentration, and intima-media thickness (IMT) were measured as well. The EAT volume was lower in all measured locations in the ultrarunners' group compared to control group ($p < 0.001$ for all). Ultrarunners had lower BMI and fat percentage (FAT%) and more favorable lipid profile compared to the control group ($p < 0.05$ for all). Ultrarunners had lower rate of pathologically high levels of plasma IL-6 (>1 pg/mL) compared to the control group (17% vs. 56%, $p < 0.05$). IMT was similar in both groups. In the ultrarunners' group, there was a positive correlation between EAT surrounding left anterior descending artery, circumflex artery, and RV and FAT%, and between EAT around circumflex artery and LDL and non-HDL cholesterol ($p < 0.05$ for all). In summary, extremely intensive training may decrease the risk of cardiovascular events in adult population of amateur athletes by reducing the amount and pro-inflammatory activity of EAT. However, more research is needed to draw firm conclusions regarding the anti- and pro-inflammatory effects of intensive training.

Keywords: epicardial adipose tissue; ultrarunners; inflammation; cardiac magnetic resonance; cardiovascular disease

1. Introduction

Despite the constant progress in pharmacological and interventional treatment, the incidence of cardiovascular diseases (CVDs) is still growing. In 2012 and 2013, CVDs were responsible for 17.3 million deaths worldwide, which makes them the leading cause of mortality [1–3]. A number of risk factors for CVDs have already been described and many of them are potentially reversible. In the worldwide INTERHEART study, there were nine potentially modifiable factors in patients with myocardial infarction: smoking, dyslipidemia, arterial hypertension, diabetes mellitus, abdominal obesity, psychosocial factors, daily consumption of fruits and vegetables, regular alcohol consumption, and regular physical activity [4].

Obesity and physical activity are potentially easily reversible risk factors. Particularly, the abdominal type of obesity is one of the most important factors predisposing to atherosclerosis and cardiovascular events [5,6]. The volume and thickness of epicardial adipose tissue (EAT) was showed to correlate with intra-abdominal fat mass, severity of obesity [7,8], and the incidence of cardiovascular events [9]. EAT is a visceral fat with high metabolic activity, located between the pericardium and the myocardium and not separated from myocardium and coronary vessels [10,11]. EAT activity solely depends on the metabolic state. In patients with metabolic syndrome, perivascular fat surrounding coronary arteries (pericoronary adipose tissue, PCAT), which is a subtype of EAT, loses its protective capability and becomes an aggressive, pro-inflammatory tissue, releasing cytokines and chemokines. Recently, it was showed that the carotid intima-media thickness (IMT), which is a very strong indicator of systemic atherosclerosis, was associated with EAT thickness measured by echocardiography, independently from body mass index (BMI) and waist circumference (WC) [11]. Hence, PCAT may be directly involved in the pathogenesis of coronary artery disease (CAD) [12–14].

Moderate physical activity has a protective effect against CAD and decreases all-cause mortality [4,15–19]. This effect can be caused by increasing HDL concentration in serum, lowering blood pressure, weight reduction, and a reduction in insulin resistance. Recently, the number of people practicing sports in developed countries has been growing geometrically. Especially, endurance running has gained popularity and probably is the most popular sport worldwide in middle-age adults [17].

Since little is known about the influence of extreme high-intensity training on cardiovascular risk factors, we sought to compare EAT volume assessed with cardiac magnetic resonance (CMR) among ultra-marathon runners and in the sedentary control group, and assess the correlations between EAT and risk factors of CAD (body composition, venous blood lipid profile, interleukin-6 (IL-6) plasma concentration, and IMT).

2. Materials and Methods

The study included a group of 30 healthy, male, amateur experienced ultra-marathon runners and 9 gender- and age-matched sedentary controls [20]. All study participants underwent CMR with 3T scanner (Siemens, Erlangen, Germany) in which the EAT area was measured in 4 points: (1) in the vicinity of the free right ventricle (RV) wall, (2) surrounding right coronary artery (RCA) and (3) circumflex artery (Cx) on 4-chamber cine balanced steady-state free processing (b-SSFP) images, and (4) in the neighborhood of the left anterior descending (LAD) coronary artery on the basal short axis b-SSFP image. Figure 1 shows an example of PCAT calculation with CMR by measurement of the area around the main coronary arteries and over the free wall of the RV.

Body composition was analyzed by Tanita body composition analyzer (Tanita Europe BV, Amsterdam, The Netherlands). Venous blood lipid profile was measured at fasting in the accredited hospital laboratory. Interleukin-6 (IL-6) plasma concentration were analyzed using the commercially available enzyme-linked immunosorbent assay (ELISA; Roche Diagnostics, Indianapolis, IN, USA). Intima-media thickness (IMT) was measured using carotid ultrasonography (Siemens ACUSON S1000 Ultrasound System, HELX Evolution with Touch Control). IMT was determined by B-mode ultrasound with a 5–14 MHz linear transducer. The measurements were performed using sagittal imaging of the common carotid artery (CCA). At least five measurements on each side were taken to obtain an average value. IMT was assessed on the posterior wall of the CCA on the right and on the left, 1 cm from the carotid bifurcation. According to the European Society of Cardiology

(ESC) Cardiovascular Disease (CVD) Prevention in Clinical Practice Guidelines 2016, we set the cut-off point to values >0.9 mm [21].

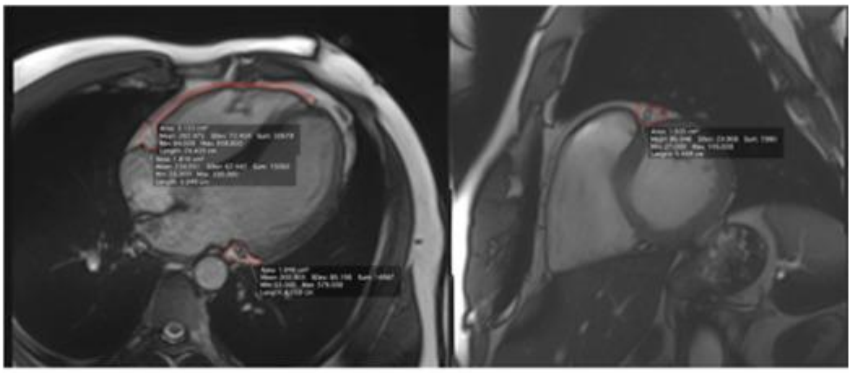

Figure 1. Example of pericoronary adipose tissue calculation with cardiac magnetic resonance by measurement of the area around the main coronary arteries and over the free wall of the right ventricle (epicardial adipose tissue).

3. Results

Description of ultra-marathon runners is shown in Table 1.

Table 1. Description of ultra-marathon runners. IQR—interquartile range.

Parameter	Ultra-Marathon Runners n = 30, Median (IQR)
Years of running	9 (7–15)
Age at start of ultra-running	34 (29–39)
Total covered distance (km)	25,000 (20,000-40,000)
Weekly running distance (km)	80 (70–90)
Number of ultra-races completed	15 (10–27.5)
Number of ultra-races during last 2 years	5.5 (4–9)
Number of completed ultra-races >100 km	3.5 (2–7)
Best place achieved in an ultra-race	5 (1–13)

The participants' baseline characteristics and results are shown in Table 2.

The EAT volume was smaller in all localizations in the ultra-marathon runners as compared to control group (p <0.001 for all). Regarding body composition, the control group had higher mean body weight, BMI, and fat mass (FAT) ($p < 0.001$) as compared to ultra-marathon runners. The concentrations of total cholesterol, low-density lipoprotein (LDL) cholesterol, non-high-density lipoprotein (non- HDL) cholesterol, and triglycerides were higher, and the concentration of high-density lipoprotein (HDL) cholesterol was lower in the control group compared to ultra-marathon runners ($p < 0.05$ for all parameters). There were no differences in the IL-6 plasma concentration between the groups ($p = 0.16$). However, in the runner's group, the rate of pathologically high levels of plasma IL-6 (>1 pg/mL) was 3-fold lower compared to the control group (17% vs. 56%; $p < 0.05$). IMT was similar in both groups.

Table 2. Participants' baseline characteristics and results.

Baseline Characteristics			
Parameter	**Ultra-marathon Runners (n = 30)**	**Control Group (n = 9)**	p
Age (years)	40.93 ± 6.57	40.78 ± 8.32	0.95
Heigth (cm)	172.02 ± 32.49	179.89 ± 6.43	0.48
Weight (kg)	72.73 ± 5.19	96.10 ± 19.39	<0.001
BMI (cm/m^2)	23.09 ± 1.54	30.00 ± 5.41	<0.001
FAT % (%)	10.78 ± 4.01	23.17 ± 5.91	<0.001
FAT mass (kg)	8.02 ± 3.32	23.16 ± 10.16	<0.001
FFM (kg)	65.30 ± 3.44	74.34 ± 14.86	0.004
TBW (kg)	47.81 ± 2.52	53.40 ± 7.17	<0.001
Blood Test Results			
Parameter	**Ultra-marathon Runners (n = 30)**	**Control Group (n = 9)**	p
TC (mg/dl)	189.66 ± 23.38	230.22 ± 28.60	<0.001
HDL (mg/dl)	70.52 ± 15.96	56.33 ± 8.99	0.016
LDL (mg/dl)	102.68 ± 22.45	144.86 ± 23.12	<0.001
non-HDL (mg/dl)	119.14 ± 25.07	173.89 ± 27.10	<0.001
TG (mg/dl)	82.27 ± 24.73	145.07 ± 41.82	<0.001
IL-6 (pg/mL)	1.29 ± 0.75	1.70 ± 0.72	0.156
Results of Imaging Tests			
Cardiac Magnetic Resonance			
Parameter	**Ultra-marathon Runners (n = 30)**	**Control Group (n = 9)**	p
LAD (cm^2)	1.12 ± 0.4	1.86 ± 0.41	<0.001
RCA (cm^2)	0.88 ± 0.39	1.78 ± 0.34	<0.001
Cx (cm^2)	0.90 ± 0.36	1.74 ± 0.49	<0.001
RV (cm^2)	2.07 ± 0.97	5.23 ± 2.77	<0.001
Ultrasonography			
Left IMT (cm)	0.07 ± 0.02	0.07 ± 0.01	0.99
Right IMT (cm)	0.08 ± 0.03	0.08 ± 0.01	0.68

FAT%—body fat percentage, BMI—body mass index, FFM—fat free mass, TBW—total body water, TC—total cholesterol concentration; HDL—high-density lipoprotein concentration; LDL—low-density lipoprotein concentration; non-HDL—non-high-density lipoprotein concentration; TG—triglyceride concentration; IL-6—interleukin-6 concentration; LAD—PCAT area around left anterior descending artery; RCA—PCAT area around right coronary artery; Cx—PCAT area around circumflex artery; RV—EAT area over right ventricle; IMT—carotid intima-media thickness.

Figure 2 shows the correlations between body FAT% and PCAT, and between PCAT around the Cx artery and lipidogram parameters in the ultra-marathon runners and control group.

There was an intermediate, positive correlation between FAT% and PCAT around LAD, Cx, and RV in the runners' group (R ≥ 0.42 for all; $p \leq 0.02$ for all; Figure 2A) and a strong, positive correlation between FAT% and PCAT around Cx and RCA in the control group (R ≥ 0.71; $p \leq 0.03$ for both; Figure 2B). Moreover, there was an intermediate position correlation between the PCAT around Cx artery and the concentration of LDL and non-HDL in the runners' group (R ≥ 0.41; $p \leq 0.03$ for both; Figure 2C), which was not observed in the control group (group (Figure 2D). There were no significant correlations between PCAT and FAT% with IL-6 plasma concentrations and IMT in both studied groups.

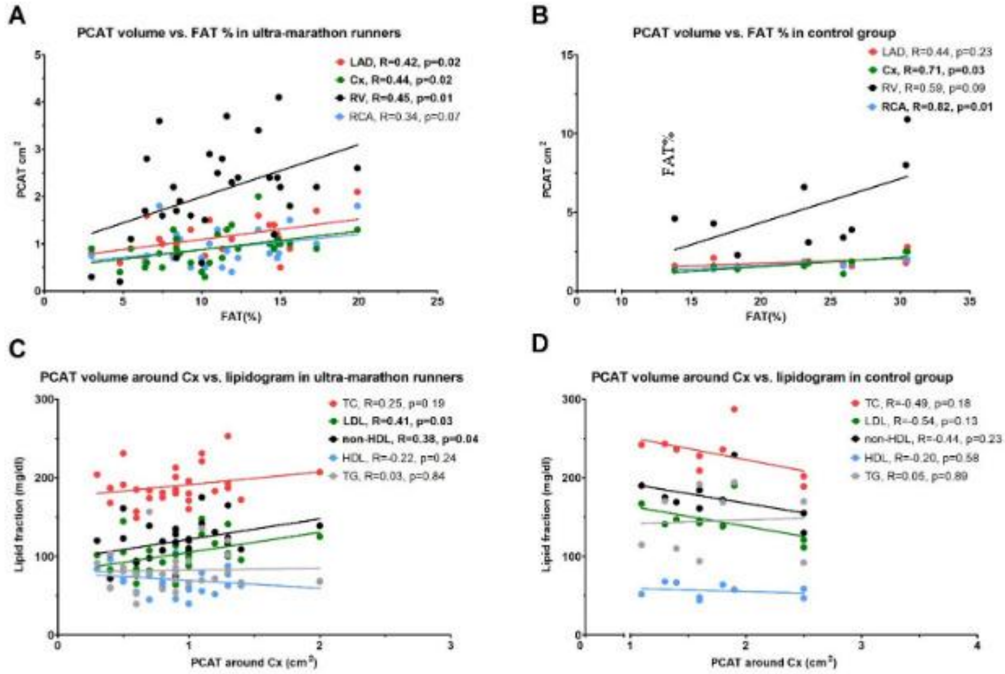

Figure 2. (**A**,**B**) Correlations between body fat percentage (FAT%) and pericoronary adipose tissue (PCAT) in the ultra-marathon runners and control group. (**C**,**D**) Correlations between PCAT around the circumflex artery (Cx) and lipidogram parameters. LAD—left anterior descending artery; RV—right ventricle; RCA—right coronary artery; LDL—low-density lipoproteins; HDL—high-density lipoproteins; non-HDL—non-high-density lipoproteins; TC—total cholesterol; TG—triglycerides.

4. Discussion

The purpose of this study was to compare the EAT volume assessed with CMR among ultra-marathon runners and in the sedentary control group, and assess the correlations between EAT and risk factors of CAD, including body composition, venous blood lipid profile, IL-6 plasma concentration, and IMT. The main findings of this study are that (i) EAT volume is smaller in all measured localizations in the ultra-marathon runners compared to the control group, and (ii) there is a positive correlation between FAT% and PCAT surrounding LAD artery, Cx artery, and RV in the ultra-marathon runners, and a positive correlation between LDL and non-HDL concentration and PCAT around Cx artery.

CVD and obesity are significant health problems in developed and developing countries [1–3,5,6]. Strong connections between obesity, insulin resistance, and atherogenic lipids profile are well-documented [22]. Whereas subcutaneous adipose tissue (SAT) is not associated with cardiovascular risk, visceral fat seems to play a major role in atherosclerosis [23]. Recently, there is a growing interest in PCAT, which is a specific visceral tissue located in the direct neighborhood of the coronary arteries and therefore, in addition to the systemic effect, it also has paracrine and vasocrine effect on the surrounding vessels [10,24,25]. There are many differences at the anatomic, histologic, embryologic, and biomolecular levels between EAT/ PCAT and other fat depots [12,13]. Inflammatory processes underlying atherosclerosis are well-documented [26]. In addition, EAT was showed to participate in the pathogenesis of coronary atherosclerosis by secreting multiple cytokines and chemokines, thus serving as an active pro-inflammatory tissue [14,27]. In patients with advanced CAD undergoing coronary artery bypass grafting, EAT released more inflammatory markers than the SAT [27]. Moreover, the thickness of EAT measured using computed tomography reflected the stage of CAD. There was also a positive correlation between PCAT and vulnerability of atherosclerotic plaques based on their composition, assessed by intravascular ultrasound imaging [28]. Mazurek et al. analyzed the composition of atherosclerotic plaques using virtual histology intravascular ultrasonography and quantitatively assessed PCAT using positron emission tomography/computed tomography among patients with acute coronary syndrome without persistent ST-segment elevation. The inflammatory activity was measured by maximal standardized uptake value of 18-fluorodeoxyglucose. Inflammatory activity within PCAT was higher than in subcutaneous, visceral thoracic or epicardial adipose tissue, and there was a strong, positive correlation with pro-inflammatory activity of PCAT and both coronary plaque burden and its necrotic core component [29]. The association between EAT, atrial fibrillation [30], and impairment of cardiac function was also demonstrated [31]. Recently, it was demonstrated that among patients without known cardiovascular disease, the mean EAT thickness evaluated in magnetic resonance was higher in those with subclinical left ventricle impairment compared to those with normal left ventricle function, independently of traditional risk factors [32]. It has been also shown that patients with a familiar hypercholesterolemia had increased EAT thickness, and there was a positive correlation between EAT and plasma LDL concentration in these patients [33]. Volume and thickness of EAT correlates with intra-abdominal fat mass, obesity extent [7,8], and is independently associated with cardiovascular events [9]. Further, EAT is an independent predictor for the presence of carotid plaque [11]. It has been emphasized that EAT has a stronger association with systemic inflammation and subclinical atherosclerosis (IMT than BMI and waist circumference) [34,35].

The effect of very intense physical activity on physiological processes is still unknown. Both short-term and long-term endurance sport can induce both anti- and pro-inflammatory response. Long-term physical exercise was shown to reduce the level of C-reactive protein (CRP), especially when accompanied by a decrease in weight, especially in individuals with initially elevated CRP [36]. On the other hand, long-term exercise may also stimulate inflammatory processes, vascular oxidative stress, and fatigue of the elastic components of the vessel wall [37]. For example, obese non-elite runners had higher CRP plasma concentration than lean elite runners, measured 24 h after marathon. In addition, obese non-elite runners had a higher level of IL-6 and a lower level of IL-10 at baseline than lean elite and lean non-elite runners [38]. These results indicate the association between adipose tissue and inflammation.

In our population of extreme amateur runners, EAT volume was smaller in all measured locations compared to control group. As expected, runners had lower weight, BMI, fat free mass, body FAT%, concentrations of LDL and TG, higher concentration of HDL, and less frequently pathologically high levels of plasma IL-6 compared to controls. These findings suggest that high intensity training may have a favorable effect on cardiovascular risk factors. On the other hand, there was a correlation between EAT/PCAT surrounding LAD artery, Cx artery, and RV, and between PCAT surrounding Cx artery and lipid profile in the ultra-marathon runners' group, which might potentially reflect increased cardiovascular risk in this population.

5. Limitations

The main limitation of this study is the small sample size. For example, likely the correlations between FAT% and PCAT around coronary arteries, which were not significant in our control group, would become significant in a larger cohort. The second limitations are the limited inclusion criteria of the study population, including homogenous individuals regarding gender (only male) and ethnicity (only Caucasian, Polish). Therefore, our results are not applicable to female and to any other ethnic group. Another limitation is the control group comprising sex- and age-matched sedentary controls with higher body weight, BMI, and FAT levels, which are known risk factors of CVD. Ideally, the control group would comprise individuals with moderate physical activity and similar body composition to the study group. However, such group was not available when we performed this study. Finally, the correlations showed in our study do not prove any causal relationship between intensive training, EAT, and CVD. Due to high risk of bias in this study, we may only speculate about the benefit and risk of extremely intensive training on cardiovascular health, and further research is required to ultimately answer this question.

6. Conclusions

In summary, extremely intensive training may decrease the risk of cardiovascular events in adult population of amateur athletes by reducing the amount and pro-inflammatory activity of EAT. More research is needed to draw firm conclusions regarding the anti- and pro-inflammatory effects of intensive training.

Author Contributions: Conceptualization, Ł.A.M. and M.P.; methodology, Ł.A.M. and M.P.; validation, Ł.A.M.; formal analysis, Ł.A.M. and M.K.; investigation, M.B.-F. and A.M. and A.C. and K.W. and W.B.; resources, Ł.A.M. and M.P.; data curation, Ł.A.M.; writing—original draft preparation, M.K.; writing—review and editing, M.K. and A.G. and T.M.; visualization, M.K. and A.G.; supervision, Ł.A.M. and T.M.; project administration, Ł.A.M.; funding acquisition, Ł.A.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of the Regional Medical Chamber in Warsaw, Poland (protocol no. 52/17 and date of approval 12 October 2017).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to patients' privacy.

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Conflicts of Interest: The authors report no conflict of interest related to this study.

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6.2. Atherosclerosis Pathways are Activated in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease.

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Atherosclerosis Pathways are Activated in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease

Michał Konwerski[1]
Agnieszka Gromadka[2]
Adam Arendarczyk[3]
Marta Koblowska[2]
Roksana Iwanicka-Nowicka[2]
Radosław Wilimski[3]
Paweł Czub[3]
Krzysztof Jerzy Filipiak[1]
Piotr Hendzel[3]
Piotr Zielenkiewicz[2]
Grzegorz Opolski[1]
Aleksandra Gąsecka[1]
Tomasz Mazurek[1]

[1]1st Chair and Department of Cardiology, Medical University of Warsaw, Warsaw, Poland; [2]Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland; [3]Department of Cardiac Surgery, Medical University of Warsaw, Warsaw, Poland

Purpose: Perivascular release of inflammatory mediators may accelerate coronary lesion formation and contribute to plaque instability. Accordingly, we compared gene expression in pericoronary adipose tissue (PCAT) in patients with advanced coronary artery disease (CAD) and non-CAD controls.

Patients and Methods: PCAT samples were collected during coronary bypass grafting from CAD patients (n = 21) and controls undergoing valve replacement surgery, with CAD excluded by coronary angiography (n = 19). Gene expression was measured by GeneChip™ Human Transcriptome Array 2.0. Obtained list of 1348 transcripts (2.0%) that passed the filter criteria was further analyzed by Ingenuity Pathway Analysis software, identifying 735 unique differentially expressed genes (DEGs).

Results: Among the CAD patients, 416 (30.9%) transcripts were upregulated, and 932 (69.1%) were downregulated, compared to controls. The top upregulated genes were involved in inflammation and atherosclerosis (chemokines, interleukin-6, selectin E and low-density lipoprotein cholesterol (LDL-C) receptor), whereas the downregulated genes were involved in cardiac ischaemia and remodelling, platelet function and mitochondrial function (miR-3671, miR-4524a, multimerin, biglycan, tissue factor pathway inhibitor (TFPI), glucuronidases, miR-548, collagen type I, III, IV). Among the top upstream regulators, we identified molecules that have proinflammatory and atherosclerotic features (High Mobility Group Box 2 (HMGB2), platelet-derived growth platelet (PDGF) and evolutionarily conserved signaling intermediate in Toll pathways (ESCIT)). The activated pathway related to DEGs consisted of molecules with well-established role in the pathogenesis of atherosclerosis (TFPI, plasminogen activator, plasminogen activator, urokinase receptor (PLAUR), thrombomodulin). Moreover, we showed that 22 of the altered genes form a pro-atherogenic network.

Conclusion: Altered gene expression in PCAT of CAD patients, with genes upregulation and activation of pathway involved in inflammation and atherosclerosis, may be involved in CAD development and progression.

Keywords: adipose tissue, inflammation, gene expression, atherosclerosis

Introduction

Obesity, particularly abdominal obesity, is one of the most important risk factors for atherosclerosis and cardiovascular events.[1–3] The volume and thickness of epicardial adipose tissue (EAT) correlate with intra-abdominal fat mass and the severity of obesity[4,5] and are independently associated with cardiovascular events.[6] Many studies have shown that inflammation plays a key role in the development of

Correspondence: Tomasz Mazurek
1st Chair and Department of Cardiology, Medical University of Warsaw, Banacha Street 1a, Warsaw, 02-097, Poland
Tel +48 22 599 19 58
Fax +48 22 599 19 57
Email tmazurek@kardia.edu.pl

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atherosclerosis and the destabilization of atherosclerotic coronary plaques, but the exact mechanism of this phenomenon is still unknown.[7–9] EAT is an active inflammatory tissue that releases cytokines and chemokines[10,11] and plays a role in the pathogenesis of coronary artery disease (CAD). EAT is a part of the visceral fat that lies between the pericardium and the myocardium[12] and is not separated from the myocardium and vessels by fascia, allowing paracrine or vasocrine interactions.[13] Pericoronary adipose tissue (PCAT), in turn, is a part of EAT that directly neighbors coronary arteries. In patients with CAD who undergo coronary artery bypass grafting (CABG), PCAT releases higher levels of inflammatory markers than subcutaneous adipose tissue (SAT),[14] which further correlates with insulin resistance. The presence of inflammatory cells in the adventitia among patients who died due to acute coronary syndrome was confirmed in autopsy studies.[15] There is also a correlation between the degree of coronary artery narrowing and the degree of inflammatory infiltration in the adventitia.[15] EAT volume (EATV), assessed by computed tomography (CT), is associated with the total coronary plaque burden, and there is a positive correlation between EATV and the amount of necrotic tissue in atherosclerotic plaques, evaluated using intravascular ultrasound imaging (IVUS), indicating a relationship between EATV and the vulnerability of atherosclerotic plaques.[16] Qualitative assessment of PCAT using positron emission tomography/computed tomography (PET/CT) was also performed in patients with acute coronary syndrome without persistent ST-segment elevation (NSTE-ACS).[17] In these patients, the inflammatory activity of PCAT, as measured by maximum fludeoxyglucose (FDG) uptake, was greater than that adipose tissue in other locations. Moreover, it correlated with the severity of atherosclerosis and the necrotic core volume of coronary plaque, as assessed by virtual histology IVUS. Similarly, in patients with stable CAD, PCAT maximum FDG uptake was greater than in healthy volunteers.[18]

There are many anatomical, histological, embryological and molecular features that distinguish EAT from the other fat depots.[8] There are studies showing that gene expression in adipose tissue depends not only on its location in the human body[19] but also on its location within the heart.[20] Moreover, gene expression in adipose tissue may differ between a healthy population and patients with CAD. Currently, there are a few studies which evaluated gene expression in EAT.[19–24] In one of the latest studies, it was demonstrated that the EAT transcriptome is (i) unique compared to the SAT transcriptome, and (ii) different among patients with and without CAD. Emerging data supports the participation of EAT and/or PCAT in the pathophysiology of CAD.[24] Moreover, it has been postulated that EAT or PCAT may be novel therapeutic targets in obesity-related atherosclerosis.[25,26] Previously, we showed that EAT/PCAT is a source of inflammatory mediators in high-risk cardiac patients.[10] Here, we hypothesized that PCAT has specific gene regulation patterns in patients with CAD, compared to those without CAD, and that the dysregulated genes form specific networks. The aim of this study was to compare gene expression in PCAT samples collected during open heart surgery in patients with and without CAD.

Materials and Methods

Forty PCAT samples were collected during CABG from patients with severe, symptomatic CAD. (study group, n = 21 samples) and heart valve replacement after excluding CAD by coronary angiography (control group, n = 19 samples). Qualification for CABG was performed by the Heart Team consisting of a general cardiologist, interventional cardiologist and thoracic surgeon. All collected samples were coded with a unique number and analyzed by operators blinded to patient data. The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (IRB) at Medical University of Warsaw (approval number: KB 91/2012) and all participants gave written informed consent.

RNA Isolation

After harvesting, tissue pieces were immediately submerged in RNAlater (Thermo Fisher, US) and stored at −20 °C. Prior to RNA purification, tissue samples were homogenized using the MagNA Lyser instrument and the MagNA Lyser Green Beads tubes, together with 100–400 µL MagNA Pure DNA tissue lysis buffer (Roche Diagnostics, Germany). After transferring the samples to the MagNA Pure Compact System, automated isolation of total RNA was performed using the MagNA Pure Compact RNA isolation kit (Roche Diagnostics, Germany). The yield and purity of the isolated RNA were calculated by measuring the absorbance at 260 nm and 280 nm with a Nanodrop spectrometer. RNA integrity checks were performed using an Agilent BioAnalyzer 2100 (Agilent, US).

Microarray Analysis

Microarray expression analysis was performed using the Affymetrix Gene GeneChip® Instrument System according to the manufacturer's instructions (Thermo Fisher, US). A total of 10 ng of RNA that passed the initial quality control screen was then processed using the GeneChip™ WT Pico Kit, which was designed specifically to process small amounts of input RNA, according to the standard protocol provided by Affymetrix. Labeled samples were hybridized to the GeneChip™ Human Transcriptome Array 2.0. After hybridization, the arrays were washed and stained using a GeneChip Fluidics Station 450 and the Affymetrix GeneChip hybridization wash and stain kit. Microarrays were scanned on the Affymetrix GeneChip Scanner 3000 7G using Command Console Software.

Data from the microarrays were normalized and analyzed using Transcriptome Analysis Console 4.0. Apart from the tested condition difference (CAD presence), the main sources of variation included inter-subject variability and sample source, while patient sex and the protocol used were less important. However, to overcome variation driven by additional factors, additional normalization and corrections were applied (such as "batch effect" normalization for used protocol), following the TAC user guide.

The analysis of variance was performed by one-way ANOVA (CAD group vs control group), followed by false discovery rate (FDR) correction by the Benjamin–Hochberg procedure. To determine the significance of differentially expressed genes (DEGs), a cut-off for the fold change value ±1.5 and FDR < 0.05 was applied.

Downstream Analysis

The list of detected differentially expressed transcripts was analyzed by Ingenuity Pathway Analysis (IPA, version: 51963813) software to identify significant interactions and pathways.[27]

To quantify biological activity of pathways and main pathway regulators, the gene expression z-scores were calculated.[28] Briefly, a z-score is defined as the difference between the error-weighted mean of the expression values of the genes in each pathway and the error-weighted mean of all genes in a sample after normalization. Positive and negative z-scores indicate activation or inhibition of pathways and regulators, respectively, based on the relationships with DEGs. All analyses were performed by limiting the IPA database information only to molecules and relationships where the information was experimentally observed among humans. The list of DEGs was also used to establish custom IPA networks to further reveal the connections between discovered genes (Figure 1). All analysis and corresponding plots were executed following software guide.

Results

Table 1 shows the baseline clinical characteristics of the study participants, which were comparable in both groups. Among 67,528 transcripts, 1348 (2%) were identified as DEGs, with 416 (30.9%) and 932 (69.1%) transcripts that were up- and downregulated, respectively, in patients versus controls. Figure 2 shows DEGs in CAD patients, compared to controls, generated using Transcriptome Analysis Console 4.1. Although the majority of DEGs were downregulated, the upregulated DEGs showed the highest difference in expression. Table 2 shows the list of top 20 upregulated genes in PCAT of patients with CAD, compared to controls. The top upregulated genes included those involved in inflammation and atherosclerosis: chemokines (CXCL8, CXCL2), interleukin (IL)-6, E-selectin, low-density lipoproteins receptor (LDL-R). The genes which were upregulated more than 20-fold in patients compared to controls were coding CXCL8 (+193 fold change, FC), selectin E (+91 FC), IL-6 (+49 FC) and ADAM metallopeptidase with thrombospondin type 1 motif 4 (ADAMTS4; +28 FC).

Table 3 shows the list of top 20 downregulated genes in PCAT of patients with CAD, compared to controls. Among the top downregulated genes, signaling proteins (ATM serine/threonine kinase), different types of collagen (type IV, I and III), enzymes (histone acetyltransferase KANSL1, glucuronidase) and miRNAs (miR-548, miR-4524, miR-1299 and miR-3671) were found. The most downregulated genes were coding collagen type IV alpha 4 chain (−9 FC), histone acetyltransferase KANSL1 (−7 FC), miR-579 (−7 FC) and miR-4524a (−6 FC).

Table 4 shows the list of top upstream regulators identified by IPA, based on the relationships with DEGs in PCAT of patients with CAD, compared to controls. The top upstream regulators were high mobility group box 2 (HMGB2), platelet-derived growth factor BB (PDGF BB), evolutionarily conserved signaling intermediate in Toll pathway, mitochondrial (ECSIT), cluster of differentiation (CD) 24 and cyclin-dependent kinase 9 (CDK9).

Figure 3 shows the main canonical pathways found by IPA, based on the relationships with DEGs. Among top canonical pathways, coagulation system pathway consisting of genes involved in atherosclerosis (tissue factor

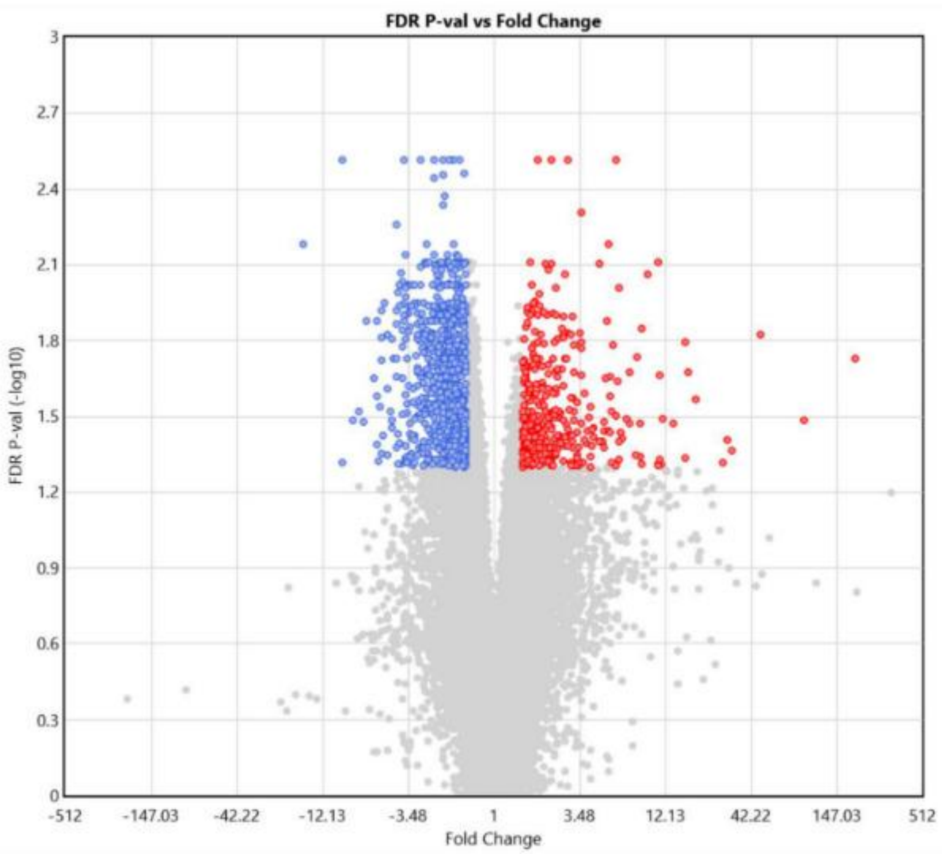

Figure 1 Differentially expressed genes (DEGs) in coronary artery disease (CAD) patients, compared to controls, generated using Transcriptome Analysis Console 4.1. Colored dots represents up- and down-regulated transcripts (red and blue, respectively). Grey dots represents transcripts not classified as DEGs due to either high FDR p-value (y-axis) or low fold change (FC; x-axis). The significance threshold was determined at FC > 1.5 and FDR p-value < 0.05.

pathway inhibitor (TFPI), plasminogen activator, urokinase receptor (PLAUR) and thrombomodulin) was activated (z-score +0.45), whereas the senescence pathway and intrinsic prothrombin activation pathway (collagen type I and III, coagulation factor XIII, different types of anaphase-promoting complex subunits, calpain 7) were inhibited (z-score −1.5 and −2.0, respectively).

Among the most significant networks automatically identified by IPA, many were associated with the pathogenesis of atherosclerosis. Figure 4 presents an example of such pathway, termed "coagulation system" by IPA (significance score 22).

Discussion

In this study, we compared gene expression in PCAT of patients with advanced CAD and non-CAD controls. We found that (i) the top upregulated genes in CAD are involved in inflammation and atherosclerosis,[14–17] (ii) the top downregulated genes are responsible for cardiac ischaemia and remodelling,[29,30] platelet function,[31,32] mitochondrial function,[33] obesity,[34] and encode different types of collagen, and (iii) the top upstream regulators related with DEGs include molecules that have proinflammatory and atherosclerotic profile (HMGB2, PDGF and ECSIT). We identified one activated pathway related with

Table 1 Baseline Clinical Characteristics

Baseline Clinical Characteristics	Total (n=40)	CAD (n=21)	CON (n=19)	P
Age	66.6 ± 1.8	64.4 ± 4.4	67.9 ± 1.2	NS
Male (%)	68	71	63	NS
BMI	30.3 ± 1.0	29.0 ± 1.3	30.8 ± 1.2	NS
Risk factors (%)				
Prior MI	18	50	0	NS
Prior stroke	0.06	0	0.08	NS
Hypertension	89	83	92	NS
Diabetes	0	0	0	NS
Dyslipidemia	56	67	50	NS
Smoking	60	71	54	NS
Medications (%)				
ACEi/ARB	89	83	92	NS
β-blocker	79	83	77	NS
Statin	78	83	75	NS
ASA	89	100	83	NS
Indication for cardiosurgery (%)				
CABG		100	0	n.a.
AS	29	0	50	n.a.
MR	19	0	26	n.a.
AOA	18	0	24	n.a.
Mean ejection fraction (%)	54.3	47	58.3	NS

Abbreviations: ACEI, angiotensin-converting-enzyme inhibitors; AOA, aortic aneurysm; ARB, angiotensin receptor blockers; AS, aortic stenosis; ASA, acetylsalicylic acid; BMI, body mass index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CON, control group; MI, myocardial infarction; MR, mitral regurgitation; n.a, not applicable; NS, not significant.

DEGs, associated with coagulation system, and two inhibited pathways related with DEGs, associated with senescence and intrinsic prothrombin activation pathway. In addition, this is one of the first studies showing that the upregulated DEGs and activated pathways form a pro-atherosclerotic network (Figure 1) in patients with CAD, which proves the involvement of PCAT in the atherosclerosis progression.

Previously, we showed that genes coding multiple inflammatory mediators are upregulated in EAT/PCAT in patients with advanced CAD, compared to SAT.[10] In line with our previous results, here we also showed the upregulation of genes coding IL-6, CXCL2 and thrombomodulin in PCAT of CAD patients. In the present study, the genes which were most upregulated in patients with CAD compared to controls were coding several proinflammatory and proatherogenic molecules, including chemokines, IL-6, selectin E and LDL-C receptor. The contribution of IL-6, selectin E and LDL-C receptor in the pathogenesis of atherosclerosis is well established.[35–37] There is no doubt that IL-6 plays one of the key roles in atherosclerosis at various stages of plaque development. IL-6 stimulates the synthesis of acute-phase proteins, activates endothelial cells and hypothalamic–pituitary–adrenal axis, increased coagulation (primarily through inducing monocyte expression of tissue factor), causes lymphocyte proliferation and differentiation and intensifies the influx of inflammatory cells.[35] E-selectin belongs to the Selectin family which mainly participate in the rolling adhesion of leukocytes. Activated endothelial cells express different types of molecules including E-selecting which attract lymphocytes and monocytes that bind to the endothelium and infiltrate arterial wall contributing to inflammation process.[35] The LDL receptor family consists of multiple transmembrane proteins that play an important role in a wide range of biological process including lipid metabolism, thrombosis and atherogenesis. There is increasing evidence that members of this family are involved not only in lipid metabolism but also as signal transducing receptors and thus may contribute to the progression of atherosclerosis.[37] We also showed increased level of expression of metalloproteinase ADAMTS4 compared to the control group. The

Figure 2 Main canonical pathways identified by Ingenuity Pathway Analysis, based on the relationships with differentially expressed genes. The pathways are ordered and listed by the -log (p-value), with threshold (orange line) representing p-value of 0.05. The colors of bar charts represent predicted dictionality described by z-score. Positive z-score indicates activation (orange bars), whereas negative z-score indicates inhibition (blue bars) of the pathway in pericoronary adipose tissue of patients with coronary artery disease, compared to controls. Bars marked as white and grey represent pathways with neutral z-score or pathways with no activity pattern available, respectively.

involvement of this proteinase in the pathogenesis of CAD, especially acute coronary syndromes, has been described in both humans and mice.[38–40] Very interesting results were presented by Zha et al who identified the expression level of ADAMTS4 in plasma and monocytes as a marker of plaque instability. In their observation, the ADAMTS4 level increased successively from the control group, through unstable angina, NSTEMI to STEMI.[38,39] It was also showed that the release of adipokines by EAT is dysregulated in obesity and CAD, and that EAT-derived adipokines induce pathophysiological changes in human monocytes and coronary artery endothelial cells.[42] Several studies showed that the immune cell phenotype in PCAT is important in the pathogenesis of CAD.[43,44] Altogether, numerous studies in mouse and human models confirmed that EAT has more proinflammatory profile of gene

Table 2 Top 20 Upregulated Genes in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease, Compared to Controls

Gene Symbol	Fold Change	FDR p-value	Description
CXCL8	193.15	0.0186	Chemokine (C-X-C motif) ligand 8
SELE	91.14	0.0326	Selectin E
IL6	48.60	0.0150	Interleukin 6
ADAMTS4	28.12	0.0480	Metallopeptidase with thrombospondin type 1 motif 4
CXCL2	18.79	0.0269	Chemokine (C-X-C motif) ligand 2
KLF10	16.13	0.0160	Kruppel like factor 10
AREG	16.02	0.0459	Amphiregulin
TRIB1	11.18	0.0216	Tribbles pseudokinase 1
LDLR	11.11	0.0466	Low density lipoprotein receptor
PLAUR	10.80	0.0492	Plasminogen activator, urokinase receptor
PPP1R15B	8.56	0.0141	Protein phosphatase 1 regulatory subunit 15B
CD55	8.32	0.0453	CD55 molecule (Cromer blood group)
ATP1B3	7.84	0.0452	ATPase Na+/K+ transporting subunit beta 3
mir-3156	7.12	0.0338	microRNA 3156–2
TNFAIP6	7.12	0.0211	TNF alpha induced protein 6
DDX21	6.83	0.0324	DExD-box helicase 21
ERRFI1	6.47	0.0386	ERBB receptor feedback inhibitor 1
GPRC5A	6.25	0.0367	G protein-coupled receptor class C group 5 member A
mir-3661	6.16	0.0097	microRNA 3661
KDM6B	6.13	0.0399	Lysine demethylase 6B

Table 3 Top 20 Downregulated Genes in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease Compared to Controls

Gene Symbol	Fold Change	FDR p-value	Description
COL4A4	−9.10	0.003	Collagen type IV alpha 4 chain
KANSL1	−7.19	0.0301	KAT8 regulatory NSL complex subunit 1
MIR548X	−6.67	0.0332	microRNA 548x
MIR4524A	−6.39	0.0131	microRNA 4524a
SLC40A1	−5.70	0.022	Solute carrier family 40 member 1
COL1A2	−5.52	0.0132	Collagen type 1 alpha 2 chain
TFPI	−5.26	0.0132	Tissue factor pathway inhibitor
HERC2P2	−5.21	0.0288	Hect domain and RLD 2 pseudogene 2
mir-1299	−5.17	0.0155	microRNA 1299
MMRN1	−5.16	0.0189	Multimerin 1
BGN	−5.14	0.012	Biglycan
CCL21	−5.02	0.0377	Chemokine (C-C motif) ligand 21
MIR3671	−4.73	0.0237	microRNA 3671
F13A1	−4.70	0.0452	Coagulation factor XIII A
FLRT2	−4.44	0.0326	Fibronectin leucine rich transmembrane protein 2
HERC2P3	−4.41	0.0156	Hect domain and RLD 2 pseudogene 3
SMA4	−4.18	0.020	Glucuronidase beta pseudogene
ATM	−4.16	0.0135	ATM serine/threonine kinase
ART4	−4.15	0.0186	ADP-ribosyltransferase 4
COL3A1	−4.13	0.0221	Collagen type III alpha 1 chain

Table 4 Top Upstream Regulators Identified by Ingenuity Pathway Analysis, Based on the Relationships with Differentially Expressed Genes

Regulator	Type	State	Z-Score	Description
HMGB2	Transcription regulator	-	-	High Mobility Group Box 2
PDGF BB	Complex	Activated	3,46	Platelet Derived Growth Factor BB
ECSIT	Transcription regulator	Activated	2,65	Evolutionarily Conserved Signaling Intermediate in Toll pathway
CD24	Other	Inhibited	−2,67	CD24 molecule
CDK9	Kinase	Activated	2,21	Cyclin-dependent kinase 9

Notes: Positive z-score indicates activation, whereas negative z-score indicates inhibition of the regulator in pericoronary adipose tissue of patients with coronary artery disease compared to controls. Lack of z-score activation pattern is indicated by "-".

expression compared to subcutaneous tissue, mediastinal adipose tissue or perirenal fat.[10,14,19,41]

The top downregulated genes included those responsible for cardiac ischaemia and remodelling (miR-3671, miR-4524a),[29,30] platelet function (multimerin, TFPI),[31,32] mitochondrial function (glucuronidases),[33] obesity (miR-548),[34] and encode different types of collagens (type I, III, IV). In line with our finding, other authors also found specific miRNA and gene signatures of EAT in CAD, with downregulation of genes involved in mitochondrial function, lipid metabolism, nuclear receptor transcriptional activity, and upregulation of those involved in chemokine signalling and inflammation.[20] Other downregulated genes included the gene for multimerin 1 and biglycan, which are known to be involved in platelet function and in the pathogenesis of atherosclerosis, respectively.[31,45] Biglycan is one the most important proteoglycans in the extracellular matrix of the vascular intima. There are many studies that directly link traditional cardiovascular risk factors such as hypertension, smoking or diabetes with increased expression of biglycan.[45] TFPI also has an established position in CAD pathogenesis,[32] and it seems that TFPI effect on the development of atherosclerosis may depend on the genetic polymorphism.[46] Tissue factor (TF) by promoting thrombus formation, inflammation, migration and proliferation of vascular smooth muscle cells (VSMC) is directly involved in CAD pathogenesis. TFPI's role is to inhibit these unfavorable processes. It should be emphasized that TFPI works not only by affecting the coagulation system but also by inhibiting the activity of endothelial cells, inhibiting the proliferation and migration of VSMC, promoting apoptosis of macrophages at plaques or inhibiting the secretion of proinflammatory factors.[32] Thus, the decreased expression of TFPI in the group of CAD patients confirms its protective role. MiR-548, which was among the downregulated microRNAs, was recently showed to regulate the expression of HMGB1.[47] HMGB1 is a nonhistone chromatin-binding protein that is involved in the regulation of transcription, DNA replication, and repair.[48] HMGB1 is secreted by cells either actively, in response to stimulation by proinflammatory cytokines or endotoxins,[49,50] or passively from necrotic or damaged cells.[51,52] In the extracellular environment, HMGB1 induces the release of proinflammatory cytokines and chemokines and exposure of the adhesion molecules on the endothelium and macrophages, thereby taking part in the pathogenesis of CAD.[53] Among the different types of downregulated types of collagens, type III was found, which facilitates platelets aggregation and plays an important role in blood clotting.[54]

The top upstream regulator related with DEGs were HMGB2, PDGF and ESCIT. HMGB2 is a member of HMGB family and has a similar role in atherosclerosis as HMGB1, which has been discussed before. In the extracellular compartment, HMGB2 acts as a chemokine and may promote proliferation and migration of endothelial cells.[55] Overexpression of PDGF may contribute to the development of atherosclerosis.[56] PDGF and its receptor are an intensively studied therapeutic target in patients with CAD.[57] Especially interesting is activated status of PDGF in our study in patients with CAD. This is in the line with literature which reports that overactivity of PDGF is associated with atherosclerosis.[56] Under normal conditions, its expression in arteries is low but increases after the inflammatory fibroproliferative response.[56] This is confirmed by the results of animal studies, in which the use of anti-PDGF-B antibodies reduced the incidence of atherosclerosis and restenosis after angioplasty.[58,59] ESCIT, activated in our study, is an adapter protein of the Toll-like and IL-1 receptor signaling pathway that is involved in the activation of nuclear factor (NF)-kappa β,[60] which in turn is a pivotal mediator of inflammatory response.[61] Toll-like receptor 4 (TLR4) can activate the transcription factors nuclear factor-κB leading to the production of proinflammatory cytokines.[36] Activation

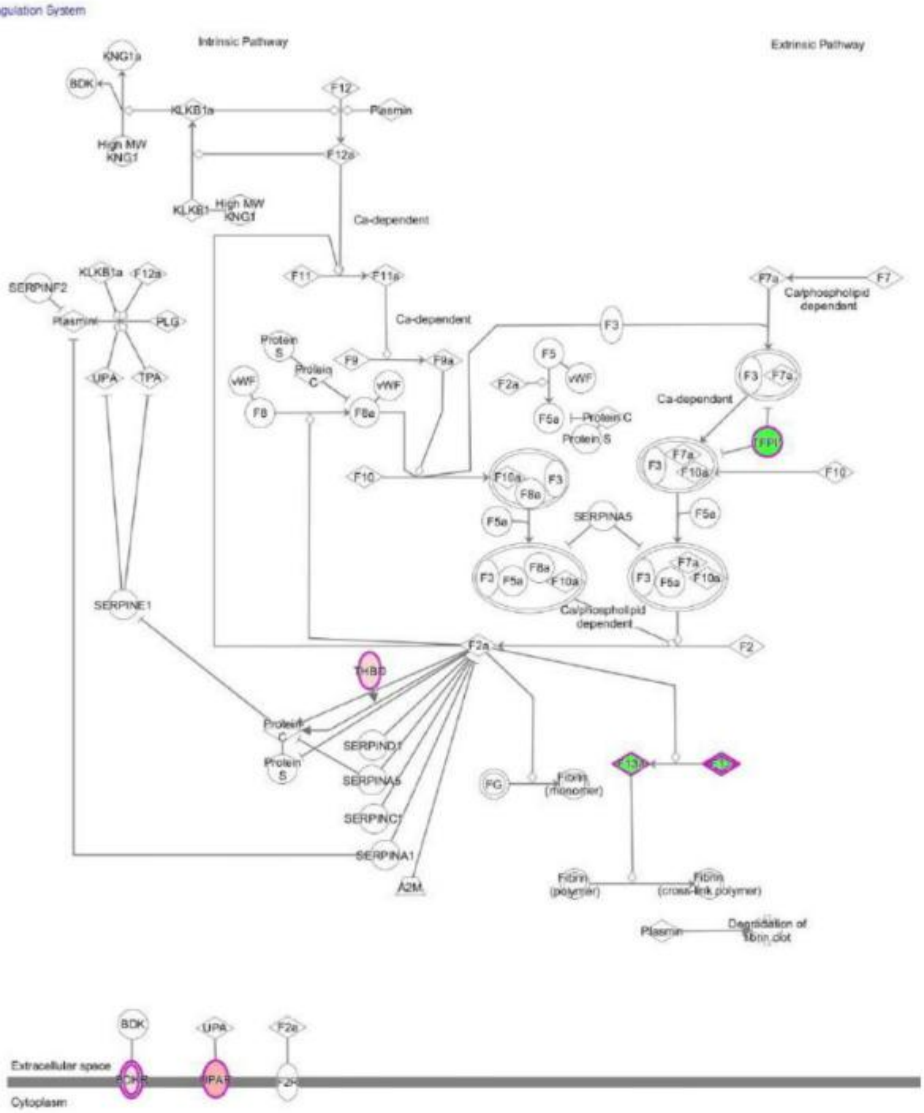

Figure 3 The coagulation pathway discovered by Ingenuity Pathway Analysis. The upregulated and downregulated genes are marked red and green, respectively.

of the NF-κB pathway has a crucial role in inflammation and can be induced by many factors including cytokines, adhesion molecules, chemokines, growth factors, and monocytes bound to the endothelium.[36,61] The activation of TLR4/NF-κB pathway promotes the plaque growth and instability.[62] These observations further confirm the proinflammatory gene expression profile in EAT in patients with CAD.

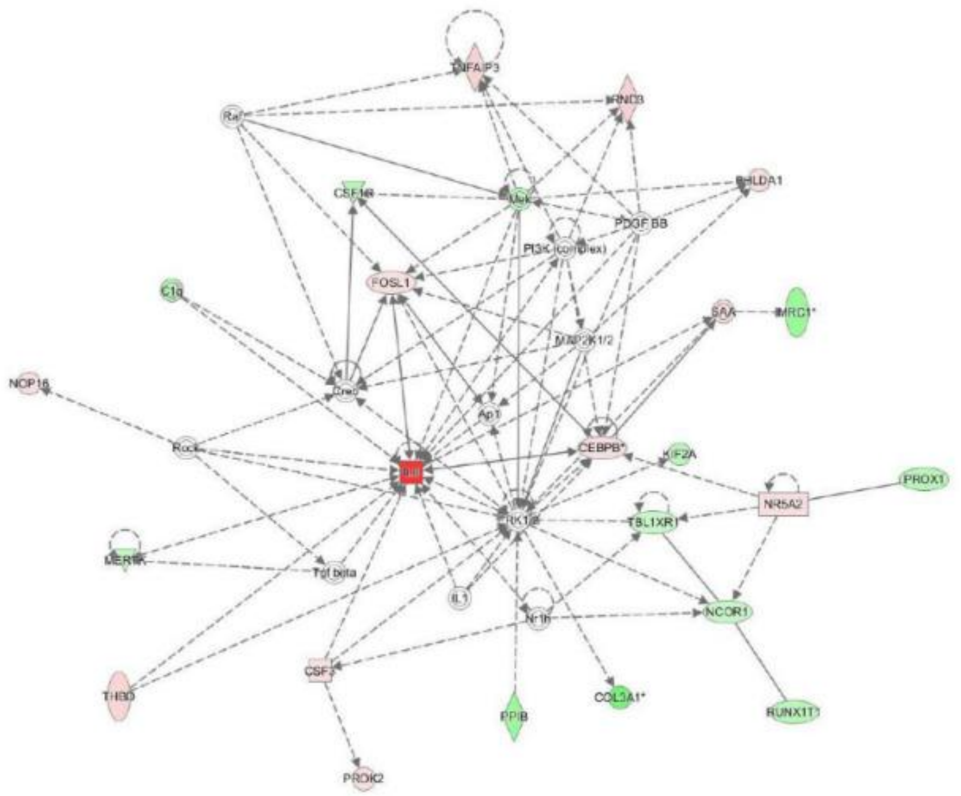

Figure 4 Interactions between differentially expressed genes found by Ingenuity Pathway Analysis, forming the network involved in atherosclerosis progression. The upregulated and downregulated genes are marked red and green, respectively. Grey arrows indicate the direction of regulation.

We identified one activated pathway related with DEGs, associated with coagulation system, and two inhibited pathways related with DEGs, associated with senescence and intrinsic prothrombin activation pathway. The pathway associated with coagulation system included TFPI, plasminogen activator, urokinase receptor (PLAUR) and thrombomodulin. The role of TFPI in the pathogenesis of CAD has been mentioned before. There are also reports in the literature about the important role of PLAUR in this process.[63] For example, the functional genetic variation of the PLAUR gene may affect the susceptibility to myocardial infarction.[64] The involvement of thrombomodulin in CAD pathogenesis is confirmed as well.[65] Moreover, as with PLAUR, there are reports about potential association of thrombomodulin gene polymorphisms with susceptibility to atherosclerosis.[66,67]

Finally, to the best of our knowledge, this is one of the first studies which showed not only the altered expression of individual genes in PCAT of CAD patients but also the entire pro-atherogenic pathways and networks formed by these genes, providing a much stronger proof that PCAT is a metabolically active source of proinflammatory molecules, involved in atherosclerosis progression.

Limitations

The main limitation of the study is the lack of data validation using polymerase chain reaction, which was not feasible due to the insufficient quantity of the collected biological samples. Since the samples were collected during open-heart surgery, validation would require setting up a new study, which we are planning to do in the near future. Moreover, since our study group included patients with severe, symptomatic CAD qualified for CABG, the results cannot be extrapolated to patients with less severe CAD. Third, based on our relatively small, observatory study, it is difficult to identify one specific pathway associated with the development and progression of CAD. Although we were able to show that the DEGs and upstream regulators contribute to the entire pathways and networks, our data still should be seen as a snapshot in time based on the most recent knowledge, but will most likely be expanded by further updated of the bioinformatic database used in our study. Finally, differences in gene expression between CAD and control group does not prove the causality between DEGs and CAD, neither the mechanisms how DEGs directly contribute to CAD development and progression.

Conclusion

PCAT of CAD patients has specific gene expression pattern, with upregulation of genes involved in inflammation and atherosclerosis (chemokines, IL-6, selectin E and LDL-C receptor), and downregulation of genes involved in cardiac ischaemia and remodelling, platelet function and mitochondrial function (miR-3671, miR-4524a, multimerin, biglycan, TFPI, glucuronidases, miR-548, collagen type I, III, IV). Among the top upstream regulators, we identified molecules that have proinflammatory and atherosclerotic features (HMGB2, PDGF and ESCIT). The activated pathway related to DEGs consisted of molecules with well-established role in the pathogenesis of atherosclerosis (TFPI, plasminogen activator, PLAUR, thrombomodulin). Most importantly, we showed the presence of entire pathways and networks composed of these genes. Altogether, the proinflammatory gene expression profile in EAT in patients with CAD seems involved in CAD development and progression.

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Disclosure

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6.3. Role of Epicardial Adipose Tissue in Cardiovascular Diseases: A Review.

biology MDPI

Review

Role of Epicardial Adipose Tissue in Cardiovascular Diseases: A Review

Michał Konwerski, Aleksandra Gąsecka, Grzegorz Opolski, Marcin Grabowski and Tomasz Mazurek *

1st Chair and Department of Cardiology, Medical University of Warsaw, 02-097 Warszawa, Poland; konwerski.mich@gmail.com (M.K.); aleksandra.gasecka@wum.edu.pl (A.G.); grzegorz.opolski@wum.edu.pl (G.O.); marcin.grabowski@wum.edu.pl (M.G.)
* Correspondence: tmazurek@kardia.edu.pl; Tel.: +48-(22)-5991958

Simple Summary: Cardiovascular diseases (CVDs) are the leading causes of death worldwide. Epicardial adipose tissue (EAT) is one of the most important risk factors for cardiovascular events and a promising new therapeutic target in CVDs. Here, we summarize the currently available evidence regarding the role of EAT in the development of CVDs, including coronary artery disease, heart failure and atrial fibrillation; compile data regarding the association between EAT's function and the course of COVID-19; and present new potential therapeutic possibilities, aiming at modifying EAT's function. The development of novel therapies specifically targeting EAT could revolutionize the prognosis in CVDs.

Abstract: Cardiovascular diseases (CVDs) are the leading causes of death worldwide. Epicardial adipose tissue (EAT) is defined as a fat depot localized between the myocardial surface and the visceral layer of the pericardium and is a type of visceral fat. EAT is one of the most important risk factors for atherosclerosis and cardiovascular events and a promising new therapeutic target in CVDs. In health conditions, EAT has a protective function, including protection against hypothermia or mechanical stress, providing myocardial energy supply from free fatty acid and release of adiponectin. In patients with obesity, metabolic syndrome, or diabetes mellitus, EAT becomes a deleterious tissue promoting the development of CVDs. Previously, we showed an adverse modulation of gene expression in pericoronary adipose tissue in patients with coronary artery disease (CAD). Here, we summarize the currently available evidence regarding the role of EAT in the development of CVDs, including CAD, heart failure, and atrial fibrillation. Due to the rapid development of the COVID-19 pandemic, we also discuss data regarding the association between EAT and the course of COVID-19. Finally, we present the potential therapeutic possibilities aiming at modifying EAT's function. The development of novel therapies specifically targeting EAT could revolutionize the prognosis in CVDs.

Keywords: atherosclerosis; cardiovascular diseases; epicardial adipose tissue; EAT; inflammation

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1. Introduction

Cardiovascular diseases (CVDs) remain one of the leading causes of death worldwide [1,2], entailing enormous costs for healthcare systems [3]. Many of them could be avoided, as cardiovascular risk factors are largely reversible.

Epicardial adipose tissue (EAT) is defined as a fat depot localized between the myocardial surface and the visceral layer of the pericardium, and is a type of visceral fat [4]. Therefore, as is the case with abdominal obesity, EAT is one of the most important risk factors for atherosclerosis and cardiovascular events [5–7]. Moreover, the volume and thickness of EAT correlate with intra-abdominal fat mass and severity of obesity [8,9] and are independently associated with cardiovascular events [10]. Additionally, there are reports that EAT may be a new therapeutic target in CVDs [11,12].

The most common classification of the adipose tissue surrounding the heart includes (i) epicardial adipose tissue, (ii) pericardial adipose tissue, (iii) paracardial adipose tissue, and (iv) perivascular adipose tissue (Figure 1). Epicardial fat is located below the visceral pericardium. Pericardial fat consists of adipose tissues between the visceral and parietal pericardial layers and the fat depot on the external surface of the parietal pericardium. Paracardial fat involves fat deposits outside the parietal pericardium. The perivascular adipose tissue is a fat around the coronary arteries, irrespective of location [13].

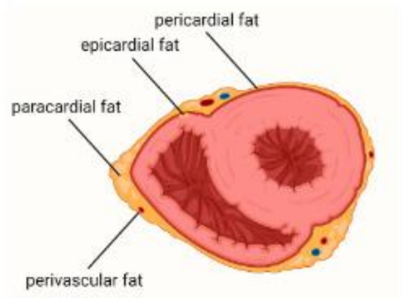

Figure 1. Adipose tissue surrounding the heart.

There are many differences between EAT and other types of adipose tissue, including anatomical, histological, embryological, and genetic differences [14,15]. EAT is located between the pericardium and the myocardium [5] and is not separated from the myocardium and vessels by fascia, allowing paracrine or vasocrine effects [16] via cytokines and chemokines [17]. In health conditions, EAT has a protective function, including protection against hypothermia [18] or mechanical protection for the coronary circulation [19]. Additionally, EAT has an important role in energy supply to the myocardium [20]. Thanks to the ability to use free fatty acid (FFA) quickly, EAT may protect the myocardium from the cardiotoxic effect of a large amount of FFA [21]. The secretion of adiponectin from epicardial adipocytes is also an important function of EAT. Adiponectin protects coronary circulation, improves endothelial function, reduces oxidative stress, and indirectly decreases the level of interleukin-6 (IL-6) and C-reactive protein (CRP) [22–24]. However, under specific conditions such as obesity, metabolic syndrome, or diabetes mellitus, the protective properties may be destroyed and EAT becomes a deleterious tissue promoting the development of CVDs. The most data on the transition of EAT's role is based on the observations of patients with coronary artery disease (CAD). For example, in one of our studies we showed a difference in gene expression in pericoronary adipose tissue in patients with and without CAD [25].

In this article, we summarize the currently available evidence regarding the role of EAT in the development of CVDs, including CAD, heart failure (HF) and atrial fibrillation (AF). Due to the rapid development of the COVID-19 pandemic, we also summarize data regarding the association between EAT's function and the course of COVID-19. Finally, we present the potential therapeutic possibilities aiming at modifying EAT's function in CVD. The role of EAT in the development of CVDs and COVID-19 is summarized in Figure 2.

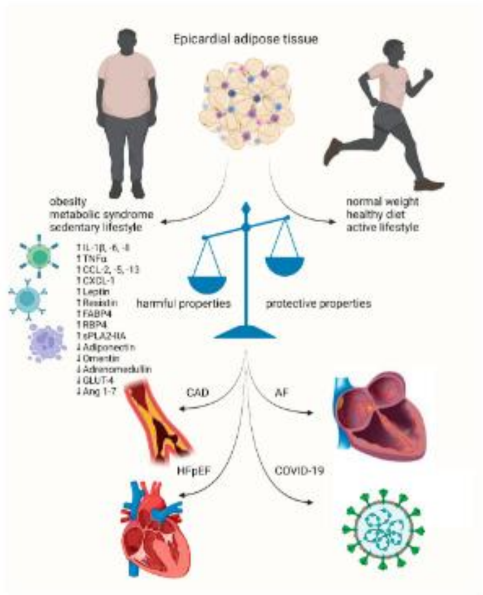

Figure 2. The role of epicardial adipose tissue in the development of cardiovascular diseases and cardiovascular complications in the course of COVID-19. AF—atrial fibrillation; Ang 1–7—angiotensin 1–7; CAD—coronary artery disease; CCL-2, -5, -13—chemokine ligand-2, -5, -13; CXCL-1— chemokine ligand 1; FABP4—fatty acid binding protein 4; GLUT-4—glucose transporter type 4; HFpEF—heart failure with preserved ejection fraction; IL-1β, -6, -8—interleukin-1β, -6, -8; RBP4— retinol binding protein 4; sPLA2-IIA—secretory phospholipase A2; TNFα—tumor necrosis factor α.

2. Coronary Artery Disease

It has been shown that EAT and CAD are closely related at different levels: (i) in patients with CAD, the secretion of adipokines from EAT is altered; (ii) EAT has a proinflammatory features in patients with CVD risk factors and/or CAD; (iii) the amount of EAT and/or its proinflammatory state correlate with the severity of CAD and the instability of the atherosclerotic plaques; (iv) there is a relationship between EAT's function and coronary microvascular dysfunction and artery spasm [14,17,26–49].

In patients with obesity, metabolic syndrome, or CAD, the epicardial adipocytes secrete less adiponectin and more leptin than in healthy people [26,27]. The decreased adiponectin expression attenuates endothelial function and leads to increased tumor necrosis factor-α (TNF-α) production, which increases inflammation and oxidative stress. The increased leptin level promotes adhesion of monocytes, macrophage-to-foam cell transformation, and unfavorable changes in lipid and inflammatory cytokine levels in adipose tissue [28]. All these processes result in the development and destabilization of atherosclerotic plaques [29].

Inflammation plays a crucial role in atherosclerosis, and EAT as a tissue with proinflammatory properties provides a huge contribution to coronary plaque formation [14,17,30,31]. For example, Mazurek et al. showed that plasma inflammatory biomarkers did not adequately reflect local tissue inflammation [17]. Proinflammatory properties of EAT were noted irrespective of clinical variables (diabetes, body mass index, and chronic use of statins or angiotensin receptor enzyme inhibitors/angiotensin II receptor blockers) or plasma concentrations of circulating biomarkers. [17]. Autopsy studies have shown the presence of inflammatory cells in the adventitia in patients with acute coronary syndrome [32].

Furthermore, there was a correlation between the degree of severity of the lesion and the intensification of the inflammatory infiltration in the adventitia [32].

Wang et al. assessed EAT in computed tomography (CT) in patients with and without diabetes, showing that EAT volume (EATV) is higher in diabetic patients and is associated with components of metabolic syndrome, coronary atherosclerosis, and coronary calcium scores [33]. In the Framingham Study, a significant association was found between epicardial fat and coronary artery calcification, which was significant after adjustment for traditional cardiovascular risk factors [34]. Alexopoulos et al. showed that EATV increased significantly with the severity of luminal coronary stenosis and was larger in patients with mixed or noncalcified plaques, compared to patients with calcified plaques or no plaques, indicating the association between EAT and the most dangerous plaque phenotype [35]. This association has been confirmed in other studies [36–38]. Yamashita et al. showed that EATV, assessed by CT, is associated with the total coronary plaque burden. Higher EATV was associated with a higher vulnerability of atherosclerotic plaques, based on the evaluation of atherosclerotic plaque composition by intravascular ultrasound imaging (IVUS) [36]. Mazurek et al. made a qualitative assessment of pericoronary adipose tissue (PCAT) using positron emission tomography/computed tomography (PET/CT) among patient with acute coronary syndrome without persistent ST-segment elevation [37] and with stable CAD [38]. In the first study, the inflammatory activity of PCAT, reflected by maximum fluorodeoxyglucose (FDG) uptake, was greater than the activity of adipose tissue in other locations [37]. There was also a correlation between the severity of atherosclerosis and the necrotic core volume of coronary plaque, as assessed by virtual histology IVUS [37]. Similar results were obtained in patients with stable CAD [38]. In another study, EAT thickness was associated with the Thrombolysis in Myocardial Infarction risk score in unstable angina and non-ST-elevation myocardial infarction [39]. Otsuka et al. demonstrated that EATV is associated with the presence of high-risk plaques, the so-called low attenuation plaques in CT, regardless of the presence of abdominal obesity [40]. Patients with higher visceral fat had a greater total plaque volume and a greater level of low-attenuation plaques [40]. Another study assessed the PCAT mean attenuation (PCAT-MA) based on CT as a measure of inflammation in EAT in patients with CAD [41]. PCAT-MA was higher in coronary arteries with plaque compared to vessels without plaque. The lesion-specific PCAT-MA was higher in noncalcified and mixed plaques compared to calcified plaques. These results suggest that lesion-specific PCAT-MA is related to plaque development and vulnerability [41]. In patients with acute myocardial infarction (MI), PCAT attenuation did not differentiate between the coronary segments with and without culprit lesions, but PCAT volume was strongly and independently associated with culprit lesions [42]. In contrast, Goeller et al. showed that PCAT attenuation was increased around culprit lesions compared with nonculprit lesions among patients with acute coronary syndrome [43]. In stable CAD patients, an increase in PCAT attenuation was associated with progression of noncalcified plaque burden and vice versa [44]. Nogic et al. reported that a higher lesion-specific PCAT attenuation baseline may predict in-stent restenosis among patients undergoing elective percutaneous coronary intervention [45]. Altogether, these results confirm that the amount of EAT and/or EAT proinflammatory state correlate with the severity of CAD and plaque vulnerability.

In women with chest pain and angiographically normal coronary arteries, there was a correlation between EAT thickness and reduced coronary flow reserve [46]. Kanaji et al. showed that in CAD patients with a single de novo lesion, PCAT attenuation is significantly associated with global coronary flow reserve [47]. Pasqualetto et al. suggested an association between PCAT attenuation in CT with coronary microvascular dysfunction; however, a significant correlation was found only in patients without severe obstructive CAD [48]. Another study found that EAT might be associated with coronary artery spasms [49]. Further studies are needed to investigate the relationship between EAT/PCAT and coronary microvascular dysfunction and vasospastic angina.

Finally, attention should be paid to the current tendency to study the relationship of CAD not only with the thickness and volume of EAT, but also with its structure and size of adipocytes [50,51]. One study has found that the size and degree of hypertrophy of the epicardial adipocytes are related to CAD severity [51].

3. Heart Failure

Among patients with HF, approximately 50% have preserved ejection fraction (HFpEF). HFpEF is a heterogeneous disease with a complex pathogenesis which is not fully understood. This complexity is due to the fact that it can be caused or exacerbated by a variety of comorbidities, including cardiac and extracardiac abnormalities. Thus, the group of patients with HFpEF is very diverse [52,53]. HFpEF is the most common myocardium disorder among obese patients [54]. Savji et al. showed that higher body mass index (BMI) is associated with higher risk of HFpEF than with HF with reduced ejection fraction (HFrEF), and that it was most pronounced among women [55]. Similarly, cardiometabolic features, including insulin resistance, were associated with a higher risk of future HFpEF than with HFrEF [55].

Based on the hitherto studies, it can be concluded that: (i) there is an association between EATV and the development of HfpEF; (ii) patients with HFpEF and obesity represent a distinct phenotype of the disease; (iii) EAT thickness or volume may have a greater impact on HFpEF than obesity per se; (iv) EAT participates in the pathogenesis of HfpEF due to EAT's proinflammatory properties, intensification of fibrosis, and influence on myocardial substrate utilization.

There were several studies that showed a correlation between the severity of left ventricle (LV) diastolic dysfunction and the volume of EAT [56–58]. A meta-analysis of 22 studies including 5682 patients also confirmed the correlation between EATV with myocardial diastolic function [59].

Patients with coexisting obesity and HFpEF had a different clinical phenotype than patients with HFpEF without obesity [60]. Obokata et al. compared patients with HFpEF and obesity, HPpEF without obesity, and a non-obese control group without HF [60]. Among obese HFpEF patients, diabetes and sleep apnea were more prevalent, whereas in the non-obese HFpEF patients, atrial fibrillation was more common. Additionally, the obese HFpEF patients had lower concentrations of N-terminal prohormone of brain natriuretic peptide (NT-proBNP), compared to the non-obese cohort. Furthermore, subjects with the obese HFpEF phenotype had increased plasma volume, a higher rate of concentric LV remodeling, greater right ventricular (RV) dilatation, and a higher rate of RV dysfunction. Obese patients also displayed worse exercise capacity, more pronounced hemodynamic abnormalities during exercise, and impaired pulmonary vasodilation. EAT thickness assessed by echocardiography was 20% higher in the obese HF group compared to non-obese HF, and 50% higher compared to the control group [60]. This, along with much greater biventricular hypertrophy, causes an increase in the total heart volume in obese patients, followed by pericardial dilation. If pericardial dilation is insufficient, there is greater coupling between the pericardium, right heart and left heart (interventricular dependance), and pericardial restraint, as showed by greater septal flattening and higher ratio of pressure in the right atrium to pulmonary capillary wedge pressure at rest and during exercise in patients with the obese HFpEF phenotype [60]. Increased EAT thickness may change myocardial substrate utilization, including increased oxygen consumption, impaired oxygen use, and increased dependance on fatty acid oxidation, and thus contributes to a reduction in cardiac reserve and aerobic capacity among obese HFpEF [60–62]. In addition, space limitations in the cardiac fossa due to increased EAT thickness may aggravate RV dysfunction and contribute to an increase in intracardiac pressures, especially during exercise [60].

Koepp et al. examined patients with the obese phenotype of HfpEF and divided them into increased EAT thickness ($\geq$9 mm in echocardiography) and normal EAT thickness [63]. It has been demonstrated that obese HfpEF patients with increased EAT thickness have more pronounced hemodynamic derangements at rest and during exercise, including greater elevation in cardiac filling pressures, more severe pulmonary hypertension, and greater pericardial restraint than the obese HFpEF group with normal EAT thickness [63]. Additionally, peak oxygen consumption was 20% lower in patients with increased EAT compared to the normal EAT group [63]. Van Woerden et al. examined EAT in patients with the HF and LV ejection fraction (EF) > 40% (HFpEF and HF with mildly reduced EF) and in the healthy control group using cardiac magnetic resonance (CMR) [64]. It was shown that despite similar BMI, the HF group has significantly higher total and ventricular EATV compared to the control group, and there were no differences in atrial EATV between the groups. These results show that not obesity per se, but rather fat distribution, may contribute to HF development. Additionally, HF patients with AF and/or diabetes had more EAT than HF patients without these disorders. Patients with higher total EATV had higher plasma levels of troponin T, creatine kinase muscle-brain fraction and glycated hemoglobin, and worse kidney function. There were no significant associations between EATV and NT-proBNP concentration. In the HF group, total EATV was positively correlated with LV end-systolic volume and with left and right atrial volume. On the contrary, global longitudinal and circumferential strain were negatively correlated with total EATV [64].

In obese patients with increased plasma volume, the ability of LV to dilate is insufficient, leading to cardiac overfilling and disproportionate increases in cardiac filling pressures. It seems that the inadequate ventricular distensibility is caused by cardiac fibrosis and microvascular rarefaction [65,66]. Moreover, the quantity of fibrosis assessed in CMR is associated with prognosis and outcomes in HFpEF [67]. Obese patients displayed more EAT [8,9] and therefore it seems likely that they were more exposed to cytokines released from the EAT reservoir. As previously noted, in patients with CVDs, the EAT reservoir becomes a site of deranged adipogenesis and a source of proinflammatory factors with deleterious effects on myocardium, including fibrosis. Packer et al. postulated that epicardial fat is a transducer of systemic metabolic disorders and a systemic inflammatory state caused by obesity on the heart [68]. Some researchers pointed to myocardial accumulation of triglycerides and myocardial fibrosis as the main causes of LV diastolic dysfunction [69–71]. Additionally, it has been shown that EAT can release vasoactive agents which, via vasa vasorum, reach the microvascular network and may reduce coronary flow reserve [72,73]. One of the most recent studies showed that EAT thickness was of prognostic value in patients with HFpEF, which may be due to increased mechanical restraint and secretion of proinflammatory and proatherogenic adipokines [74]. In contrast, in HFrEF, greater EAT thickness seems to have a protective role, while EAT thinning is associated with a worse prognosis [74]. It is suggested that measuring EAT thickness can be useful to classify patients with or at increased risk of heart failure [75].

Further studies are needed to better understand the influence of EAT on the pathogenesis of HFpEF and EAT's potential applicability as a target for novel drugs.

4. Atrial Fibrillation

AF is the most common arrythmia in the adult population in the world, and its prevalence is increasing. It is estimated that AF affects up to 4% of the population in Australia, Europe, and the USA [76]. The involvement of hemodynamic stress in the pathogenesis of AF is well-documented, and hypertension is the most common risk factor [77]. Valvular diseases also significantly contribute to the development of this arrhythmia [78]. These disorders cause the remodeling of heart chambers, including enlargement of the left atrium (LA) and an increase in LA pressure. Alleviation of hemodynamic stresses can reduce AF's burden [77,78]. However, there is a large group of patients with AF who do not have hypertension or valvular disease but do have the features of atrial myopathy (LA enlargement, increased LA pressure), as observed in imaging studies [79]. It is known that inflammation is associated with the development of atrial myopathy [80], including both inflammation in course of systemic inflammatory diseases [81–84] and metabolic disorders accompanied by adipose tissue inflammation [85,86]. The risk of developing AF is especially increased in rheumatoid arthritis [81] and psoriasis [82]. Among the metabolic diseases, special attention should be paid to obesity [85] and diabetes mellitus [86]. In these states, AF's burden was proportional to the severity of metabolic disorders, such as glycemic control [85–88].

The literature indicates several potential mechanisms linking EAT with AF, including: (i) proinflammatory status of EAT; (ii) reactive oxygen species (ROS) released from EAT; (iii) fatty infiltration of the atrium; (iv) dysfunction of the autonomic nervous system in EAT.

AF and inflammation are closely associated [80–86]. EAT can release inflammatory factors and contribute to inflammation and fibrosis in the adjacent myocardium via paracrine signaling. It should be emphasized that EAT has some features of brown adipose tissue, such as the presence of the uncoupling protein-1, which is a thermogenic protein specific to brown adipocytes [89]. These properties are mainly expressed in conditions of health and low oxidative stress [90–92]. The healthy EAT is a source of adiponectin, which may reduce inflammation and fibrosis [91–94]. In obesity, EAT loses its protective properties and becomes a tissue with a proinflammatory profile, subsequently increasing the risk of atrial myopathy and AF [95–99]. Mazurek et al. showed that inflammatory activity of EAT reflected by maximal standardized uptake value of FDG in PET/CT was higher in patients with AF than in the control group and it was not related to BMI [100].

It has been suggested that ROS play an important role in the pathogenesis of AF [101,102]. EAT has been shown to be richer in ROS than other fat depots [103], but at the same time, this effect was reduced by adiponectin [93].

It also seems that fatty infiltration into atrial myocardium plays an important role in the pathogenesis of AF, as demonstrated by histological examinations [104]. Fatty infiltration was more pronounced in persistent AF, compared with paroxysmal AF [105]. EATV and fatty infiltration were associated with cardiac conduction abnormalities [106]. It was postulated that EAT can change electrophysiological features and ion currents by cytokine, adipokine, and adipocyte infiltration, causing electrical substrate formation for AF [107]. Opolski et al. showed that increased EATV along the LA assessed by CT was associated with AF after coronary artery bypass grafting [108].

It should be noted that EAT contains significant amounts of ganglionated plexi which are a part of the autonomic nervous system (ANS), which may play a role in the pathogenesis of AF [109,110]. The thickness of the EAT was related to ANS dysfunction [111], and catheter ablation of epicardial fat-reduced cardiac ANS activity, which makes it an interesting therapeutic perspective [112].

There are also several other less-understood potential mechanisms which may explain the involvement of EAT in the pathogenesis of AF, such as the local aromatase effect [113–115]. A significant positive correlation was determined between the total aromatase content of EAT and the occurrence/duration of triggered atrial arrhythmias [114]. Further mechanisms are pending investigation.

In clinical terms, the relationship between epicardial adipose tissue and AF is extremely interesting. Obesity is a well-known risk factor for AF [85] and every 1 kg/m^2 reduction in BMI reduces the risk of AF by about 7% [116]. There is an association between the severity of obesity and the volume and thickness of EAT [8,9]. The relationship between AF and EAT has been investigated in many studies using noninvasive imaging methods such as transthoracic echocardiography, CT, or CMR, showing that: (i) the prevalence of AF is related to the volume/thickness of the EAT; (ii) EAT promotes AF persistence; (iii) higher EATV is associated with lower catheter ablation efficacy.

Results from the Framingham Heart Study involving 2317 patients who underwent CT showed that higher EATV was associated with 40% higher odds of AF and remained significant regardless of traditional risk factors including age, sex, MI, or HF [117]. Interestingly, there was no association between AF prevalence and adipose tissue elsewhere [117].

Chekakie et al. showed a relationship between EATV and both persistent and paroxysmal AF using CT imaging [118]. Patients with persistent AF had a higher EATV than patients with paroxysmal AF or sinus rhythm [118]. The same conclusions were reached by Batal et al., who suggested that EAT can promote AF persistence [119]. Muhib et al.

showed similar results in patients with hypertrophic cardiomyopathy using CMR to assess EAT [120].

From a clinical point of view, Wong et al. [121] and Tsao et al. showed a strong relationship between EAT and AF recurrence after catheter ablation. Patients with higher EATV had worse outcomes and early AF recurrence after ablation [121,122].

Finally, two meta-analyses confirmed a relationship between AF and EAT [123,124]. Wong et al. showed the strongest association between persistent AF and EAT, but the association with paroxysmal AF was also significant [123]. Interestingly, the strength of associations between AF with EAT was greater than for between AF and abdominal or overall adiposity [123]. Gaeta et al., based on the analysis of seven imaging studies, demonstrated a 32 mL higher EATV between the AF group and patients with sinus rhythm [124], further indicating that EAT plays a crucial role in AF development and persistence.

5. COVID-19

COVID-19 is a complex multisystem infectious disease caused by the SARS-CoV-2 virus, which predominantly affects the lungs [125]. Since COVID-19 was first diagnosed in December 2019, it has caused a significant burden to healthcare systems worldwide. Therefore, there is an urgent need to investigate the pathophysiological mechanisms underlying COVID-19.

After more than a year of the COVID-19 pandemic, there are reports indicating a potential relationship between EATV and cardiovascular complications of SARS-CoV-2 infection. Systemic inflammation has a central role in the development and progression of COVID-19 [126,127], and there are several studies which have shown that inflammatory and thrombotic biomarkers such as D-dimer or ferritin predict the clinical severity of COVID-19 [128–130]. Growing evidence shows that obesity adversely affects the course of mortality due to COVID-19 [131]. In one study, it has been postulated that EAT may have immunomodulatory properties and may be a reservoir for SARS-CoV-2, thus facilitating the spread of the virus and enhancing the inflammatory response [132].

Studies conducted so far on a small number of patients suggest that EATV and attenuation in CT: (i) were independent predictors of severe and unfavorable COVID-19 courses, including death; (ii) may be associated with cardiovascular complications in patients with COVID-19.

Abrishami et al. assessed inflammatory parameters (including CRP) and EAT (volume and density) by CT on admission in 100 patients with COVID-19 [133]. Patients were followed until death or discharge. The mortality rate was 17% and was higher in obese patients. Among laboratory tests, increased lactate dehydrogenase (LDH) and decreased platelet count were significantly associated with death. EATV was similar in patients who died and in those who survived, but EAT density was significantly lower in patients who died ($p = 0.79$ and $p = 0.008$, respectively) [133]. Similar results were obtained by Deng et al. among patients aged 18 to 40. In addition, patients with severe COVID-19 had significantly higher EATV [134].

Iacobellis et al. assessed EAT thickness and density depending on the COVID-19 severity [135]. Patients with most severe course of COVID-19 had significantly greater EAT attenuation than those presenting with mild and moderate COVID-19 ($p \leq 0.01$), but EAT thickness was similar in all patients [135].

Grodecki et al. examined the relationship of EAT quantified on CT with the severity of pneumonia and adverse outcomes among patients with COVID-19 [136]. The primary outcome was clinical deterioration (intensive care unit admission, invasive mechanical ventilation, or vasopressor therapy) or in-hospital death. Among 109 patients, the primary outcome occurred in 21.1% of patients, and both EATV and attenuation were independent predictors of clinical deterioration or death ($p = 0.011$ and $p = 0.003$, respectively). The severity of pneumonia was also associated with these EAT parameters. Further, there was a correlation between EATV and CRP level and LDH level [136]. The results of the above-mentioned studies indicate that the assessment of EAT by CT may be useful in risk stratification in patients suffering from SARS-CoV-2 infection. However, all these studies were performed in small groups of patients (n = 41–109) and studies on larger groups are needed.

COVID-19 can cause myocardial injury and other cardiovascular complications, including acute myocarditis, pulmonary embolism, or acute heart failure [137–141]. The exact mechanism of heart damage in the course of SARS-CoV-2 infection is not fully understood, but it may occur directly or indirectly, or in both ways [137]. Some cardiovascular complications are asymptomatic during acute infection, but emerging data have reported on post-COVID-19 heart syndrome [142]. It has been suggested that low EAT density in CT may indicate myocardial injury, as it occurs mainly in severe and critical COVID-19 patients [143]. Wei et al. showed in a group of 400 patients with laboratory-confirmed COVID-19 that patients with COVID-19-associated myocardial injury had a history of CVDs, primarily hypertension, diabetes, hypercholesterolemia, and CAD. These patients had higher plasma concentrations of IL-6 and higher risk of adverse in-hospital events (death, invasive mechanical ventilation, admission to an intensive care unit). A chest CT performed on admission showed that these patients also had a higher EATV (139.1 vs. 92.6 cm^2, $p = 0.036$) and that EATV over 137.1 cm^2 was a strong independent predictor for myocardial injury in patients with COVID-19 [144].

Based on hitherto studies, EATV and EAT density seem to either reflect or affect the overall course of COVID-19, including pulmonary and cardiovascular complication, but more studies are needed to elucidate the mechanisms underlying this association.

6. Therapeutic Options to Affect EAT

Since EAT affects the development and progression of CVDs, EAT is a promising therapeutic target in cardiovascular patients. However, none of the therapeutic tools available to date have been specifically developed for EAT. However, it has been shown that (i) lifestyle changes, (ii) bariatric surgery, and (iii) pharmacotherapy can reduce EATV [145,146] by a pleiotropic or an off-target effect (Figure 3).

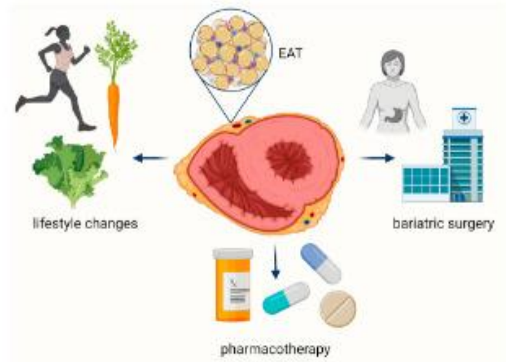

Figure 3. Therapeutic options to affect epicardial adipose tissue.

Both resistance [147,148] and endurance training reduce EATV [147,149,150]. Christensen et al. showed that physical activity can reduce EATV up to 32%, as assessed by CMR [147]. The results of our pilot study in 30 amateur ultramarathon runners are in line with these data [151]. We found that ultrarunners have significantly lower CMR-assessed EATV than the sedentary control group, lower rate of pathologically high levels of plasma IL-6 (>1 pg/mL) and better lipid profile [151]. Therefore, the benefits of regular physical activity to reduce cardiovascular risk may extend beyond the traditional risk factors, as physical activity seems to modulate the EATV and activity.

Another way to reduce EATV is diet [152–155]. Twenty severely obese subjects followed a 6-month weight-loss program with a very low-calorie diet, achieving a 33% reduction in echocardiographic EAT thickness [155].

There are several small-group studies ($n = 23$ to 65) investigating the effect of bariatric surgery on EAT [156–159]. Two years after bariatric surgery, EAT thickness was reduced by 31% in a group of 51 operated patients, as assessed by echocardiography [159].

Although the positive effect of lifestyle modifications of EAT and overall cardiovascular health is clear, the compliance remains a concern. Therefore, pharmacotherapy remains a field of great interest regarding the modulation of EATV and function. The following groups of drugs were shown to affect EAT: (i) statins, (ii) antidiabetic drugs, (iii) anti-inflammatory drugs.

Statins have been shown to decrease EATV [160,161]. Alexopoulos et al. demonstrated that statin therapy leads to a reduction in EATV, and intensive therapy was more effective than moderate-intensity therapy in a group of 420 postmenopausal women with hyperlipidemia [160]. There was no correlation between EATV and lipid-lowering effect. Hence, this effect may have been secondary to anti-inflammatory effects of statins [160], which is consistent with the reports on EAT in patients with severe aortic stenosis [162]. Parisi et al. showed a relationship between statin therapy, EAT thickness reduction, and EAT inflammatory status, both in vivo and in vitro [162]. Raggi et al. indicated that statins reduce EAT attenuation in CT independent of their lipid-lowering effect, which indirectly indicates a reduction in EAT inflammation [163]. The clinical benefit of statin therapy in patients with CAD has been known for a long time [164]. However, the range of statin pleiotropic effects is growing, including their anti-inflammatory effects and modulation of EAT [164]. Tawakol et al. showed that statin therapy resulted in rapid, dose-dependent reductions in FDG uptake in PET/CT, representing changes in atherosclerotic plaque inflammation [165]. Even short-term intensive statin therapy significantly reduced the volume of EAT compared to placebo in patients with AF who underwent pulmonary vein isolation [161]. Hence, the anti-inflammatory effect of statins on EAT seems to reduce the risk of atrial myopathy, as demonstrated both in animal and human models [166,167]. The antiarrhythmic effect of statins was also confirmed in randomized trials and was most pronounced in the secondary prevention of AF [168–170]. Statins also ameliorate cardiac fibrosis, as demonstrated in animal models of HFpEF [171,172]. The positive effect of statin therapy on LV's diastolic function was also seen in clinical settings [173,174]. Statin use is also associated with a reduced risk of morbidity and mortality in patients with HF [69,175]. In patients with HFpEF, statin use was associated with reduced mortality [176,177], which was confirmed in meta-analyses [178,179]. On the contrary, no benefit on clinical outcomes was observed in patients with HFrEF [180]. It has been suggested that statins can reduce EAT's metabolic activity [163]. With the exception of statins, Rivas Galvez et al. showed a significant reduction in thickness of EAT after 6 months of treatment with other lipid-lowering drugs, proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors [181]. All these data may suggest that statins and PCSK-9 inhibitors may exert their pleiotropic effects at least partly through EAT, although the underlying mechanisms of action are yet incompletely understood.

Another group of drugs that affect EAT are antidiabetic drugs, including thiazolidinediones, metformin, sodium-glucose cotransporter 2 (SGLT2) inhibitors, and incretin-based agents. Interest in antidiabetic drugs particularly increased after the publication of the results of the EMPA-REG and LEADER trials, which showed that SGLT2 inhibitors and glucagon-like peptide-1 receptor (GLP-1) agonists could have a cardioprotective effect by a mechanism independent of blood glucose level reduction [182,183]. The older groups of drugs (pioglitazone, metformin) also reduced the risk of cardiovascular complications in patients without diabetes, but with insulin resistance or pre-diabetes [184,185].

Metformin has been the most widely used antidiabetic drug for over 60 years. There is evidence of its anti-inflammatory effects on adipose tissue in diabetic and obese patients [186,187]. The anti-inflammatory effect of metformin has been confirmed by both its anti-aging and antitumor properties [188,189]. Similarly, pioglitazone was shown to reduce mast cells and inflammatory macrophages in adipose tissue [190,191]. These properties seem to be independent of the presence of diabetes mellitus [192]. Ziyrek et al. has shown that metformin monotherapy for 3 months reduced EAT thickness by 10% [193]. The exact mechanism by which metformin interacts with EAT is not clear, but it appears to shift the metabolism into fat oxidation and upregulate the thermogenesis [193,194]. It has recently been shown to be effective against endothelial dysfunction [195]. Chen et al. reported that metformin reduced the secretion of the proinflammatory cytokine, activin A, from epicardial fat [196]. Sardu et al. showed that metformin reduced the inflammatory burden in PCAT and improved prognosis in prediabetic patients with acute MI treated with coronary artery bypass grafting [197]. Metformin through its pleiotropic effect influences the pathogenesis of many cardiovascular diseases [198,199]. One of the mechanisms underlying metformin's mode of action is the activation of adenosine monophosphate-activated protein kinase, which has an anti-inflammatory effect [198,200]. Studies performed in animal models indicated that SGLT2 inhibitors, GLP-1 agonists, and dipeptidyl peptidase-4 (DPP-4) inhibitors [201–203] have similar anti-inflammatory effects in adipose tissue, but further studies are needed to draw firm conclusions.

Thiazolidinediones are another group of drugs which reduce inflammation and the release of proinflammatory cytokines from EAT [204–206]. These effects may contribute to the reduced the risk of MI and stroke in patients treated with thiazolidinediones [207,208].

SGLT2 inhibitors were shown to reduce EATV assessed by CMR, among patients with type 2 diabetes, both with and without obesity [209–211]. SGLT2 inhibitors also improved the inflammatory status of EAT [209]. They have been documented to be effective against HF and endothelial dysfunction [212,213]. This group of drugs causes a significant reduction in body weight, and one of the mechanisms of action is the stimulation of visceral fat burn [214]. In one study, dapagliflozin caused a reduction in EAT thickness independent of weight loss [215], perhaps thanks to the improvement of EAT cells' sensitivity to insulin and a reduction in local proinflammatory chemokines secretion [216]. Although SGLT2 inhibitors are relatively new drugs, the first meta-analyses have already confirmed their beneficial impact on EAT [217].

Finally, incretin-based drugs, which include GLP-1 agonists and DPP-4 inhibitors, were also shown to reduce EAT thickness, measured by echocardiography [218] and EATV, assessed by CMR [219]. This effect was mainly weight-loss-dependent [219]. Importantly, despite the reduction in body weight, incretin-based drugs did not reduce the proinflammatory properties of adipose tissue [220,221], although they inhibited the development of atherosclerosis in animal models [222]. It has been shown that GLP-1 receptors are present in EAT, in contrast to subcutaneous adipose tissue [223]. Hence, it has been suggested that GLP-1 agonists affect EAT by stimulating pre-adipocyte differentiation, thermogenesis, and adipocyte browning [224,225]. DPP-4 inhibitors also reduce EAT thickness [226] but can have an adverse effect on its inflammatory status, which may stimulate myocardial fibrosis [227–229]. On the other hand, several studies have shown the anti-inflammatory properties of DPP-4 inhibitors [230–232]. These studies have suggested that these drugs downregulate the receptor for advanced glycation end-products [233], activate cyclic adenosine monophosphate/protein kinase A signaling and IL-6 production [234], and reduce ROS generation and intercellular adhesion molecule-1 expression [235].

Taking into account the inflammatory nature of atherosclerosis, three anti-inflammatory drugs are important in the context of EAT modulation: (i) canakinumab, (ii) methotrexate, and (iii) colchicine.

Canakinumab is a human anti-interleukin-1-beta (IL-1β) monoclonal antibody which was shown to improve cardiovascular outcomes in over 10,000 patients with a history of CAD and elevated CRP levels in the CANTOS study [236]. Inhibition of the IL-1β/IL-6 signaling cascade with canakinumab led to a significant reduction in cardiovascular risk (nonfatal MI, nonfatal stroke, or cardiovascular death), independent of lipid-level lowering, but with an increased risk of serious infections [236]. Subgroup analysis showed that cardiovascular benefits were achieved due to a reduction in CRP concentration during canakinumab therapy [237].

Methotrexate reduces the proinflammatory effects of IL-6, IL-12, and TNF-alfa and increases the anti-inflammatory effects of IL-10 and IL-1 receptor antagonists [238]. In patients treated with methotrexate for rheumatoid conditions, it reduced the risk of MI by 18%, according to a recent meta-analysis [239]. In the CIRT study that investigated the benefits of methotrexate on outcomes in patients with a history of acute coronary syndrome, MI, and diabetes or metabolic syndrome, no benefits regarding the composite endpoint including nonfatal MI, nonfatal stroke, and cardiovascular death were shown [240]. This is probably due to the fact that elevated CRP levels were not taken into account as an inclusion criterion, in contrast to the CANTOS study.

The efficacy of colchicine in patients with stable CAD was demonstrated by LoDoCo and LoDoCo2 studies [241,242]. In the LoDoCo study, a reduction in a composite endpoint consisting of MI, cardiac arrest, or noncardioembolic stroke was observed in colchicine group, compared to the placebo group [241]. In LoDoCo2 study, a significant reduction in the primary composite endpoint of cardiovascular death, nonprocedural MI, ischemic stroke, or ischemia-driven coronary revascularization was achieved in colchicine group, compared to the placebo [242]. The benefits of adding colchicine to standard therapy were also shown in COLCOT study, which included patients with a history of MI within the last month [243]. The primary efficacy endpoint (a composite of cardiovascular death, resuscitated cardiac arrest, MI, stroke, or urgent hospitalization for angina requiring coronary revascularization) occurred in 5.5% in the colchicine group compared to 7.1% in the placebo group ($p = 0.02$) [242]. Adding colchicine to standard therapy led to a lower risk of ischemic cardiovascular events, compared to the placebo.

Hitherto, there have been no studies evaluating the effects of canakinumab, methotrexate, and colchicine on EAT. Further reports on new drugs affecting EAT and inflammatory mechanisms are expected in the near future. Potential pharmacological therapeutic options are summarized in Table 1.

Table 1. Pharmacological therapeutic options to affect epicardial adipose tissue.

Pharmacological Therapeutic Options	
Group of Drugs	**Potential Mechanisms of Action**
Statins	anti-inflammatory [160,162–165] modulation of EAT [164] ↓ EAT metabolic activity [163]
PCSK-9 inhibitors	unknown
Metformin	anti-inflammatory [186,187,196–198,200] ↑ fat oxidation and thermogenesis [193,194] ↓ endothelial dysfunction [195] activation of adenosine monophosphate-activated protein kinase [198,200]
Thiazolidinediones	anti-inflammatory [190,191,204–206]
SGLT2 inhibitors	anti-inflammatory [210,217] ↓ endothelial dysfunction [213] stimulation of visceral fat burn [215] ↑ EAT cells sensitivity to insulin [217]
GLP-1 agonists	↑ pre-adipocyte differentiation [225] ↑ thermogenesis [226] ↑ adipocyte browning [226]
DPP-4 inhibitors	anti-inflammatory [231–233] downregulation of the receptor for advanced glycation end-products [234] ↑ cyclic adenosine monophosphate/protein kinase A signaling and IL-6 production [235] ↓ ROS generation and intercellular adhesion molecule-1 expression [236]

Table 1. *Cont.*

Pharmacological Therapeutic Options	
Group of Drugs	**Potential Mechanisms of Action**
Canakinumab	anti-inflammatory [237]
Methotrexate	anti-inflammatory [239]
Colchicine	anti-inflammatory [242,243]

EAT—epicardial adipose tissue; DPP-4—dipeptidyl peptidase-4; GLP-1—glucagon-like peptide-1 receptor; ROS—reactive oxygen species; SGLT2—sodium-glucose cotransporter 2.

7. Conclusions

EAT is not only an adipose tissue in the histological sense, but is above all a metabolically active tissue, modulating numerous pathophysiological processes in the course of CVDs. In presence of cardiovascular risk factors, the protective properties of EAT are destroyed and it becomes a pro-inflammatory tissue promoting the development and progression of CVDs including CAD, heart failure, arrhythmias, and cardiovascular complications of COVID-19. EAT's function can be modulated and potentially restored by changing the lifestyle and anti-inflammatory drugs. The development of novel therapies specifically targeting EAT might revolutionize the prognosis in patients with CVD. The search for potential drug targets in EAT is an exciting challenge we currently face.

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7. Podsumowanie i wnioski

W pierwszej z prezentowanych prac oceniono wpływ ekstremalnie intensywnego wysiłku fizycznego na ilość nasierdziowej tkanki tłuszczowej, wyrażoną jako pole powierzchni w cm^2 oraz jej korelację z czynnikami ryzyka choroby wieńcowej. Do badania włączono 30 zdrowych ultramaratończyków amatorów i 9 ochotników prowadzących siedzący tryb życia. Ilość nasierdziowej tkanki tłuszczowej oceniano przy użyciu rezonansu magnetycznego (Siemens, 3T) w 4 lokalizacjach: dookoła 3 głównych tętnic wieńcowych (gałąź przednia zstępująca [GPZ], gałąź okalająca [GO], prawa tętnica wieńcowa [PTW]) oraz na powierzchni prawej komory [PK]. Dodatkowo oceniano skład ciała przy użyciu analizatora Tanita, lipidogram, osoczowe stężenie interleukiny 6 oraz grubość kompleksu intima-media (ocena metodą ultrasonograficzną z punktem odcięcia > 0.9 mm). Ilość nasierdziowej tkanki tłuszczowej w grupie ultramaratończyków była istotnie mniejsza we wszystkich badanych lokalizacjach w porównaniu z grupą kontrolną (GPZ: 1.12 ± 0.4 vs 1.86 ± 0.41; PTW: 0.88 ± 0.39 vs 1.78 ± 0.34; GO: 0.90 ± 0.36 vs 1.74 ± 0.49; PK: 2.07 ± 0.97 vs 5.23 ± 2.77 [cm^2]; $p < 0.001$ dla wszystkich). Zgodnie z oczekiwaniami, ultramaratończycy mieli mniejszy odsetek tłuszczu trzewnego (10.78 ± 4.01 vs 23.17 ± 5.9 [%]; $p < 0.001$) oraz lepszy profil lipidowy niż grupa kontrolna (nie-HDL: 119.14 ± 25.07 vs 173.89 ± 27.10, LDL: 102.68 ± 22.45 vs 144.86 ± 23.12 [mg/dl]; $p < 0.001$ dla wszystkich). Nie zaobserwowano natomiast różnic w grubości kompleksu intima-media w obrębie tętnic szyjnych (p=0.99 dla lewej tętnicy szyjnej, p= 0.68 dla prawej tętnicy szyjnej). Nie było także istotnej statystycznie różnicy w stężeniu interleukiny 6 pomiędzy grupami (p= 0.16), jednak w grupie biegaczy częstość występowania patologicznie wysokiego stężenia interleukiny 6 (jako punkt odcięcia przyjęto stężenie > 1 pg/ml) była 3-krotnie niższa niż w grupie kontrolnej (17% vs 56%, $p < 0.05$). Dodatkowo dla grupy biegaczy uzyskano dodatnią korelację pomiędzy objętością tkanki tłuszczowej otaczającej gałąź przednią zstępującą, gałąź okalającą oraz prawą komorę a odsetkiem całkowitego tłuszczu trzewnego ($R \geq 0.42$ dla wszystkich, $p \leq 0.02$ dla wszystkich) oraz pomiędzy objętością tłuszczu wokół gałęzi okalającej a stężeniem frakcji LDL i nie-HDL cholesterolu ($R \geq 0.41$ dla wszystkich, $p \leq 0.03$ dla wszystkich). Nie obserwowano natomiast korelacji pomiędzy ilością okołowieńcowej tkanki tłuszczowej a stężeniem interleukiny 6.

Wyniki badania wskazują, że ekstremalnie intensywny trening fizyczny może obniżać ryzyko sercowo-naczyniowe poprzez redukcję ilości i prozapalnej aktywności nasierdziowej tkanki tłuszczowej, jednak potrzebne są dalsza badania potwierdzające zależności wynikające z badań prowadzonych w ramach niniejszej pracy doktorskiej. Do ograniczeń badania należy fakt, że grupy ultramaratończyków oraz ochotników prowadzących siedzący tryb życia różniły się w zakresie wyjściowej charakterystyki (masa ciała, wskaźnik masy ciała, procentowa zawartość tkanki tłuszczowej w organizmie). Ponadto do badania włączono wyłącznie mężczyzn rasy kaukaskiej, a badane kohorty były małe. Na podstawie uzyskanych wyników możemy zatem jedynie spekulować nad potencjalnymi korzyściami z uprawiania sportów o ekstremalnej intensywności dla układu sercowo- naczyniowego. Docelowo, aby uzyskać bardziej wiarygodne wyniki należy przeprowadzić badanie w większej populacji oraz z odpowiednio dobraną grupą kontrolą (umiarkowanie aktywna fizycznie, z porównywalną masą i składem ciała).

W kolejnej pracy porównano ekspresję genów w okołowieńcowej tkance tłuszczowej u pacjentów z zaawansowaną chorobą wieńcową i w grupie kontrolnej. Próbki tkanki uzyskano w czasie operacji pomostowania aortalno- wieńcowego (n = 21, grupa badana) lub kardiochirurgicznej operacji niewieńcowej u chorych z wcześniej wykluczoną chorobą wieńcową (n = 19, grupa kontrola: stenoza aortalna, niedomykalność mitralna, tętniak aorty piersiowej zakwalifikowane do leczenia kardiochirurgicznego). Badane grupy nie różniły się istotnie pod względem wieku, płci, współchorobowości (w tym nadciśnienie tętnicze, zaburzenia lipidowe, nikotynizm), frakcji wyrzutowej lewej komory czy przyjmowanych leków. Spośród 67 528 transkryptów, 1348 zostało zidentyfikowanych jako tzw. geny o zróżnicowanej ekspresji (ang. *differentially expressed genes*, DEGs). W celu określenia znaczenia genów o zróżnicowanej ekspresji zastosowano wartość „fold change" (FC). Spośród nich, 416 (30,9%) wykazywało nadekspresję, a 932 (69,1%) zaklasyfikowano do grupy o zmniejszonej ekspresji w porównaniu z grupą kontrolą. Wśród genów wykazujących zwiększoną ekspresję znalazły się m.in. te kodujące molekuły o działaniu prozapalnym i proaterogennym, takie jak: chemokiny CXCL8, CXCL2, interleukina 6, selektyna E, receptor dla lipoprotein o niskiej gęstości, metaloproteinazy z grupy ADAMTS. Największą różnicę pośród nich wykazano dla chemokiny CXCL8 (FC +193), selektyny E (FC +91), interleukiny 6 (FC +49) i metaloproteinazy ADAMTS4 (FC +28). Wśród genów o zmniejszonej ekspresji zidentyfikowano m.in. geny kodujące białka sygnałowe, enzymy, mikroRNA czy różne typy kolagenu. Są wśród nich molekuły powiązane z niedokrwieniem i remodelingiem mięśnia sercowego, funkcją płytek krwi i mitochondriów (np. miR- 3671, miR- 4524a, multimeryna, biglikan, TFPI, glukuronidazy, miR- 548, kolagen typu I, III i IV).

Dodatkowo wyróżniono grupę tzw. „upstream regulators" związanych z genami o zróżnicowanej ekspresji. Termin ten odnosi się do dowolnej molekuły, która może wpływać na ekspresję, transkrypcję czy fosforylację innej cząsteczki. W tej niejednorodnej grupie uwagę zwracają m.in. geny kodujące płytkopochodny czynnik wzrostu (ang. *platelet-derived growth factor*, PDGF), białko grupy 2 o wysokiej mobilności (ang. *high mobility group box 2*, HMGB2) czy ESCIT (ang. *evolutionarily conserved signaling intermediate in toll pathway*), które literatura wskazuje jako potencjalne cząsteczki prozapalne i promiażdżycowe. Co więcej, użyte oprogramowanie Ingenuity Pathway Analysis (IPA) powiązało geny o zróżnicowanej ekspresji w całe sieci powiązań i zależności, tzw. ścieżki kanoniczne i sieci. Wśród aktywowanych szlaków znalazła się m.in. „ścieżka układu krzepnięcia" zawierająca molekuły znane ze swojego promiażdżycowego i prozapalnego charakteru (inhibitor szlaku czynnika tkankowego, aktywator plazminogenu, receptor dla urokinazy, trombomodulina).

Podsumowując, przeprowadzone badanie było jednym z pierwszych, które wykazało zmienioną ekspresję nie tylko pojedynczych genów w PCAT, ale całych sieci i ścieżek z nich stworzonych, dostarczając kolejnych dowodów na to, że badana tkanka jest aktywnym źródłem promiażdżycowych molekuł mogących przyspieszać rozwój choroby wieńcowej. Głównym ograniczeniem badania jest brak walidacji danych za pomocą reakcji łańcuchowej polimerazy, co nie było możliwe ze względu na niewystarczającą ilość materiału biologicznego pozostałego po wykonaniu analizy DEGs. Ponadto, ponieważ w grupie badanej znajdowali się pacjenci z ciężką, objawową chorobą wieńcową, zakwalifikowani do CABG, wyników nie można ekstrapolować na pacjentów z mniej zaawansowaną chorobą wieńcową. Co więcej, na podstawie powyższego badania obserwacyjnego trudno jest zidentyfikować jedną konkretną ścieżkę związaną z rozwojem i progresją choroby wieńcowej. Wreszcie, różnice w ekspresji genów między grupą badaną i kontrolną nie dowodzą związku przyczynowego między DEGs w obrębie PCAT i rozwojem miażdżycy tętnic wieńcowych. Podsumowując, profil ekspresji genów prozapalnych w EAT u pacjentów z chorobą wieńcową wydaje się mieć związek z rozwojem i progresją miażdżycy, jednak powyższe obserwacje wymagają walidacji w kolejnych badaniach.

Trzecią pracę, artykuł poglądowy, poświęcono podsumowaniu aktualnego stanu wiedzy na temat udziału EAT w patogenezie chorób układu sercowo-naczyniowego, w tym choroby wieńcowej, niewydolności serca i migotania przedsionków. Zwrócono także uwagę na możliwy związek pomiędzy EAT a przebiegiem COVID-19. Na koniec zaprezentowano EAT jako potencjalny cel terapeutyczny w leczeniu chorób układu sercowo-naczyniowego w przyszłości.

Podsumowując, cykl prezentowanych prac demonstruje, że okołosercowa tkanka tłuszczowa jest jednym z najważniejszych elementów biorących udział w patogenezie chorób układu sercowo-naczyniowego, jednak kolejne badania na poziomie molekularnym są kluczowe, aby zidentyfikować konkretne geny i kodowane przez nie białka, których modulacja mogłaby zmienić niekorzystny fenotyp EAT u pacjentów z chorobami sercowo-naczyniowymi. Negatywny wpływ EAT na układ krążenia może być ograniczony już teraz poprzez interwencje niefarmakologiczne, a dzięki dalszemu rozwojowi badań na poziomie ekspresji genów, istnieje szansa identyfikacji nowych celów terapeutycznych w obrębie EAT.

8. Piśmiennictwo

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9. Opinia Komisji Bioetycznej

Konwerski M, Postuła M, Barczuk-Falęcka M, Czajkowska A, Mróz A, Witek K, Bakalarski W, Gąsecka A, Małek ŁA, Mazurek T. Epicardial Adipose Tissue and Cardiovascular Risk Assessment in Ultra-Marathon Runners: A Pilot Study. Int J Environ Res Public Health. 2021;18(6):3136. doi: 10.3390/ijerph18063136. Badanie przeprowadzono za zgodą Komisji Bioetycznej przy Okręgowej Izbie Lekarskiej w Warszawie (protokół numer 52/17 z dnia 12.10.2017 r.).

Konwerski M, Gromadka A, Arendarczyk A, Koblowska M, Iwanicka-Nowicka R, Wilimski R, Czub P, Filipiak KJ, Hendzel P, Zielenkiewicz P, Opolski G, Gąsecka A, Mazurek T. Atherosclerosis Pathways are Activated in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease. J Inflamm Res. 2021;14:5419-5431. doi: 10.2147/JIR.S326769. Badanie przeprowadzono za zgodą Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym w Warszawie (protokół numer KB 91/2012 z dnia 17.04.2012 r.).

10. Oświadczenia wszystkich współautorów publikacji określające indywidualny wkład każdego z nich w ich powstanie

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Kierownik Kliniki: prof. dr hab. med. Marcin Grabowski

Oświadczenie o współautorstwie

Tytuł artykułu: Epicardial Adipose Tissue and Cardiovascular Risk Assessment in Ultra-Marathon Runners: A Pilot Study.

Autorzy: Konwerski M, Postuła M, Barczuk-Falęcka M, Czajkowska A, Mróz A, Witek K, Bakalarski W, Gąsecka A, Małek ŁA, Mazurek T.

Dane bibliometryczne: Int J Environ Res Public Health. 2021;18(6):3136.

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 80%, a wkład merytoryczny dotyczy zadań A, B, C, D, E, zgodnie z poniższą legendą.

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Podpis Współautora (Postuła M)

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Podpis Współautora (Czajkowska A)

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 1%, a wkład merytoryczny dotyczy zadań B, E zgodnie z poniższą legendą.

Podpis Współautora (Mróz A)

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 1%, a wkład merytoryczny dotyczy zadań B, E zgodnie z poniższą legendą.

Podpis Współautora (Witek K)

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Podpis Współautora (Bakalarski W)

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Podpis Współautora (Gąsecka A)

Aleksandra Gąsecka

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Podpis Współautora (Małek ŁA)

hab. n. med. Małek
specjalista kardiolog
kardiolog sportowy
PWZ 2214266

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Podpis Współautora (Mazurek T)

hab. n. med. Tomasz Mazurek
specjalista chorób wewnętrznych
KARDIOLOG
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Oświadczenie o współautorstwie

Tytuł artykułu: Atherosclerosis Pathways are Activated in Pericoronary Adipose Tissue of Patients with Coronary Artery Disease.

Autorzy: Konwerski M, Gromadka A, Arendarczyk A, Koblowska M, Iwanicka-Nowicka R, Wilimski R, Czub P, Filipiak KJ, Hendzel P, Zielenkiewicz P, Opolski G, Gąsecka A, Mazurek T.

Dane bibliometryczne: J Inflamm Res. 2021;14:5419-5431. doi: 10.2147/JIR.S326769.

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 80%, a wkład merytoryczny dotyczy zadań A, B, C, D, E, zgodnie z poniższą legendą.

Podpis Współautora (Konwerski M)

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 4%, a wkład merytoryczny dotyczy zadań B, C, D, E, zgodnie z poniższą legendą.

Podpis Współautora (Gromadka A)

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 1%, a wkład merytoryczny dotyczy zadań B, E, zgodnie z poniższą legendą.

Podpis Współautora (Arendarczyk A)

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 1%, a wkład merytoryczny dotyczy zadań B, E, zgodnie z poniższą legendą.

Podpis Współautora (Koblowska M)

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Podpis Współautora (Iwanicka-Nowicka R)

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Podpis Współautora (Zielenkiewicz P)

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Podpis Współautora (Opolski G)

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Podpis Współautora (Mazurek T)

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Kierownik Kliniki: prof. dr hab. med. Marcin Grabowski

Oświadczenie o współautorstwie

Tytuł artykułu: Role of Epicardial Adipose Tissue in Cardiovascular Diseases: A Review. Biology.

Autorzy: Konwerski M, Gąsecka A, Opolski G, Grabowski M, Mazurek T.

Dane bibliometryczne: Biology. 2022;11(3):355. doi: 10.3390/biology11030355.

Oświadczam, że mój wkład procentowy w przygotowanie powyższej publikacji wyniósł 90%, a wkład merytoryczny dotyczy zadań A, B, C, D, E, zgodnie z poniższą legendą.

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Podpis Współautora (Gąsecka A) Aleksandra Gąsecka

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