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Receptor końcowych produktów glikacji jako punkt wspólny szlaków metabolicznych dla otyłości i jej powikłań

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

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Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Medycznych
Warszawskiego Uniwersytetu Medycznego

Warszawa 2025 r.

Słowa kluczowe

otyłość, RAGE, AGE, lnc-AGER1, cukrzyca typu 2, ostry zespół wieńcowy

Keywords

obesity, RAGE, AGE, lnc-AGER1, type 2 diabetes mellitus, acute cardiac syndrome

Badania prowadzone w ramach niniejszej pracy doktorskiej zostały sfinansowane z budżetu następujących projektów badawczych:

- Grant OPUS Narodowego Centrum Nauki 2018/31/B/NZ5/01556 *„Epigenetyczna regulacja metabolizmu estrogenów w tkance tłuszczowej osób otyłych”*
  Kierownik projektu: dr hab. Alina Kuryłowicz

- Grant z Funduszu Badań Własnych IMDIK PAN FBW/05/2020 *„Rola długich niekodujących RNA (ang. long non-coding RNA, lncRNA) w patogenezie dysfunkcji tkanki tłuszczowej u osób otyłych”*
  Kierownik projektu: dr hab. Alina Kuryłowicz

Przede wszystkim chciałabym podziękować mojej Promotor
dr hab.n.med. Alinie Kuryłowicz
za jej ogromne zaangażowanie, wysiłek i motywowanie mnie do pracy na każdym etapie.

Chciałabym podziękować mojemu Promotorowi
Panu Profesorowi Krzysztofowi Czajkowskiemu
za możliwość wykonania projektu, dzielenie się wiedzą i doświadczeniem.

Z całego serca dziękuje moim Rodzicom
za ich nieustanną wiarę we mnie i wsparcie w każdej, nawet trudnej sytuacji.

Pracę dedykuje Ukochanemu Mężowi, który jest moim Aniołem, Miłością, moim Wszystkim

w każdy dzień.

Wykaz publikacji stanowiących pracę doktorską

Gutowska, K., Czajkowski, K., & Kuryłowicz, A. (2023). Receptor for the Advanced Glycation End Products (*RAGE*) Pathway in Adipose Tissue Metabolism. *International journal of molecular sciences*, *24*(13), 10982. https://doi.org/10.3390/ijms241310982
IF 2023 = 4,9; 5-letni IF = 5,7; MNiSW = 140 pkt

Gutowska, K., Koźniewski, K., Wąsowski, M., Jonas, M. I., Bartoszewicz, Z., Lisik, W., Jonas, M., Binda, A., Jaworski, P., Tarnowski, W., Noszczyk, B., Puzianowska-Kuźnicka, M., Czajkowski, K., & Kuryłowicz, A. (2023). AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus. *International journal of molecular sciences*, *24*(24), 17447. https://doi.org/10.3390/ijms242417447;
IF 2024 = 4,9; 5-letni IF = 5,7; MNiSW = 140 pkt

Gutowska, K., Ambroziak, M., Podraza, J. Puzianowska-Kuźnicka, M., Czajkowski, K., Budaj, A., & Kuryłowicz, A. (2025). Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population. *BMC Medical Genomics*, 2025;18(1):143. Published 2025 Sep 30. doi:10.1186/s12920-025-02215-3
IF 2025 = 2,7; 5-letni IF = 2,4; MNiSW = 100 pkt

Spis treści

1. Wykaz stosowanych skrótów

ACS	ostry zespół wieńcowy
AGEs	zaawansowane produkty glikacji
BMI	indeks masy ciała
ERK	kinazy regulowane zewnątrzkomórkowo
HDL	lipoproteina o dużej gęstości
HMGB1	białka o wysokiej ruchliwości elektroforetycznej B1
JNK	kinaza c-JunN-terminalna
LDL	lipoproteina o małej gęstości
lncAGER-1	długi niekodujący AGER-1
MAPK	kinazy białkowe aktywowane mitogenem
NF-kB	czynnik jądrowy kappa B
PDGF	płytkopochodnego czynnika wzrostu
RAGE/AGER	receptor dla zaawansowanych produktów glikacji
ROS	reaktywne formy tlenu
sRAGE	rozpuszczalny receptor dla zaawansowanych produktów glikacji
WHO	Światowa Organizacja Zdrowia

2. Streszczenie w języku polskim

Otyłość to przewlekła choroba charakteryzująca się nadmiernym nagromadzeniem i nieprawidłowym działaniem tkanki tłuszczowej, co prowadzi do szeregu niekorzystnych konsekwencji zdrowotnych. Obecnie stanowi jedno z kluczowych wyzwań zdrowotnych jako czynnik ryzyka wystąpienia około 200 powikłań, w tym chorób metabolicznych i układu sercowo-naczyniowego co przekłada się na skrócenie oczekiwanej długości życia średnio o 6 lat w przypadku mężczyzn, a u kobiet – o około 7 lat. Za podstawowy mechanizm patogenetyczny łączący nadmierną masę ciała z rozwojem powikłań narządowych uważa się przewlekły stan zapalny, którego źródłem jest dysfunkcyjna tkanka tłuszczowa (zapalenie metaboliczne). W pracach eksperymentalnych, przeprowadzonych przede wszystkim na modelach zwierzęcych, stwierdzono, że jednym z kluczowych szlaków komórkowych zaangażowanych w rozwój związanego z otyłością zapalenia metabolicznego jest szlak receptora końcowych produktów zaawansowanej glikacji (RAGE*, ang. receptor for advanced glycation end products*).

Celem niniejszej rozprawy doktorskiej składającej się z cyklu 3 opublikowanych prac było opisanie roli szlaku RAGE w rozwoju związanych z otyłością powikłań kardiometabolicznych u ludzi. Cykl publikacji obejmuje jedną pracę przeglądową oraz dwie prace oryginalne.

Pierwsza praca pt. „Receptor for the Advanced Glycation End Products (RAGE) Pathway in Adipose Tissue Metabolism" przedstawia aktualny stan wiedzy dotyczący roli szlaku sygnałowego RAGE w dysfunkcji tkanki tłuszczowej w przebiegu otyłości i powiązanych powikłań metabolicznych. Na wstępie przedstawiono tkankę tłuszczową jako narząd endokrynny zwracając uwagę na mechanizmy prowadzące do zaburzenia jej aktywności w przebiegu otyłości. Następnie opisano rolę końcowych produktów zaawansowanej glikacji (AGE, *ang. advanced glycation end products*) oraz szlaku RAGE i przedstawiono ich udział w regulacji aktywności tkanki tłuszczowej. Dodatkowo opisano aktualne badania szlaku RAGE na modelach zwierzęcych i u ludzi analizujące znaczenie szlaku w rozwoju otyłości i towarzyszących jej powikłań. Ostatecznie przedstawiono perspektywy terapeutyczne mające na celu ograniczenie puli AGE oraz hamowanie szlaku RAGE jako potencjalną strategię w leczeniu otyłości i jej powikłań.

Druga praca pt. „AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus" analizuje ekspresję genu receptora dla produktów zaawansowanej glikacji (*AGER*) oraz długiego

niekodującego RNA AGER-1 (lncAGER-1, *ang. long non-coding AGER-1*) w tkance tłuszczowej w kontekście otyłości i cukrzycy typu 2 oraz bada ich korelację z nasileniem stanu zapalnego. W pracy tej wykazano, że otyłość wiąże się z ze zwiększoną ekspresją mRNA *AGER* w podskórnej tkance tłuszczowej, która ulega obniżeniu w przypadku redukcji masy ciała. Taki sam trend zaobserwowano dla lncAGER-1, którego poziom dodatkowo korelował z ekspresją *AGER* w tkance tłuszczowej kobiet chorujących na otyłość i cukrzycę typu 2. Ponadto w tej grupie stwierdzono dodatnią korelację pomiędzy poziomem mRNA *AGER* a ekspresją genów kodujących mediatory stanu zapalnego.

Trzecia praca pt. „Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population" koncentruje się na wpływie polimorfizmów pojedynczego nukleotydu (SNP, *ang. single nucleotide polymorphism*) w genie kodującym RAGE (rs2070600 G/A i rs184003 G/T) na predyspozycję do wystąpienia ostrego zespołu wieńcowego oraz choroby niedokrwiennej serca w populacji polskiej. W pracy zaobserwowano wyższą częstość genotypów zawierających allel T (GT + TT) polimorfizmu rs184003 u pacjentów z ostrym zespołem wieńcowym. Dodatkowo nosiciele allelu T (rs184003) charakteryzowali się istotnie niższym stężeniem cholesterolu HDL (*ang. high density lipoprotein)* oraz wyższym stężeniem troponiny I w chwili wystąpienia ostrego zespołu wieńcowego. Dla polimorfizmu rs2070600 nie wykazano istotnego związku z ryzykiem ostrego zespołu wieńcowego. Co ważne nie zaobserwowano istotnych korelacji między badanymi polimorfizmami a stopniem zwężenia tętnic wieńcowych w ocenie angiograficznej.

Podsumowując, uzyskane wyniki sugerują potencjalną rolę lncAGER-1 w regulacji ekspresji genu kodującego RAGE (*AGER*) w tkance tłuszczowej chorych z otyłością oraz na związek podwyższonej ekspresji *AGER* z nasileniem zapalenia metabolicznego i ryzykiem rozwoju cukrzycy typu 2. Ponadto, w niniejszej pracy wykazano, że obecność allelu T polimorfizmu rs184003 *AGER* może predysponować do rozwoju miażdżycy naczyń wieńcowych i jej powikłania w postaci ostrego zespołu wieńcowego w polskiej populacji. Niemniej jednak, w analizowanej populacji polimorfizmy rs184003 G/T i rs2070600 G/A w *AGER* nie wiązały się z występowaniem ostrego zespołu wieńcowego w młodym wieku, tj. przed 50. rokiem życia.

3. Streszczenie w języku angielskim

The receptor for advanced glycation end products as a common point of metabolic pathways for obesity and its complications

Obesity is a chronic disease characterized by the excessive accumulation and abnormal functioning of adipose tissue. This results in several adverse health consequences. Currently, it is one of the key health challenges, as it is a risk factor for approximately 200 complications, including metabolic and cardiovascular diseases. These complications result in an average reduction in life expectancy of 6 years for men and 7 years for women. The basic pathogenic mechanism linking excessive body weight to organ complications is chronic inflammation caused by dysfunctional adipose tissue (metabolic inflammation). Previous studies based on animal models have shown the significant contribution of the receptor for advanced glycation end products (RAGE) pathway to obesity-related metabolic inflammation.

This doctoral dissertation consists of three published papers that aim to describe the role of the RAGE pathway in the development of obesity-related cardiometabolic complications in humans. The series includes one review paper and two original papers.

The first paper, titled "Receptor for Advanced Glycation End Products (RAGE) Pathway in Adipose Tissue Metabolism", summarizes the current knowledge about the role of the RAGE signaling pathway in adipose tissue dysfunction in obesity and related metabolic complications. The introduction describes adipose tissue as an endocrine organ and highlights the mechanisms that lead to its dysfunction in obesity. It is then followed by a description of the role of advanced glycation end products (AGEs) and the RAGE pathway in regulating adipose tissue activity. Additionally, the dissertation describes current research on the RAGE pathway in animal models and humans, analyzing the pathway's importance in the development of obesity and its accompanying complications. Finally, we present therapeutic perspectives aimed at reducing the AGE pool and inhibiting the RAGE pathway as a potential strategy for treating obesity and its complications.

The second paper, titled "AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus,"

analyzes the expression of the advanced glycation end-product receptor gene (*AGER*) and long non-coding RNA AGER-1 (lncAGER-1) in adipose tissue in the context of obesity and type 2 diabetes, examining their correlation with inflammation severity. The study revealed that obesity is associated with increased *AGER* mRNA expression in subcutaneous adipose tissue, which decreases with weight loss. The same trend was observed for lncAGER-1, whose level additionally correlated with *AGER* expression in the adipose tissue of women with obesity and type 2 diabetes. Furthermore, a positive correlation was found in this group between *AGER* mRNA levels and the expression of genes encoding inflammatory mediators.

The third paper, titled "Advanced Glycation End-Product Receptor Gene (RAGE) Polymorphism in Patients with Acute Coronary Syndrome: A Case-Control Study in the Polish Population," focuses on the impact of two single nucleotide polymorphisms (SNPs) in the RAGE-encoding gene – *AGER* (rs2070600 G/A and rs184003 G/T) on predisposition to acute coronary syndrome and ischemic heart disease in the Polish population. The study observed a higher frequency of genotypes containing the T allele (GT + TT) of the rs184003 polymorphism in patients with acute coronary syndrome. Additionally, carriers of the rs184003 T allele were characterized by significantly lower high-density lipoprotein (HDL) cholesterol levels and higher troponin I levels at the time of acute coronary syndrome onset. No significant association was found between the rs2070600 polymorphism and the risk of acute coronary syndrome. Notably, no significant correlations were observed between the studied polymorphisms and the degree of coronary artery stenosis as assessed by angiography.

In summary, the results suggest a potential role for lncAGER-1 in regulating the expression of the RAGE gene (*AGER*) in adipose tissue of obese patients, as well as a link between increased *AGER* expression, metabolic inflammation, and the risk of developing type 2 diabetes. Furthermore, this study demonstrated that the presence of the T allele of the rs184003 *AGER* polymorphism may predispose individuals in the Polish population to the development of coronary artery atherosclerosis and its complication, acute coronary syndrome. However, the rs184003 G/T and rs2070600 G/A *AGER* polymorphisms were not associated with the occurrence of acute coronary syndrome at a young age (before 50 years old) in the analyzed population.

4. Wstęp

4.1 Otyłość i jej powikłania jako problem społeczno-ekonomiczny

Otyłość to przewlekła choroba charakteryzująca się nadmiernym nagromadzeniem i nieprawidłowym działaniem tkanki tłuszczowej, co prowadzi do szeregu niekorzystnych konsekwencji zdrowotnych [1,2]. Zalecanym przez Światową Organizację Zdrowia (WHO, *ang. World Health Organization*) narzędziem do oceny stanu odżywienia jest wskaźnik masy ciała (BMI, *ang. body mass index*), definiowany jako iloraz masy ciała (wyrażonej w kilogramach) i wzrostu (wyrażonego w metrach). Przyjmuje się, że wynik BMI ≥ 30 kg/m^2 wskazuje na otyłość, a wartości w zakresie 25-29,9 kg/m^2 – na nadwagę [3]. Na podstawie wartości BMI oceniono, że w 2020 roku u 2,6 mld osób na świecie można rozpoznać zbyt wysoką masę ciała, a do 2030 roku liczba mieszkańców świata żyjących z nadwagą lub otyłością wzrośnie do 3,0 mld [4]. Z kolei, zgodnie z danymi Głównego Urzędu Statystycznego w 2019 roku 56,6% osób w Polsce powyżej 15 roku życia miało rozpoznaną nadwagę lub otyłość i prognozuje się, że w 2035 roku problem otyłości dotknie w naszym kraju co trzeciego mężczyznę i co czwartą kobietę [4]. Biorąc pod uwagę, że BMI nie uwzględnia kluczowych dla rozpoznania nadwagi i otyłości zmian w składzie ciała, prognozy te mogą być niedoszacowane [5].

Otyłość jest rozpatrywana obecnie jako jedno z kluczowych wyzwań zdrowia publicznego, ponieważ stanowi czynnik ryzyka przedwczesnej śmierci – rozpoznanie tej choroby u osoby po 40 roku życia przekłada się na skrócenie oczekiwanej długości życia średnio o 6 lat w przypadku mężczyzn, a u kobiet – o około 7 lat [6]. Jest to spowodowane przede wszystkim zwiększeniem ryzyka wystąpienia chorób metabolicznych i układu sercowo-naczyniowego, choć lista powikłań związanych z nadmierną masą ciała jest długa i obejmuje blisko 200 jednostek chorobowych [7]. Oszacowano, że ryzyko wystąpienia cukrzycy typu 2 u osób z otyłością I i II stopnia wg WHO (BMI w zakresie 30-34,9 kg/m^2 i BMI w zakresie 35-39,9kg/m^2) wzrasta o 100% w stosunku do osób o prawidłowej masie ciała. Natomiast przy wartości BMI ≥ 40 kg/m^2 prawdopodobieństwo zachorowania na ten typ cukrzycy wzrasta o 150% u kobiet i o 180% u mężczyzn [8]. Nadmierna masa ciała predysponuje również do rozwoju miażdżycy naczyń, zwłaszcza wieńcowych [9].

Powikłania otyłości przyczyniają się znacząco do pogorszenia jakości życia chorych, a ich konsekwencje społeczne i koszty związane z leczeniem stanowią istotny problem socjo-ekonomiczny [10]. Zrozumienie mechanizmów łączących nadwagę i otyłość z rozwojem powikłań kardiometabolicznych nie byłoby możliwe bez poznania procesów patofizjologicznych zachodzących w tkance tłuszczowej na skutek wzrostu masy ciała. Podstawową rolę odgrywa tu zapalenie metaboliczne wywołane nadmierną kumulacją materiału energetycznego w adipocytach prowadzącą do ich dysfunkcji – w tym – zmiany wydzielanych mediatorów na takie o profilu prozapalnym [11].

4.2 Szlak receptora końcowych produktów zaawansowanej glikacji (AGE)

Jednym z kluczowych szlaków komórkowych zaangażowanych w rozwój zapalenia metabolicznego jest szlak receptora końcowych produktów zaawansowanej glikacji (RAGE, *ang. receptor for advanced glycation end products*) [12].

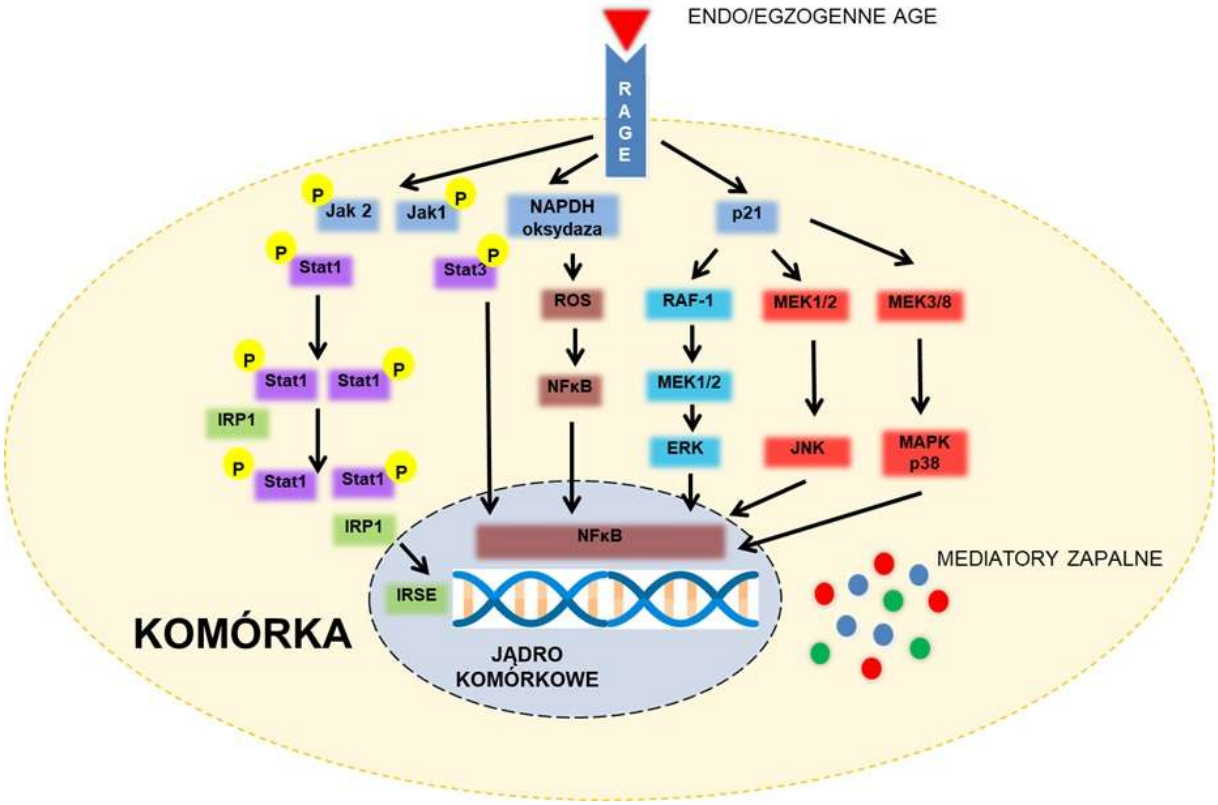
4.2.1. Identyfikacja i mechanizm powstawania AGEs

W warunkach patofizjologicznych, takich jak hiperglikemia dochodzi do formowania i kumulacji w organizmie końcowych produktów zaawansowanej glikacji (AGE, *ang. advanced glycation end products*). Glikacja (czyli nieenzymatyczna glikozylacja reszt aminowych), zwana także reakcją Maillarda, to spontaniczny, dwuetapowy proces, którego nasilenie wzrasta wraz ze wzrostem stężenia cukrów prostych we krwi [13,14]. Jeżeli proces ten dotyczy aminokwasów, to jego skutkiem jest zaburzenie struktury i funkcji białek będących nie tylko budulcem komórek, ale także enzymów pełniących istotną rolę w regulacji procesów metabolicznych. W przypadku kwasów nukleinowych proces glikacji może powodować pękanie łańcuchów nukleinowych i powstawanie mutacji. Z kolei reakcja angażująca lipidy wchodzące w skład błony komórkowej prowadzi do ich zwiększonej przepuszczalności [15]. W trakcie pierwszego etapu reakcji Maillarda, w wyniku reakcji grup karbonylowych cukrów redukujących z wolnymi grupami aminowymi kwasów nukleinowych, białek lub lipidów powstają niestabilne zasady Schiffa, przeformowywane następnie do bardziej stabilnych produktów Amadori. W drugim etapie, produkty Amadori mogą być przekształcane poprzez proces utleniania lub hydrolizy w AGE lub tworzyć prekursory AGE (takie jak np. glioksal) [13]. Co istotne, AGEs mogą występować również w organizmach osób niedoświadczających hiperglikemii, ponieważ ich obecność stwierdza się w produktach o dużej zawartości cukrów

prostych, jak również mogą powstawać w procesie przygotowywania pożywienia na drodze m.in. niewłaściwej obróbki cieplnej [16,17]. AGE mogą powstawać również na drodze egzogennej i być dostarczane do organizmu z pożywieniem oraz dymem tytoniowym [11].

4.2.2 Receptor końcowych produktów zaawansowanej glikacji

Receptor końcowych produktów zaawansowanej glikacji (RAGE, *ang. receptor for advanced glycation end-products*) należący do nadrodziny immunoglobulin zlokalizowany jest na powierzchni wielu typów komórek, w tym komórek śródbłonka, mięśni gładkich, podocytach jak i na komórkach budujących tkankę tłuszczową: adipocytach, ich prekursorach, komórkach naczyń krwionośnych oraz naciekających makrofagach [18]. Posiada zdolność wiązania wielu typów ligandów – oprócz AGEs wiąże białka należące do rodziny S100, peptydy amyloidu (Aβ), priony oraz białka o wysokiej ruchliwości elektroforetycznej B1 (HMGB1, *ang. high mobility group box 1*) [18-21]. Przyłączenie ligandu do receptora staje się sygnałem do wewnątrzkomórkowego tworzenia reaktywnych pochodnych tlenu (ROS, *ang. reactive oxygen species*), które w mechanizmie dodatniego sprzężenia zwrotnego nasilają ekspresję genu kodującego RAGE. W wyniku rozwijającego się stresu oksydacyjnego dochodzi do aktywacji prozapalnego czynnika jądrowego κB (NF-κB, *ang. nuclear factor κB*), a także innych szlaków sygnałowych, w tym, na przykład: kinaz białkowych aktywowanych mitogenem (MAPK, *ang. mitogen-activated protein kinases*), oksydazy NADPH (a*ng. nicotinamide adenine dinucleotide phosphate hydrogen*), kinaz regulowanych sygnałem zewnątrzkomórkowym 1 i 2 (ERK 1/2, *ang. extracellular signal-regulated kinase 1/2*), kinazy fosfoinozytydowej 3 (PI3K, *ang. phosphatidylinositol 3-kinase*) oraz kinazy białkowej B (AKT, *ang. kinase protein B*), co prowadzi do zwiększonej transkrypcji genów kodujących cytokiny i cząsteczki adhezyjne [22-24]. Rozwijający się w ten sposób proces zapalny sprzyja uszkodzeniom narządów i rozwojowi przewlekłych powikłań związanych z otyłością. Uproszczony schemat szlaków sygnałowych pobudzanych przez RAGE przedstawiono na Rycinie 1.



Rycina 1. Uproszczony schemat wewnątrzkomórkowych szlaków sygnałowych pobudzanych przez RAGE.

Interakcja między zaawansowanymi produktami glikacji (AGE) a ich receptorem (RAGE) stymuluje kilka kaskad sygnałów wewnątrzkomórkowych, np. Jak/Stat, oksydaza NADPH, kinazy białkowe aktywowane mitogenem (MAPK)/p38, kinazy regulowane zewnątrzkomórkowo (ERK)-1/2 oraz kinaza c-JunN-terminalna (JNK). Zjawiska te powodują aktywację czynników transkrypcyjnych, takich jak czynnik jądrowy (NF-κB) lub elementy odpowiedzi stymulowane przez IFN (ISRE), wzmacniając ekspresję mediatorów prozapalnych. Zmodyfikowano na podstawie Hudson BI i wsp [25], rozwinięcie skrótów zawarto w tekście.

Poza formą obecną na powierzchni komórek, RAGE występuje także w formie rozpuszczalnej (sRAGE, *ang. soluble RAGE*) powstającej w wyniku proteolitycznego rozszczepienia receptora zakotwiczonego w błonie komórkowej lub alternatywnego splicingu mRNA. sRAGE, który nie posiada domeny przezbłonowej i cytoplazmatycznej, pełni rolę receptora „wabikowego" (*ang. decoy receptor*) uniemożliwiając wiązanie się ligandów z pełną formą receptora RAGE obecną na komórkach [26].

Co ciekawe, aktywność RAGE może być determinowana przez warianty kodującego ten receptor genu (*AGER*). W badaniach asocjacyjnych zaobserwowano, że obecność polimorfizmów zamiany pojedynczych nukleotydów (SNP, *ang. single nucleotide polymorphism*) AGER może predysponować do rozwoju szeregu chorób w tym cukrzycy i jej

powikłań, choroby niedokrwiennej serca, ale także nowotworów i chorób autoimmunologicznych [27].

4.2.3. Związek RAGE z rozwojem odpowiedzi zapalnej i zapalenia metabolicznego

Aktywacja szlaku RAGE stanowi ważny mechanizm zarówno w rozwoju prawidłowej odpowiedzi zapalnej jak i w patogenezie wielu chorób i stanów patologicznych. Obecność RAGE stwierdzono na komórkach układu odpornościowego, takich jak np. neutrofile, limfocyty T, limfocyty B, monocyty, makrofagi oraz komórki dendrytyczne [28-30]. Szlak RAGE jest zaangażowany, między innymi, w proces różnicowania limfocytów T w kierunku podtypu Th1, a także w rekrutację leukocytów w miejsce reakcji zapalnej, gdzie pełni on rolę receptora adhezyjnego [31-33]. Do chwili obecnej dokładny mechanizm patofizjologiczny indukcji ostrej vs przewlekłej odpowiedzi zapalnej przez RAGE nie został jednak dokładnie poznany. Postulowane są dwie hipotezy. Pierwsza z nich mówi o związku stanu oligomeryzacji ligandów z potencjałem wywołania typu reakcji zapalnej: ligandy o strukturze oligomerycznej wykazują wyższe powinowactwo do RAGE i przez to wywołują odpowiedź zapalną o charakterze przewlekłym, a ligandy monomeryczne charakteryzujące się niskim powinowactwem do receptora są zdolne do rozwoju „jedynie" ostrej reakcji zapalnej [34]. Druga hipoteza mówi, że krytycznym elementem determinującym typ odpowiedzi zapalnej indukowanej przez RAGE jest pochodzenie ligandu [35]. Zgodnie z nią ligandy o pochodzeniu endogennym prowadzą do rozwoju przewlekłego zapalenia, przyłączenie zaś do RAGE ligandu egzogennego aktywuje ostrą reakcję [36-38].

Jak wspomniano już wcześniej, aktywacja szlaku RAGE wydaje się kluczowa dla rozwoju związanej z otyłością dysfunkcji tkanki tłuszczowej, której przejawem jest, między innymi, zapalenie metaboliczne. Tkanka tłuszczowa poza swoją rolą w regulacji bilansu energetycznego organizmu działa jednocześnie jako aktywny narząd endokrynny wydzielający wiele czynników bioaktywnych – adipokin, które biorą bezpośredni udział w regulacji szeregu procesów fizjologicznych, w tym, między innymi, wrażliwości na insulinę czy odpowiedzi odpornościowej [39-41]. Nadmierna kumulacja lipidów w tkance tłuszczowej u chorych z nadwagą i otyłością prowadzi do zaburzenia aktywacji sekrecyjnej adipocytów na rzecz produkcji adipokin o niekorzystnym profilu metabolicznym, a także powoduje rozwój stresu oksydacyjnego i procesu zapalnego, w czym pośredniczy RAGE [42-47]. Potwierdzają to badania na modelach zwierzęcych otyłości, gdzie wykazano, że wyłączenie (knock-out) genu kodującego RAGE chroni gryzonie przed kumulacją tkanki tłuszczowej i rozwojem zapalenia

metabolicznego nawet w przypadku wytworzenia dodatniego bilansu energetycznego [48]. Dodatkowo, aktywacja RAGE w tkance tłuszczowej prowadzi do rekrutacji monocytów i ich polaryzacji w kierunku prozapalnych makrofagów M1, co prowadzi do eskalacji odpowiedzi zapalnej [49-50]. Nadmierna aktywacja szlaku RAGE w tkance tłuszczowej u chorych z nadwagą i otyłością powoduje również nieprawidłowości lipolizy i termogenezy co nasila zaburzenia bilansu energetycznego.

4.2.4 Udział RAGE w patofizjologii cukrzycy typu 2 i jej powikłań

Podstawowym mechanizmem w patogenezie cukrzycy typu 2 jest insulinooporność, w której rozwoju istotną rolę odrywa indukowane przez szlak RAGE zapalenie metaboliczne. Poprzez uwalniane z dysfunkcyjnej tkanki tłuszczowej do krążenia mediatory, proces zapalny obejmuje inne narządy kluczowe dla metabolizmu węglowodanów (takie jak wątroba i mięśnie), wpływając na zmniejszenie ich wrażliwości na insulinę. Co więcej, proces zapalny obejmuje również wyspy trzustkowe, prowadząc do stopniowego upośledzenia wydzielania insuliny, a w konsekwencji – do jawnej hiperglikemii [51]. Konsekwencją przetrwałej hiperglikemii (w mechanizmie błędnego koła) jest akumulacja AGEs, aktywacja RAGE i uruchomienie opisanej wcześniej kaskady reakcji obejmujących szlaki sygnałowe prowadzące do nasilonej transkrypcji genów związanych z procesem zapalnym i stresem oksydacyjnym [52-53]. Dochodzi wówczas do glikacji białek mitochondrium i generowania ROS zwrotnie nasilających ekspresję receptora RAGE, co skutkuje pojawieniem się tzw. pamięci metabolicznej (*ang. „metabolic memory”*). Jest to, zależna od poziomu glikacji białek mitochondrialnych, kaskada reakcji pobudzających produkcję ROS i utrzymująca stałą nadekspresję genu kodującego RAGE, której nie towarzyszy stan hiperglikemii [54]. Mechanizm pamięci przyczynia się także do rozwoju typowych dla cukrzycy powikłań makro- i mikronaczyniowych (w tym neuropatii, cukrzycowej choroby oka i cukrzycowej choroby nerek) [55].

Neuropatia cukrzycowa uważana jest za najczęstsze przewlekłe powikłanie cukrzycy charakteryzujące się neurodegeneracyjnym uszkodzeniem obwodowego układu nerwowego obejmującym aksony czuciowe, autonomiczne oraz ruchowe [56]. W bioptatach skóry pobranych od pacjentów z neuropatią cukrzycową stwierdzono zwiększoną ekspresję genu kodującego RAGE (*AGER*), co korelowało z istotnym obniżeniem gęstości śródnaskórkowych włókien nerwowych [57]. Ponadto w badaniach doświadczalnych z wykorzystaniem zwierzęcego modelu cukrzycy zaobserwowano, że zahamowanie ekspresji *AGER* pozwala na

uzyskanie częściowej ochrony przed utratą czucia, poprawę w zakresie regeneracji i częściowego przywrócenia funkcji aksonów [58].

Podobnych obserwacji dostarczyły prace na mysim modelu retinopatii cukrzycowej – kolejnego powikłania długotrwałej hiperglikemii prowadzącego do zwiększenia przepuszczalności naczyń krwionośnych, postępującego wysięku i utraty naczyń włosowatych siatkówki oraz patologicznej neowaskularyzacji [59]. W badaniach tych zaobserwowano, że zahamowanie ekspresji *AGER* u myszy z cukrzycą wiąże się ze zmniejszoną degradacją naczyń włosowatych i osłabionym odczynem zapalnym w siatkówce [60]. Ponadto stwierdzono, że blokada działania RAGE poprzez jego rozpuszczalną formę sRAGE, spowalnia dysfunkcję neuronów i rozwój patologicznych zmian w kapilarach na dnie oka [61].

Szlak RAGE odgrywa również istotną rolę w patogenezie cukrzycowej choroby nerek, która jest obecnie uważana za główną przyczynę niewydolności tego narządu na świecie [62]. Podobnie jak w przypadku wyżej opisanych powikłań, również w przypadku cukrzycowej choroby nerek, wyciszenie ekspresji *AGER* u zwierząt doświadczalnych skutkowało spowolnieniem procesów patofizjologicznych i częściową ochroną przed postępującym uszkodzeniem funkcji nerek [63].

4.2.5 Udział RAGE w patofizjologii miażdżycy

Niezależnie od współwystępowania cukrzycy, AGEs mogą promować uszkodzenie naczyń krwionośnych i tworzenie blaszki miażdżycowej [64]. Proponowane są dwa mechanizmy aterogennego działania AGEs: (i) formowanie i akumulacja depozytów AGEs w naczyniach krwionośnych, które wraz z innymi białkami tworzą masę substancji zewnątrzkomórkowej promującą usztywnienia ściany naczyń oraz (ii) rozwój zmian miażdżycowych na tle aktywacji szlaków sygnałowych zależnych głównie od RAGE [65]. Zwiększoną ekspresję receptora RAGE stwierdzono na kluczowych komórkach zaangażowanych w proces aterogenezy, tj. komórkach endotelialnych, komórkach mięśni gładkich, monocytach/makrofagach oraz limfocytach, gdzie poprzez wiązanie z ligandami nasilał on, poprzez szlaki auto- i parakrynne, proces zapalny oraz stres oksydacyjny [66-67]. Wiązanie AGEs z receptorem prowadzi także do nasilenia chemotaksji monocytów w obręb wewnętrznej ściany naczynia. Dochodzi wówczas do różnicowania monocytów w makrofagi i pobudzenia czynników prozapalnych – w tym czynnika martwicy nowotworów α (TNF-α*, ang. tumor necrosis factor-α*), interleukiny-1β, prostaglandyny E2 oraz płytkopochodnego czynnika wzrostu (PDGF, *ang. platelet-derived growth factor*) [68-70]. Stwierdzono również, że

aktywacja RAGE promuje wychwyt przez makrofagi utlenionych cząsteczek cholesterolu LDL (*ang. low density lipoprotein*) co prowadzi do powstania tzw. komórek piankowatych (a*ng. foam cells*) w obrębie blaszki miażdżycowej [68,70].

Wpływ RAGE na rozwój miażdżycy jest szczególnie nasilony u chorych z cukrzycą. Stwierdzono, że blaszki miażdżycowe pacjentów z cukrzycą osiągają większe rozmiary i zawierają większe ogniska nekrozy co koreluje z wyższym stężeniem RAGE i związanych z nim ligandów [71]. Z kolei stężenie sRAGE (blokującego działanie błonowej postaci receptora) w surowicy pacjentów z cukrzycą typu 2 był istotnie niższy niż u osób zdrowych [72], a w przypadku chorych z cukrzycą typu 1 obserwowano odwrotną korelację między stężeniem sRAGE a grubością kompleksu błony środkowej i wewnętrznej tętnicy szyjnej [73].

Wykazano również, że podwyższone stężenie AGEs we krwi jest czynnikiem ryzyka ostrych zespołów wieńcowych (ACS, *ang. acute coronary syndrome*) i zgonu z powodu chorób układu krążenia u pacjentów z niewydolnością serca i cukrzycą typu 2 [74-75].

5. Uzasadnienie połączenia prac w cykl publikacji

Przedstawiona rozprawa doktorska stanowi spójny tematycznie cykl 3 publikacji przedstawiających rolę receptora końcowych produktów zaawansowanej glikacji (RAGE) jako elementu łączącego szlaki metaboliczne otyłości i jej powikłań

W **pracy nr 1,** w oparciu o obszerny przegląd literatury, przedstawiono dotychczasowy stan wiedzy dotyczący udziału szlaku RAGE w powstawaniu dysfunkcji tkanki tłuszczowej w przebiegu otyłości. Dodatkowo opisano udział RAGE w rozwoju powikłań metabolicznych otyłości oraz wskazano potencjalne strategie terapeutyczne w leczeniu otyłości i towarzyszących jej zaburzeń metabolicznych poprzez ingerencję w szlak RAGE.

W **pracy nr 2** zbadano związek otyłości i cukrzycy typu 2 ze zmienioną ekspresją genu kodującego RAGE (*AGER*) i regulującego tę ekspresję długiego niekodującego RNA AGER-1 (lncAGER-1) w tkankach tłuszczowych. Oceniono także wpływ tych zmian na lokalne stężenia wybranych cytokin prozapalnych i adipokin.

W **pracy nr 3** przeanalizowano związek rozkładu genotypów i alleli dwóch polimorfizmów pojedynczego nukleotydu (SNP) w genie kodującym RAGE (*AGER*) z predyspozycją do rozwoju choroby niedokrwiennej serca i ostrych zespołów wieńcowych.

6. Założenia i cel pracy

6.1 Założenia pracy

Otyłość stanowi jedno z kluczowych wyzwań zdrowia publicznego, ponieważ znacząco zwiększa ryzyko i przyspiesza wystąpienie szeregu chorób, takich jak cukrzyca czy choroby układu krążenia, co przekłada się na pogorszenie jakości i skrócenie czasu życia chorych. Badania z zakresu patofizjologii wskazują, że u podstaw rozwoju związanych z otyłością powikłań leży zapalenie metaboliczne wynikające z nieprawidłowego działania (dysfunkcji) tkanki tłuszczowej. W pracach eksperymentalnych, przeprowadzonych przede wszystkim na modelach zwierzęcych, stwierdzono, że jednym z kluczowych szlaków komórkowych zaangażowanych w rozwój związanego z otyłością zapalenia metabolicznego jest szlak receptora końcowych produktów zaawansowanej glikacji (RAGE).

6.2 Cel pracy

Badania przeprowadzone w ramach niniejszej pracy doktorskiej miały na celu zbadanie roli szlaku receptora końcowych produktów zaawansowanej glikacji (RAGE) w rozwoju związanych z otyłością powikłań kardiometabolicznych u ludzi, w tym:

a) Ocenę ekspresji genu kodującego RAGE (*AGER*) w tkankach tłuszczowych chorych z otyłością i współistniejącą cukrzycą typu 2 w odniesieniu do chorych z otyłością bez cukrzycy i osób o prawidłowej masie ciała a także roli długiego niekodującego RNA AGER-1 (lncAGER-1) w regulacji tego procesu.
b) Zbadanie korelacji pomiędzy ekspresją kodującego receptor RAGE (*AGER*) a ekspresją genów kodujących adipokiny i cytokiny prozapalne w tkankach tłuszczowych chorych z otyłością w zależności od współwystępowania cukrzycy.
c) Zbadanie związku między polimorfizmami zamiany pojedynczych nukleotydów (SNP) w *AGER* a wystąpieniem ostrych zespołów wieńcowych (ACS) w polskiej populacji pacjentów z miażdżycą naczyń wieńcowych.

7. Kopie opublikowanych prac

7.1 Receptor for the Advanced Glycation End Products (RAGE) Pathway in Adipose Tissue Metabolism

International Journal of *Molecular Sciences*

MDPI

Review

Receptor for the Advanced Glycation End Products (*RAGE*) Pathway in Adipose Tissue Metabolism

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Abstract: Advanced glycation end products (AGEs) are mediators in the process of cellular dysfunction in response to hyperglycemia. Numerous data indicate that the accumulation of AGEs in the extracellular matrix plays a key role in the development of obesity-related adipose tissue dysfunction. Through binding of their membrane receptor (*RAGE*), AGEs affect numerous intracellular pathways and impair adipocyte differentiation, metabolism, and secretory activity. Therefore, inhibiting the production and accumulation of AGEs, as well as interfering with the metabolic pathways they activate, may be a promising therapeutic strategy for restoring normal adipose tissue function and, thus, combating obesity-related comorbidities. This narrative review summarizes data on the involvement of the *RAGE* pathway in adipose tissue dysfunction in obesity and the development of its metabolic complications. The paper begins with a brief review of AGE synthesis and the *RAGE* signaling pathway. The effect of the *RAGE* pathway on adipose tissue development and activity is then presented. Next, data from animal and human studies on the involvement of the *RAGE* pathway in obesity, diabetes, and cardiovascular diseases are summarized. Finally, therapeutic perspectives based on interference with the *RAGE* pathway are discussed.

Keywords: advanced glycation end products (AGEs); receptor for AGE (*RAGE*); obesity; adipose tissue dysfunction; metabolic inflammation; insulin resistance; diabetes; cardiovascular diseases

Citation: Gutowska, K.; Czajkowski, K.; Kuryłowicz, A. Receptor for the Advanced Glycation End Products (*RAGE*) Pathway in Adipose Tissue Metabolism. *Int. J. Mol. Sci.* **2023**, *24*, 10982. https://doi.org/10.3390/ijms241310982

Academic Editor: Vincent Sapin

Received: 18 May 2023
Revised: 22 June 2023
Accepted: 28 June 2023
Published: 1 July 2023

1. Introduction

Over the years, the concept of the role played by adipose tissue in maintaining human homeostasis has evolved. Initially treated solely as a mechanical insulator and energy store, it is now seen as the source of a number of substances and mediators (adipokines) that have the ability to regulate the function of organs and tissues throughout the body [1]. Consequently, the dysfunction of adipose tissue impacts the homeostasis of the entire body. This situation takes place in the course of obesity, when excess nutrients accumulated in adipocytes lead to mitochondrial dysfunction and associated oxidative stress [2]. In turn, oxidative stress can increase preadipocyte proliferation, adipocyte differentiation, and the size of mature adipocytes. In obesity, oxidative stress is not limited to adipose, and reactive oxygen species (ROS) can impair the function of hypothalamic neurons that control satiety and hunger behavior in a vicious circle mechanism [3].

These phenomena lead to reprogramming of the profile of genes expressed in the adipocyte and thus to a change in the adipokines it secretes. This process, termed adipose tissue dysfunction, is thought to underlie the development of insulin resistance predisposing to glucose intolerance and a range of other chronic complications associated with obesity, affecting virtually all organs in the human body and significantly impairing quality of life [4].

However, adipose tissue dysfunction is not just about adipocyte metabolism. Changes that occur in the adipose tissue extracellular matrix (ECM) play an equally important role in this process. The ECM is a complex structure composed of various proteins, polysaccharides, and proteoglycans and constitutes a scaffold for cells to modulate their biological processes [5]. Obesity is characterized by the massive expansion of adipose tissue; therefore, remodeling and reorganization of the ECM are necessary to provide sufficient space for adipocyte enlargement (hypertrophy) and for the formation of new adipocytes through adipogenesis from precursor cells (hyperplasia). An important component of normal adipose tissue expansion is the formation of new blood vessels, as this disruption results in adipocyte necrosis, as well as hypoxia, which trigger chronic low-grade inflammation and fibrosis, exacerbating adipose tissue dysfunction and leading to increased insulin resistance [6].

Advanced glycation end products (AGEs), resulting from the non-enzymatic glycation of lipids and proteins, are mediators in the process of cell dysfunction in response to hyperglycemia. AGEs disrupt the tertiary structure of proteins and alter their function and structural interactions. In addition, acting as ligands for scavenger receptors present on many cells (such as CD36 or the receptor for advanced glycation end products: *RAGE*), they affect numerous intracellular pathways [7,8]. In the course of hyperglycemia (both in diabetes and prediabetes), the concentrations of AGEs increase in several locations, including the ECM of adipose tissue [9]. Preclinical and clinical studies indicate that the accumulation of AGEs in the ECM may interfere with the proper differentiation of adipocyte precursors [10,11] and affect the metabolism of mature cells, including their lipid sto*RAGE* capacity [12,13], insulin sensitivity [13–15], secretory activity [16], and production of inflammatory mediators [17,18]. Therefore, inhibiting the production and accumulation of AGEs, as well as interfering with the metabolic pathways they activate, may be a promising therapeutic strategy for restoring the normal function of adipose tissue and thus in combatting obesity-related comorbidities.

The aim of this narrative review is to summarize the data on the role of the *RAGE* pathway in adipose tissue dysfunction in obesity and its metabolic complications. First, the mechanisms of AGE formation and the *RAGE* signaling pathway are briefly presented. Next, we outline the role of the *RAGE* pathway in the regulation of adipogenesis and adipose tissue function. We then summarize data from animal and human studies on the involvement of the *RAGE* pathway in obesity, diabetes, and cardiovascular disease. Finally, we discuss therapeutic perspectives based on interference with the *RAGE* pathway.

2. Advanced Glycation End Products (AGEs) and the Receptor for Advanced Glycation End Products (RAGE) Pathway

2.1. Advanced Glycation End Products

AGEs are a heterogeneous group of compounds formed exogenously or endogenously from different precursors and by different mechanisms. One criterion for the division of AGEs is their origin: compounds absorbed from food or cigarette smoke are called exogenous AGEs, and those produced in tissues and body fluids are endogenous; these, in turn, can be further divided according to their precursor. High temperatures and extended food preparation times increase the formation of these molecules. Notably, exogenous AGEs are formed more rapidly and in larger quantities. Regardless of the source, AGEs can also be divided into cross-linkers, non-fluorescent cross-linker products, or non-fluorescent non-cross-linkers. However, the exact categorization of AGEs by structure has been described elsewhere and is not the subject of this article [19–21]. To date, more than 20 different AGEs have been characterized, including, for example, lysine carboxymethyl (CML), lysine carboxyethyl (CEL), pentosidines, and lysine methylglyoxal dimers. These compounds have received increased research attention due to their role in the development of chronic conditions such as diabetes, cardiovascular and neurological diseases, and cancer [19,22].

AGEs present in adipose tissue can be either exogenous (supplied by the circulation) or endogenous (formed locally as a result of adipose-tissue-dysfunction-related oxidative

stress or diabetes-related hyperglycemia). Hyperglycemia not only promotes formation the of endogenous AGEs but also their accumulation. Glycation (i.e., the non-enzymatic glycosylation of amino residues) is a spontaneous, two-stage process, the intensity of which depends on the content of simple sugars, including glucose, in the body. First, unstable Schiff bases are formed through the direct correlation between the carbonyl groups of reducing sugars and the free amino groups of nucleic acids, proteins, or lipids. If amino acids are included, this can lead to disturbances in the structure and function of proteins crucial for cells' organization and metabolism [19–21]. In the case of nucleic acids, the glycation of amino residues promotes the breaking of nucleotide chains and the formation of mutations, while the glycation of cell membrane lipids (e.g., Phosphatidylethanolamine) contributes to their increased permeability. Second, molecules known as Amadori products may be modified in two ways: converted by oxidation or hydrolysis into AGEs or by forming AGE precursor compounds, such as glyoxal (GO) and methylglyoxal (MGO). In addition to Amadori products, a heterogenous class of reactive carbonyl is created and can be converted into protein adducts or protein crosslinks. As a heterogenous group, AGEs are also generated through the Maillard pathway, the Wolff pathway (oxidation of monosaccharides), and the Namiki pathway (amino acid or lipid degradation and the cleavage of dicarbonyl compounds from aldimines) [19,21]. AGE formation depends on the concentration and reactivity of glucose, the amount of AGE precursors, and the availability of free amino groups. The Wolff (polyol) pathway is active under hyperglycemic conditions and involves the formation of sorbitol from glucose. Excessive activation of this pathway leads to increased dicarbonyl formation through the accumulation of upstream metabolites such as fructose. Endogenous lipid peroxidation, resulting from the ROS-induced lipid peroxidation of polyunsaturated fatty acids present in cell membranes, can also lead to the increased formation of dicarbonyls, and subsequently, AGEs (the Namiki pathway). AGEs formed by reactive dicarbonyls produced by lipid peroxidation are also referred to as ALEs (advanced lipoxidation end products) [23].

2.2. Receptor for the Advanced Glycation End Products (RAGE) Pathway

Both endogenous and exogenous AGEs have the ability to bind to a specific *RAGE* receptor present on the surface of, among others, endothelial cells, muscle cells, immunocompetent cells, glomerular podocytes, and neurons. *RAGE* expression has been found in many adipose-tissue-forming cells: adipocytes, their precursors, stromal cells, vasculature, or infiltrating macrophages [24].

The binding of AGEs to a specific receptor of the immunoglobulin superfamily becomes a signal for the intracellular formation of reactive oxygen derivatives and the activation of transcription factors. As a result of the oxidative stress developing in cells, the pro-inflammatory nuclear factor κB (NF-κB) is activated, as well as other signaling pathways (including, for instance, mitogen-activated protein kinases (MAPK), NADPH oxidase, extracellular regulated(ERK)-1/2, and c-JunN-terminal kinase (JNK)), leading to the increased transcription of genes encoding cytokines and adhesion molecules [19,22,25,26]. The inflammatory process that develops in this way promotes organ damage and the development of chronic complications associated with both aging and obesity. *RAGE* has been shown to engage multiple diverse ligands, not only AGEs but also damage-associated molecular pattern molecules, such as high-mobility group box 1 (HMGB1) S100 proteins and amyloid-β peptide [27,28]. The pathways activated upon *RAGE* binding are often cell- and ligand-specific. For instance, in murine macrophages (RAW cells), the stimulation of *RAGE* by glycated bovine serum albumin (BSA) leads to the activation of the Jak–Stat pathway, while the binding of *RAGE* with CML in rat mesangial cells results in the activation of angiotensin II signaling [23]. A simplified scheme of *RAGE*-mediated signaling is shown in Figure 1.

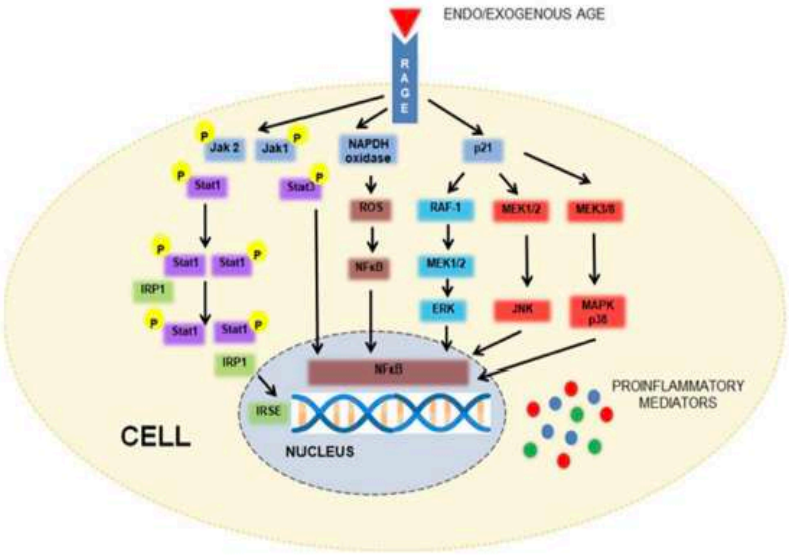

Figure 1. A simplified scheme of *RAGE*-mediated signaling. The interaction between the advanced glycation end products (AGEs) and their receptor (*RAGE*) stimulates several intracellular signaling cascades, e.g., Jak/Stat, NADPH oxidase, mitogen-activated protein kinases (MAPK)/p38, extracellular regulated (ERK)-1/2, and c-JunN-terminal kinase (JNK). These phenomena result in the activation of transcription factors, such as nuclear factor (NF-kB) or IFN-stimulated response elements (ISRE), enhancing the expression of proinflammatory mediators (modified, based on [8,23]).

Several studies have supported the hypothesis that, in addition to the full-length form at the cell surface, *RAGE* also exists in other non-membrane-bound forms. Soluble *RAGE* (s*RAGE*) is produced by either the proteolytic cleavage of the full-length form of *RAGE* (c*RAGE*) or alternative mRNA splicing and lacks trans-membrane and cytoplasmic domains. Importantly, s*RAGE* can inhibit full-length *RAGE* activation by binding to their ligands in the extracellular space [29].

3. Influence of the RAGE Pathway on Adipose Tissue Activity

Both white (WAT) and brown adipose tissue (BAT) are flexible organs that play a prominent role in regulating energy homeostasis [30]. In addition, adipocytes have been proven, inter alia, to defend internal organs from mechanical harm and secrete biologically active substances capable, in an endocrine manner, of controlling the function of several organs and tissues throughout the body [31]. Most recently, the relationship between *RAGE* and adipose tissue activity has gained more attention due to the possible modulation of adipocytes' functioning.

3.1. Influence of the RAGE Pathway on Adipogenesis and Adipose Tissue Browning

Adipose-derived stem cells (ASCs) are multipotent cells naturally occurring in adipose tissue, and which are able to differentiate into adipogenic, osteogenic, chondrogenic, and other types of cells. Although ASCs are mostly derived from white adipose tissue, they are also present in BAT but with different characteristics [32,33].

Exposure to a high-fat, high-sugar diet negatively impacts on ASCs in adipose and bone tissue. Excessive energy intake promotes AGE formation, which impairs the proliferation and differentiation potential of ASCs, manifested as the decreased cell counting kit-8 (CCK-8) protein level and alkaline phosphatase (ALP) activity. However, the effect can be species specific. In mice, a high-fat and high-carbohydrate diet leads to a lower expression of osteogenic differentiation genes (Alp, Opn, Ocn, and Runx2) in ACCs. This effect may result from DNA methylation in ASCs and the key role this process plays in the Wnt/β-catenin signaling pathway. Indeed, AGEs drive the downregulation of Wnt

signaling molecules (β-catenin, lymphoid enhancer-binding factor 1 (LEF1), and glycogen synthase kinase 3 beta GSK3β), while enhancing the expression of methyltransferase genes [34]. However, in vitro studies using human mesenchymal stem cells (MSCs) suggest that exposure to AGEs inhibits their adipogenic differentiation (assayed by oil red O staining, lipoprotein lipase production, and intracellular triglyceride content) without incurring significant impairments in osteogenic development [10].

In addition to the evidence for AGE–*RAGE* pathway involvement in the regulation of ASCs' fate, there are data on its contribution to adipose tissue expansion via the modulation of adipocyte senescence. For instance, in murine preadipocytes (3T3-L1 cells), activation of the AGE–*RAGE* axis, probably by the blockade of the p53 protein, restores the adipogenic potential of these cells. Interestingly under these experimental conditions, AGEs showed no effect on adipogenesis in young preadipocytes, and the exact mechanism of this phenomenon remains unknown [35].

Regardless of the direct impact on adipocytes, AGE formation in hyperglycemic conditions damages other components of adipose tissue. Recent studies on 3T3-L1 preadipocytes suggest that the accumulation of AGEs in the ECM, independently of the *RAGE* pathway, impairs adipocyte differentiation [36]. In addition, through ECM modification, AGEs have direct effects on cell niches and plasma membranes via loss of their plasticity and therefore induce alterations in cellular signaling and cytoskeletal organization. Collectively, with AGE accumulation, adipogenesis is reduced, with the downregulated differentiation of fibroblasts and adipocytes [36]. Moreover, the accumulation of AGEs in the ECM also results in impaired glucose uptake and may contribute to the development of insulin resistance, as was shown in human primary adipocytes isolated from diabetic and non-diabetic obese individuals [37].

Furthermore, recent research highlights the potential role of the AGE–*RAGE* axis in regulating adaptive thermogenesis. This process activates temporary changes in white adipocytes in response to certain stressors, such as cold, exercise, or excessive energy input. Technically, it results in the stimulation of β3-adrenergic receptors, leading to the overexpression of uncoupling protein 1 (UCP1) and thereafter "beiging" of white adipocytes [38]. Activation of the *RAGE* gene in mice fed high-fat diets (HFDs) was found to downregulate adipose tissue browning and thermogenic activity compared with controls on a normal chow diet. Thus, the global deletion of *RAGE* in mice protects from this effect, even during exposure to an HFD [24,39]. Experiments on *RAGE*−/− murine adipocytes strongly supports this evidence, indicating that *RAGE* inhibits thermogenesis in WAT and BAT via diminishing the protein kinase A (PKA)-mediated phosphorylation of hormone-sensitive lipase (HSL) and other pathways (e.g., p38 mitogen-activated protein kinase, MAPK) involved in the regulation of energy balance [24].

3.2. *Influence of the RAGE Pathway on Lipolysis/Lipogenesis*

Molecules of major importance involved in adipose tissue metabolism are fatty acids (FAs). They are one of the two products of the hydrolytic degradation of triglycerides (TGs): lipolysis regulated by catecholamines, insulin, and natriuretic peptides [40,41]. It is well established that an activated β3-adrenergic receptor leads to the mobilization of cAMP and mediates the PKA-dependent phosphorylation of hormone-sensitive lipase (HSL) and p38 mitogen-activated protein kinase (MAPK), enzymes directly associated with lipolysis [42]. In a *RAGE*-rich environment, this pathway is inhibited; thus, lipid droplet accumulation increases, causing obesity and its complications. This concept is based on a mouse model, in which the transplantation of adipose tissue from mice with a global overexpression of *RAGE* to wild-type (WT) mice promoted obesity and insulin resistance [24].

On the other hand, detailed analysis of preadipocyte cultures revealed that the stimulation of *RAGE* induces an increase in adipocyte size, accelerates their differentiation, and enhances higher expression of *RAGE* ligands (e.g., AGE, HMGB1, S100β, and FA). In the preadipocytes transfected with small interfering RNA (siRNA), knockout of the *RAGE* gene led to the suppression of *RAGE* ligands and thus inhibited cell hypertrophy [13]. Mor-

phological changes in adipocytes might also be mediated via crosstalk between Toll-like receptors (TLRs). This theory was followed by a series of studies on mice and cell cultures indicating that the downregulation of Toll-like receptor 2 (TLR2) and Toll-like receptor 4 (TLR4) in diet-induced obesity improves adipocyte differentiation and insulin sensitivity in mice [43,44]. However, experiments on mice fed an HFD and murine adipocytes revealed that the suppression of TLR2 enhances adipogenesis; TLR4 results in an opposite effect [44,45]. This dichotomy opens paths for future studies exploring *RAGE*–TLR linkages. In addition, the activation of *RAGE* promotes a switch in macrophages infiltrating the adipose tissue of mice with diet-induced obesity to M1-type producing inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL) 1β [13,46]. Consequently, the route to the synthesis of ROS and the blockade of the phosphoinositide 3-kinase (PI3K)–protein kinase B (AKT) pathway promoting lipogenesis is open.

Another function of AGEs relevant to lipid metabolism is the inhibition of apolipoprotein E (ApoE) expression. ApoE, as a component of very low density lipoproteins (VLDLs), remnant lipoproteins, and high-density lipoproteins, contributes to lipoprotein internalization and degradation [47,48]. Using 3T3-L1 cells and mouse models, researchers observed that in hyperglycemic conditions, oxidative stress and the NF-κB pathway mediate ApoE abolishment. Blocking ApoE could partially participate in suppressing adipocyte triglyceride synthesis because the transplantation of wild-type adipocytes in mice with a global ApoE knockout results in less triglyceride accumulation. Additionally, ApoE knockdown adipocytes have fewer VLDL receptors on their cell surface, which results in the reduced internalization of lipoproteins [48].

3.3. Influence of the RAGE Pathway on Adipose Tissue Insulin Resistance

The AGE–*RAGE* axis in adipose tissue also appears to play a key role in the development of obesity-related insulin resistance. Potential pathogenetic mechanisms include, but are not limited to, altered insulin signaling, the promotion of adipocyte hypertrophy and related functional complications, the exacerbation of macrophage-mediated inflammation, impaired adipose tissue browning, and the dysregulation of adipokine secretion [49].

Animals with global knockout of the *RAGE* gene (*RAGE*−/−) and exposed to an HFD were characterized by improved glucose tolerance compared with wild-type (WT) controls reared under the same conditions [50]. This effect may be related to increased insulin-induced protein kinase B (AKT) phosphorylation, critical for glucose–insulin crosstalk, in the adipose tissue of the $RAGE^{-/-}$ animals compared with the control mice. In addition, the $RAGE^{-/-}$ mice showed reduced levels of free fatty acids and glycerol compared with the WT mice [50]. Through *RAGE*-mediated AMPK downregulation of the AKT signaling pathway, AGEs can also decrease insulin sensitivity in other tissues crucial for glucose metabolism, such as the skeletal muscle [51]. *RAGE* lacks intracellular kinase signaling activity; therefore, the effects of receptor stimulation depend on the proteins binding to its intracellular domain. One example of such proteins is Diaphanous 1 (DIAPH1), a member of the formin family of Rho GTPase binding proteins with identified roles in cytokinesis, actin polymerization, cytoskeleton remodeling, and immune cell trafficking. Activation of the AGE/*RAGE*/DIAPH1 axis was found to be positively associated with the expression of genes reflecting impaired glucose metabolism and markers of inflammation in the subcutaneous adipose tissue of obese individuals [51]. Moreover, AGE-stimulated murine adipocyte hypertrophy, which is accompanied by downregulation of insulin sensitivity genes (e.g., glucose transporter type 4 and adiponectin), may contribute to diminished glucose uptake and impaired insulin signaling [13].

AGE-induced inflammation also plays a key role in the development of insulin resistance. Consistently, global *RAGE* knockout correlates with diminished macrophage migration to adipose tissue and lower systemic IL-6 concentrations in mice on an HFD [50]. Perigonadal adipose tissue (PGAT) and bone marrow derived from *RAGE*−/− mice on an HFD exhibit reduced expression of inflammatory markers typical for M1 macrophages compared with controls. In addition, macrophages in the PGAT of *RAGE*−/− mice were

shown to express lower levels of the CD11c marker which, together with polarization into the anti-inflammatory M2 phenotype, results in the upgraded regulation of insulin secretion [52,53]. The effect of AGEs on TLR signaling in the context of insulin sensitivity may also be relevant because mice with suppressed TLR2 presented disruption of glycometabolic control, higher levels of TGs, and inflammatory molecules [45]. However, other studies report contrasting results linking deficiency in TLR2 with improvements in glucose uptake parameters [13,54]. Another mechanism by which the AGE–*RAGE* pathway may regulate insulin sensitivity is through its effect on brown adipose tissue expansion; as described above, *RAGE* deficiency is a strong indicator of WAT browning [24,55].

Interestingly, *RAGE* impacts on glucose homeostasis were found to be sex-dependent. Female *RAGE*−/− mice on an HFD showed significantly improved glucose and insulin tolerance compared with males. This finding was accompanied by the increased polarization of M2 macrophages and the expression of genes involved in browning in adipose tissue, as well as elevated insulin-induced AKT phosphorylation [56]. Finally, the AGE–*RAGE* axis can modulate the insulin sensitivity of adipose tissue by regulating the adipokines it secretes (described in detail in the following section) [49,57].

Given the role of *RAGE* activation in the development of insulin resistance, it is conceivable that silencing the signals transmitted by this receptor may represent a therapeutic strategy to improve insulin sensitivity in patients with impaired glucose tolerance. The use of *RAGE* antagonists could potentially improve insulin receptor signaling, increase the expression of genes encoding glucose transporters, and reduce intracellular inflammation not only in adipocytes but also in hepatocytes and myocytes, etc.

3.4. Influence of the RAGE Pathway on Adipokine Secretion

Another feature of adipose tissue as a very dynamic organ is the secretion of bioactive peptides and proteins, including leptin, adiponectin, IL-6, TNF-α, and other cytokines, contributing to metabolic homeostasis. These molecules with both pro- and anti-inflammatory activity maintain insulin sensitivity and regulate cardiovascular and reproductive functions [57,58]. The obesity-related dysregulation of the AGE–*RAGE* pathway has an impact on adipokine secretion, although the exact roles in this crosstalk are still not clarified.

Leptin, mostly produced in WAT and released into the bloodstream in concentrations proportionate to fat mass, plays a crucial role in preserving energy balance as a part of the negative feedback loop. Through the blood–brain barrier (BBB), it conveys information to the central nervous system regarding energy expenditure; therefore, it is essential for maintaining metabolic homeostasis. In obesity, levels of leptin are higher and transport across the BBB is altered, while activated inflammatory pathways promote central leptin resistance. Similarly to IR in diabetes, impaired response to leptin seems to be selective. It has been shown that in mice on HFD leptin, due to activation of the renal sympathetic nervous system, the elevation of blood pressure was stimulated. However, this failed to induce anorexigenic effects [59,60]. In mice with obesity-associated diabetes, leptin downregulation leads to overexpression of *RAGE* in β-cells and therefore inhibits insulin infusion and mediates AGE-elicited pancreatic islet apoptosis [61]. It was suggested that AGE formation during prolonged hyperglycemia could cause β-cell damage through insufficient leptin action and subsequent *RAGE* induction [61]. These phenomena result in ROS generation and endoplasmic reticulum (ER) stress response. AGEs with leptin inhibitory properties include glycolaldehyde-modified BSA (which binds *RAGE*) and oxidized low-density lipoproteins which, in turn, interact with the CD36 receptor, as shown in 3T3-L1 adipocytes and mouse epididymal adipocytes [16]. An important role in the interaction between the AGE–*RAGE* pathway and leptin is played by the peroxisome proliferator-activated receptor-γ (PPAR-γ) [62]. PPAR-γ has been discovered to co-regulate leptin gene expression, as a heterozygotic deletion of PPAR-γ in HFD mice increased leptin levels [63]. Proper PPAR-γ expression in murine adipose tissue hampers AGE formation and acts as a downregulator of *RAGE*, thus contributing to the prevention of AGE–*RAGE* axis-mediated cardiovascular disorders [63]. Further studies should also consider the role

of the AGE–*RAGE* pathway in the development of hypothalamus inflammation and central leptin resistance [64]. It is also worth pointing out that post-translational modifications play an important role in the regulation of leptin function. For example, the phosphorylation of serine 491 is essential for leptin's effects on hypothalamic α2AMPK activity, neuropeptide expression, food intake, and body weight [65]. Leptin is a peptide; therefore, one would expect glycation to modify its functions. However, to date, there are no data available in the literature on the effect of glycation of the leptin molecule on its ability to regulate adipogenesis and adipose tissue function.

Leptin levels as well as the expression of adiponectin are significantly modified in obesity. In a recent preclinical study, an inverse correlation between serum adiponectin and atherosclerosis in $RAGE^{-/-}$ mice was reported [66]. Still under investigation is whether the *RAGE* impacts on adiponectin secretion are direct or occur through the regulation of adipose tissue inflammation. One study revealed that the stimulation of *RAGE* by its agonist N(ε)-(carboxymethyl)lysine (CML) resulted in a significant increase in inflammatory molecules' expression (such as plasminogen activator inhibitor (PAI)-1 and IL-6) with a simultaneous reduction in adiponectin secretion by human preadipocytes [57]. This observation was followed by another experiment. Likewise, in the case of leptin, ROS generation is recognized as a promoter of adiponectin downregulation. Indeed, Maeda et al. reported that epithelium-derived factor (PEDF), which is a multifunctional, antioxidant glycoprotein, inhibits the AGE–*RAGE*-induced suppression of adiponectin mRNA levels in human visceral adipocytes through the suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [67].

Data regarding the effect of the activation of the AGE–*RAGE* pathway on the expression of other adipokines are scarce. However, it is known that *RAGE*-mediated signaling may play a key role in the pro-inflammatory effects of visfatin [68]. In turn, resistin production by macrophages/monocytes in humans correlates with serum s*RAGE* concentrations [69].

The impact of the AGE–*RAGE* pathway on adipose tissue is summarized in Table 1.

Table 1. The impact of advanced glycation end products (AGEs) on adipose tissue.

Process	Experimental Model	Effect of AGE–*RAGE* Pathway Activation	Mechanism	References
Adipogenesis	Human MSCs	↓ differentiation potential towards adipocytes		[10]
	ASCs from diabetic osteoporotic and control C57BL/6 mice	↓ proliferation ↓ differentiation potential of ASCs	↓ Wnt signaling pathway ↑ methyltransferase genes	[34]
	Senescent murine preadipocytes	↑ senescent preadipocytes differentiation	↓ p53 protein	[35]
Browning and thermogenesis	$RAGE^{-/-}$ mice $RAGE^{-/-}$ murine adipocytes	↓ thermogenesis	↓ PKA-mediated phosphorylation of HSL and p38 MAPK	[24]
	Mice on an HFD	↓ browning ↓ thermogenesis		[39]
Lipolysis	$RAGE^{-/-}$ mice	↓ lipolysis ↑ weight gain	↓ PKA-mediated phosphorylation of HSL	[24]
Lipogenesis	DIO mice receiving a *RAGE* inhibitor	↑ lipogenesis	↑ TLR receptors ↓ PI3K protein kinase B pathway	[46] [48]
Insulin sensitivity	$RAGE^{-/-}$ mice	↓ insulin sensitivity	↓ PI3K protein kinase B pathway ↑ DIAPH1 expression ↑ metabolic inflammation	[13,50] [51] [50]
Adipokine secretion	3T3-L1 adipocytes	↓ leptin secretion	↑ ROS synthesis ↓ PPAR-γ expression	[16] [63]

Table 1. *Cont.*

Process	Experimental Model	Effect of AGE–*RAGE* Pathway Activation	Mechanism	References
	$RAGE^{-/-}$ mice	↓ adiponectin secretion	↑ ROS synthesis ↑ metabolic inflammation ↓ NADPH oxidase	[57] [67]

↓, decrease; ↑, increase; ASCs, adipose-derived stem cells; DIAPH1, diaphanous 1 protein; DIO, diet-induced obesity; HFD, high-fat diet; HSL, hormone-sensitive lipase; MAPK, mitogen-activated protein kinase; NADPH, nicotinamide adenine dinucleotide phosphate; PKA, protein kinase A; PI3K, phosphoinositide 3-kinase; PPAR-γ, peroxisome proliferator-activated receptor-γ; *RAGE*, receptor for advanced glycation end products; ROS, reactive oxygen species.

4. RAGE Pathway in Animal and Human Obesity and Related Complications

4.1. RAGE Pathway in Animal Models of Obesity

Two characteristics that distinguish obesity from other diseases are its worldwide prevalence and complexity, as well as the involvement of various organs, resulting in more than 200 complications [70]. Due to its high heterogeneity, novel therapies characterized by safe and personalized approaches are required in the treatment of obesity. Animal models, especially rodents, play an essential role in understanding the pathogenesis of obesity and finding new therapeutic solutions. Due to their susceptibility to nutritional interventions and the possibility of genetic modifications, they enable us to mimic different models of obesity and metabolic disorders. Nevertheless, to date, there is no ideal animal model to replicate human obesity. Factors that cause this imperfection include the different pathomechanisms of obesity in rodents compared with humans (e.g., different contributions of thermogenesis to energy balance) and the fact that experimental protocols are often based on diets with excessive fat contents or exposure to high temperatures, disrupting macro- and micronutrient balance and promoting bias in the methodology [71,72].

Given that the *RAGE* pathway has been proposed to participate in the development and progression of obesity, a number of preclinical studies exploring this hypothesis have emerged. As described before, $RAGE^{-/-}$ mice on an HFD exhibit improved glucose tolerance and decreased lipolysis compared with WT animals, while females are also characterized by the reduced infiltration of adipose tissue by pro-inflammatory macrophages and the more intense browning of white adipocytes [24,50]. However, in other experimental settings, $RAGE^{-/-}$ mice exposed to an HFD were characterized by the potential of developing clusters of metabolic syndrome (MetS) manifested by accelerated weight gain, increased plasma cholesterol, and higher insulin levels compared with control animals [73,74]. In addition, $RAGE^{-/-}$ mice exhibited lower expression of the genes encoding antioxidative enzymes (Mn and Cu/Zn superoxide dismutases) and ceruloplasmin in cardiac tissue [74]. In contrast, in a study by Hoffman et al., the hearts and aortic valves of $RAGE^{-/-}$ mice exposed to an HFD exhibited fewer morphometric changes, less calcification, and less AGE accumulation compared with WT C57BL/6 mice bred under the same conditions. Moreover, $RAGE^{-/-}$ mice had a more favorable high-density to low-density lipoprotein (HDL/LDL) ratio, with inflammatory and oxidative stress parameters protecting them from ventricular remodeling [75,76]. These findings are consistent with data on the contribution of the AGE–*RAGE* pathway to the activation of inflammatory pathways, and the acceleration of dendritic cell maturation and cytokine production, which leads to the overexpression of genes causing cardiomyocyte hypertrophy and heart failure [77].

Animal models of obesity also indicate that the inflammatory state resulting from the activation of the AGE–*RAGE* pathway is implicated in the development of hyperglycemia-associated complications, such as retino-, neuro-, and nephropathy, as well as an increased risk of bone fracture and osteoporosis [78,79]. These observations suggest that blockade of the AGE–*RAGE* pathway may be a valuable therapeutic strategy for the future prevention and treatment of the chronic complications of diabetes.

4.2. The RAGE Pathway in Human Obesity

The growing awareness that *RAGE* plays a pivotal role in the development and progression of human obesity, as well as the knowledge regarding adipose tissue as an active endocrine organ, has led to the design of many experiments defining the functions of *RAGE*.

Attempts have been made to assess to what extent different lifestyle interventions might influence AGE–*RAGE* pathway activity. Using an alternate-day fasting (ADF) diet as a model of weight loss, changes in soluble *RAGE* isoforms (endogenous soluble *RAGE*, es*RAGE*, and cleaved *RAGE*, c*RAGE*) and adipokines were identified. This model assumed an intake of 25% of baseline caloric needs on fasting days and 125% of individuals' energy needs on non-fasting days. As a result, es*RAGE* and the c*RAGE*:es*RAGE* ratio were significantly higher after 24 weeks of ADF in the experimental group. In addition, at this time point, changes in adipose tissue mass were negatively correlated with the es*RAGE* form. More interestingly, when considering fat deposits, a loss of subcutaneous adipose tissue was associated with a significant increase in es*RAGE* and the simultaneously constant level of c*RAGE*, suggesting es*RAGE* as an important factor in weight changes across fat deposits. As an additional effect, ADF resulted in moderate alterations in adipokine concentrations including negative correlations between IL-6, leptin, and s*RAGE* with no influence on total adiponectin level. However, changes in adiponectin concentration were inversely correlated with the c*RAGE*:es*RAGE* ratio [80]. Changes in the concentrations of soluble *RAGE* isoforms appear to be very dynamic; therefore, data from other studies revealed a positive correlation between es*RAGE* and adiponectin levels in obese female subjects [81]. Obesity-induced reductions in adiponectin and es*RAGE* levels have been conversely associated with markers of lipid peroxidation and platelet activation. Therefore, es*RAGE* may act as an antagonist of platelet activation, and its concentration may be helpful in assessing the cardiovascular risk of obesity [81]. In contrast, higher s*RAGE* levels are positively correlated with the mortality risk from cardiovascular diseases, whereas increased total *RAGE* concentrations indicate a greater possibility of death from all causes [82].

The concept of the involvement of the AGE–*RAGE* pathway in cardiovascular disease has inspired numerous studies on the association between epicardial adipose tissue (EAT) and s*RAGE* in this context. Studies have shown that in cardiometabolic diseases, the increasing EAT thickness and the increased expression of inflammatory genes are driven by the higher expression of *RAGE*; this is more pronounced in patients with diabetes. In addition, s*RAGE* as a decoy receptor is negatively associated with the EAT volume, waist circumference, and visceral adipose tissue deposits in obese but healthy women. Therefore, s*RAGE* may be a marker for cardiometabolic diseases, correlating with fat accumulation and visceral and epicardial deposits, associated with increased cardiovascular risk [83]. Subsequently, AGE and s*RAGE* concentrations and their potential as indicators of vasculopathy have been investigated in several studies across different age groups [84,85]. These studies show that the increased deposition of cholesterol and its derivatives or other markers of vascular disorders in arterial walls was associated with a decrease in s*RAGE* expression [86,87].

Considerable research has also been dedicated to the association of s*RAGE* and its forms with the risk of developing type 2 diabetes and its complications [12,78,88,89]. It was found that s*RAGE* and c*RAGE* were negatively correlated with plasma glucose levels. The decrease in c*RAGE* concentration progressed in patients with impaired glucose tolerance and diabetes mellitus, which was probably due to the hyperglycemia-induced enhanced proteolytic degradation of the *RAGE* subdomain mediated by disintegrins and metalloproteinases. The loss of es*RAGE* alone, on the other hand, was particularly marked in obese patients, in a manner proportional to BMI and percentage body fat. Age appeared to be a significant factor influencing s*RAGE* isoform concentrations. With age, differences in the concentrations of its isoforms disappear, but s*RAGE* and c*RAGE* concentrations were still reduced in people with type 2 diabetes [90]. In addition to attempts to understand the role of the AGE–*RAGE* axis in diabetes progression, the impact of this pathway on

hyperglycemia-mediated calcification has been investigated. AGEs have been shown to mediate the activation of cellular and systemic responses leading to the activation of protein kinase C (PKC), p38 mitogen-activated protein kinase (MAPK), fetuin-A, and the NF-kB and ERK1/2 pathways upregulating extant bone matrix proteins, therefore promoting vascular calcification. AGE–*RAGE*-pathway-induced oxidative stress is also implicated in a phenotype shift of vascular smooth muscle cells to osteoblast-like cells [91].

Shedding light on the relationship between the AGE/*RAGE* axis and obesity, attention needs to be paid not only to endogenous forms but also dietary AGEs (dAGEs) and their impact on anthropometric measures. Significant negative associations between dAGE consumption and BMI, waist circumference, waist-to-hip ratio, fat-free mass, and muscle mass index in non-linear models have been described [92]. Similarly, a recent meta-analysis showed a negative correlation between circulating AGEs and BMI [93]. In turn, some researchers claim that diets with low intake of AGEs reduce BMI, with no such impact on waist circumference. This paradox might be explained using CML as a marker of AGEs, which, in obesity, is trapped in adipose tissue and subsequently causes falsely reduced levels of serum CML. Therefore, the suggested AGE type for further studies is methylglyoxal-derived hydroimidazolone-1 (MG H1) [94]. Low intake of AGEs is considered a preventative measure for obesity and its associated complications. Firstly, a recent meta-analysis revealed that diets with low levels of AGEs significantly decreased insulin resistance, fasting insulin, and total and LDL cholesterol, best reflected in subjects on prolonged low-AGE diets and with metabolic syndrome risk factors [95]. Secondly, low AGE consumption lowers leptin levels, with parallel increases in adiponectin concentration, which is consistent with previous studies highlighting the role of dAGEs in insulin resistance. Due to the heterogeneity of the diet, it is difficult to make precise recommendations on the optimal intake of dAGEs, but limiting the consumption of dAGEs may be a beneficial factor in supporting a healthy lifestyle [94,95]. In addition, the heterogeneity of the signals transmitted by *RAGE* means that the therapeutic effects associated with the blockade of the AGE–*RAGE* pathway can sometimes be difficult to predict.

Figure 2 summarizes in a simplified way how AGEs, by inducing adipose tissue dysfunction, may contribute to the development of obesity-related complications.

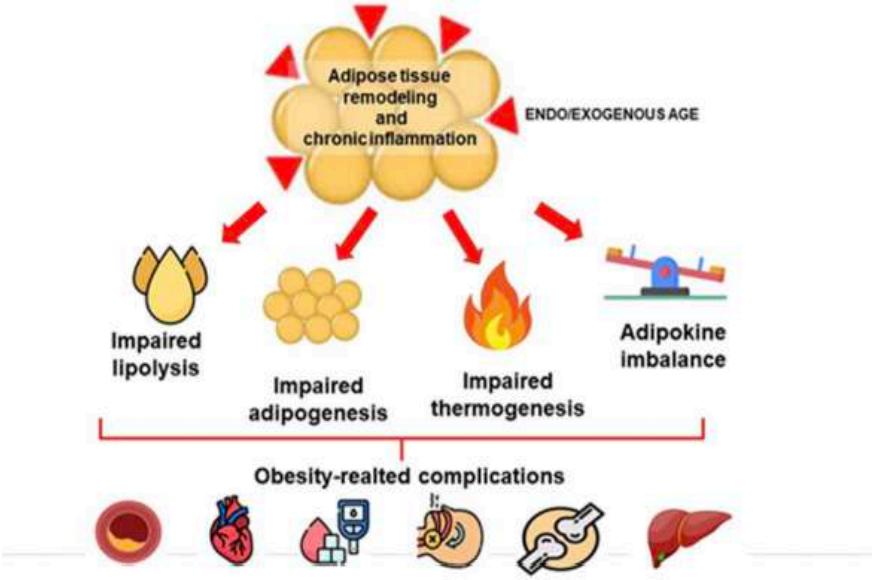

Figure 2. Effects of advanced glycation products (AGEs) on adipose tissue dysfunction and the development of obesity-related complications.

5. Therapeutic Perspective of Interfering with the RAGE Pathway to Counteract Obesity

A key feature to resolve the interplay between the *RAGE* pathway and the pathogenesis of obesity and related comorbidities is uncovering AGE receptor functions that could be exploited therapeutically. Dietary interventions remain the primary method for regulating AGE concentrations.

There is rapidly growing awareness of the impact of food nutritive value and daily calorie intake on health [96,97]. Thus, previous studies have demonstrated that reduced energy intake acts against AGE accumulation. Only approximately 10% of exogenous AGEs are absorbed from the diet; therefore, caloric restrictions have been found to be an important factor in suppressing *RAGE* mRNA levels, oxidative stress, and inflammatory responses in tissues, although the data are conflicting [98–102]. Accordingly, as described, weight loss might also have a beneficial impact on s*RAGE* concentration and adipocyte dysfunction. Importantly, the quantity as well as the quality of the diet contribute to AGE formation. Hence, Mediterranean diets based on monounsaturated fatty acids (MUFAs) and plant-based ingredients and prepared at lower temperatures and in higher humidities, resulting in lower doses of dAGEs, are preferred over Western diets rich in saturated fatty acids (SFAs) and highly processed, fried, or grilled food. In this setting, the daily intakes of PUFAs and MUFAs in the Mediterranean diet show antiglycation and antioxidant properties, such as decreased serum levels of AGEs and the expression of *RAGE* [101,102]. In addition, gene–diet interactions can modify metabolic parameters. For instance, via fatty acid desaturase 2 (FADS2) gene polymorphism, which significantly interacts with weight, adipose tissue mass, waist circumference, and cholesterol values and heterozygotes tend to exhibit lower accumulation of dAGEs [103].

Several studies have indicated that high daily concentrations of AGEs, especially in Western diets, correlate with the development and progression of multiple chronic diseases. Therefore, the effect of low dietary AGEs on biomarkers of these health disturbances was investigated. For example, there is evidence demonstrating that low intake of AGEs might decrease the level of 8-isoprostanes (markers of oxidative stress), particularly in patients with diabetes and chronic kidney disease [104,105]. Additionally, vascular cell adhesion molecule 1 (VCAM-1) and oxidized LDL concentrations, markers of cardiovascular diseases, are increased with high-AGE diets in hyperglycemic conditions, which might underpin a positive role of AGE-restricted diets. However, this effect was only observed with a small sample size, and further studies are necessary to support this consideration [106].

Actions aimed at lowering AGE concentrations do not solely result in beneficial cardiometabolic effects. AGEs also interact with periodontal tissues in diabetes and suppress the osteogenic differentiation ability of human periodontal ligament stem cells (hPDLSCs), leading to their degeneration. Wnt/β-catenin is considered to be the activated pathway; however, berberine hydrochloride, proposed as an inhibitor of this pathological process, may recover the differentiation abilities of hPDLSCs and can thus be considered an anti-AGE agent, useful in the treatment of diabetes-related periodontitis [107].

Given the clear negative correlation between *RAGE* isoforms and cardiometabolic indices, targeting their anti-inflammatory, antioxidative effects might be a step-up approach for the treatment of obesity and its comorbidities [108]. However, the current state of knowledge does not enable precise determination of the effect of long-term calorie restrictions and diets with different AGE contents on cardiometabolic risk and survival. Moreover, the results of randomized and observational studies are often contradictory. Conclusions from meta-analyses on therapeutic interventions interfering with the *RAGE* pathway to counteract obesity are summarized in Table 2.

Table 2. Therapeutic interventions interfering with the *RAGE* pathway to counteract obesity.

Intervention	Participants	Duration	Outcome	References
		Meta-Analyses		
Low-calorie diet 3 RCTs	123 overweight/obese patients with/without T2D	2–3 months	↓ AGE serum levels	[100]
Mediterranean diet rich in MUFAs and low in AGE 6 RCTs	395 overweight/obese patients with/without T2D	1–3 months	↓ AGE serum levels ↓ *RAGE* expression	[101]
Low-AGE diet 6 RCTs	172 overweight/obese patients with/without T2D	1 day to 12 weeks	↓ triglycerides ↓ fasting insulin and glucose ↓ uACR,↓ 8-isoprostanes ↓ BMI, WC, and WHR ↑ eGFR and HDLs	[102]
Low-AGE diet 13 RCTs	293 patients of different weights with/without T1D or T2D	2–16 weeks	↓ AGE, TNF-α, 8-isoprostanes, VCAM-1 and oxLDL serum levels	[106]

↓, decrease; ↑, increase; AGE, advanced glycation end products; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDLs, high-density lipoproteins; MUFAs, monounsaturated fatty acids; oxLDLs, oxidated low-density lipoproteins; *RAGE*, receptor for advanced glycation end products; RCT, randomized controlled trial; T1D, type 1 diabetes mellitus; T2D, type 2 diabetes mellitus; TNF-α, tumor necrosis factor-α; uACR, urinary albumin/creatinine ratio; VCAM-1, vascular cell adhesion molecule 1; WC, waist circumference; WHR, waist-to-hip ratio.

6. Final Remarks and Conclusions

In recent years, knowledge of the *RAGE* pathway and its crosstalk with obesity and related complications has been the source of several studies. Thus far, it has been outlined that collaboration between AGEs and their receptors affects adipose tissue through modification of the development, functionality, and cleavage of adipose cells. The mechanisms that drive such effects still require investigation; however, preclinical research indicates a dominant role in inflammatory and oxidative stress pathways. This approach is consistent with concepts indicating obesity as a chronic low-grade inflammation disease [109].

An additional key finding is the influence of AGEs on other processes such as glucose–insulin homeostasis or adipokine secretion. For example, the AGE–*RAGE* axis can serve as a provoker of insulin resistance and altered leptin secretion, leading to an increased risk of diabetes and cardiovascular diseases' prevalence. Thus, it will be challenging to identify the exact mechanism of action that could inhibit these pathological responses.

Finally, future studies should focus on expanding the therapeutical pipeline for obesity and related comorbidities with the use of the AGE–*RAGE* axis. Currently available therapeutic strategies targeting the AGE–*RAGE* pathway are based on limiting the supply of exogenous AGEs from the diet. An alternative option would be to reduce the synthesis of endogenous AGEs. In contrast, selective *RAGE* antagonists constitute one option that targets this pathway regardless of the AGE source.

Author Contributions: Conceptualization: A.K.; writing—original draft preparation: K.G. and A.K.; writing—review and editing: A.K. and K.C.; funding acquisition: A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Science Centre Poland, grant 2018/31/B/NZ5/01556.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analysis, or interpretation of the data; in the writing of the manuscript; or in the decision to publish the results.

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7.2 AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus

International Journal of
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AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus

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Citation: Gutowska, K.; Koźniewski, K.; Wąsowski, M.; Jonas, M.I.; Bartoszewicz, Z.; Lisik, W.; Jonas, M.; Binda, A.; Jaworski, P.; Tarnowski, W.; et al. AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus. *Int. J. Mol. Sci.* **2023**, *24*, 17447. https://doi.org/10.3390/ijms242417447

Academic Editor: Antonio Lucacchini

Received: 15 November 2023
Revised: 8 December 2023
Accepted: 10 December 2023
Published: 13 December 2023

Abstract: The advanced glycosylation end-product receptor (AGER) is involved in the development of metabolic inflammation and related complications in type 2 diabetes mellitus (T2DM). Tissue expression of the AGER gene (*AGER*) is regulated by epigenetic mediators, including a long non-coding RNA AGER-1 (lncAGER-1). This study aimed to investigate whether human obesity and T2DM are associated with an altered expression of *AGER* and lncAGER-1 in adipose tissue and, if so, whether these changes affect the local inflammatory milieu. The expression of genes encoding AGER, selected adipokines, and lncAGER-1 was assessed using real-time PCR in visceral (VAT) and subcutaneous (SAT) adipose tissue. VAT and SAT samples were obtained from 62 obese (BMI > 40 kg/m^2; N = 24 diabetic) and 20 normal weight (BMI = 20–24.9 kg/m^2) women, while a further 15 SAT samples were obtained from patients who were 18 to 24 months post-bariatric surgery. Tissue concentrations of adipokines were measured at the protein level using an ELISA-based method. Obesity was associated with increased *AGER* mRNA levels in SAT compared to normal weight status ($p = 0.04$) and surgical weight loss led to their significant decrease compared to pre-surgery levels ($p = 0.01$). Stratification by diabetic status revealed that *AGER* mRNA levels in VAT were higher in diabetic compared to non-diabetic women ($p = 0.018$). Elevated *AGER* mRNA levels in VAT of obese diabetic patients correlated with lncAGER-1 ($p = 0.04$, $r_s = 0.487$) and with interleukin 1β ($p = 0.008$, $r_s = 0.525$) and resistin ($p = 0.004$, $r_s = 0.6$) mRNA concentrations. In conclusion, obesity in women is associated with increased expression of *AGER* in SAT, while T2DM is associated with increased *AGER* mRNA levels and pro-inflammatory adipokines in VAT.

Keywords: advanced glycosylation end-products (AGEs); advanced glycosylation end-product receptor (AGER); long non-coding RNA (lncRNA); adipose tissue; obesity; metabolic inflammation; type 2 diabetes mellitus (T2DM)

1. Introduction

Adipose tissue is composed of heterogeneous anatomical depots with different epigenetic characteristics that determine their metabolic functions [1]. Although initially identified as a passive energy store, definitive studies have underlined its complex nature, ensuring endocrine, autocrine, and immune homeostasis [2]. Furthermore, adipose tissue is highly flexible and can therefore be remodeled according to caloric supply [1]. The excessive accumulation of energy reserves associated with obesity leads to adipocyte dysfunction, mitochondrial damage, and hypoxia, resulting in the disruption of cell metabolism and mechanical stress through stretching of the extracellular matrix [3,4]. It is widely accepted that obesity is associated with chronic low-grade inflammation, driven by various immune cells, which can result in irreversible organ damage [5]. In a state of positive energy balance, inflammatory cytokine and chemokine responses shift the immune system from an anti-inflammatory to a pro-inflammatory state and lead to an increased risk of developing metabolic disorders, including insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [5]. Although this relationship is considered controversial by some, basic and clinical research confirms that the level of inflammation associated with obesity is positively correlated with the degree of IR and T2DM [6].

A key link between adipose tissue expansion, associated comorbidities, and inflammatory signaling is the advanced glycosylation end-product receptor (AGER) [7,8]. This cell surface molecule—present in a variety of cell types—triggers the generation of reactive oxygen species and the induction of an inflammatory response. Upon binding a variety of different ligands, including advanced glycation end-products (AGEs), it mediates the activation of nuclear factor κB (NF-κB) and other pro-inflammatory pathways, leading to tissue dysfunction and the development of obesity and other metabolic diseases [9–11].

Evidence suggests that activation of the inflammatory network depends on equally important environmental, genetic, and epigenetic contributing factors [12]. Long untranslated non-coding RNAs (lncRNAs) of more than 200 nucleotides are considered to be a hallmark of epigenetic regulation in inflammatory signaling [12]. These transcripts can act as regulatory elements that affect gene expression at different stages, including modulation of transcriptional availability of chromatin and mRNA processing [13,14]. Dynamic epigenetic modulations accompany obesity, as lncRNA expression patterns in adipose tissue and sera of obese subjects differ from those of normal weight individuals [15,16]. Furthermore, IR has been shown to alter non-coding RNA expression in obese subjects, confirming the central contribution of low-grade inflammation in both conditions [17]. As lncRNAs contribute to the epigenetic shaping of inflammatory genes, and AGER is a promoter of the immune response that is enhanced in obesity and T2DM, there may be a link between the concentration of particular lncRNAs and *AGER* expression.

With this in mind, in the current study, we aimed to investigate whether human obesity and T2DM are associated with altered expression of *AGER* and long non-coding RNA AGER-1 (lncAGER-1) in adipose tissue and, if so, whether these changes affect the local inflammatory milieu.

2. Results

2.1. Clinical and Biochemical Characteristics of Study Participants

The study group consisted of 62 women with a body mass index (BMI) of >40 kg/m^2—calculated by weight in kg divided by height squared (m^2). Based on the American Diabetes Association diagnostic criteria, T2DM was diagnosed in 23 participants (37.0%) within the study group [18]. All patients in the study group (O) underwent surgical treatment

for obesity (sleeve gastrectomy or mini gastric bypass). During these surgical procedures, visceral (VAT) and subcutaneous (SAT) adipose tissue samples were collected from the lower abdomen. Fifteen additional SAT samples from the lower abdomen were obtained during abdominoplasty surgery, from previously obese (PO; BMI = 24.3–29.5 kg/m^2) study participants who were 18 to 24 months post-bariatric surgery. Five patients in the post-bariatric group had residual hypertension, three had persistent pre-diabetes, and six had hyperlipidemia, but none met the criteria for a diagnosis of metabolic syndrome. As abdominoplasty is not associated with opening the abdominal cavity, it was not possible to obtain visceral adipose tissue samples from the PO participants.

The control group (N) consisted of 20 women with a BMI within the normal range (20.7–24.93 kg/m^2). In addition, they had no history of chronic disease, and their metabolic health was confirmed as normal following a physical examination and biochemical blood tests. Although their body composition was not assessed, they were considered metabolically healthy based on their medical histories, normal BMI and biochemical parameters, and the absence of any metabolic syndrome components. None of the study participants were receiving anti-inflammatory treatment (e.g., glucocorticoids, non-steroidal anti-inflammatory drugs, or anti-cytokine drugs).

The baseline clinical and biochemical parameters of the study participants are summarized in Table 1.

Table 1. Clinical and biochemical characteristics of the study participants.

	Obese Individuals before Weight Loss (*N* = 62)		**Obese Individuals after Weight Loss (*N* = 15)**		**Normal Weight Controls (*N* = 20)**	
	Mean ± SD	**Min–Max**	**Mean ± SD**	**Min–Max**	**Mean ± SD**	**Min–Max**
Age (years)	40.48 ± 10.19	20–59	40.76 ± 8.13	29–59	44.08 ± 13.5	26–63
Weight (kg)	125.6 ± 17.11	87.8–170.0	73.11 ± 6.25	66.0–82.0	67.92 ± 10.48	54.0–74.0
BMI (kg/m^2)	45.97 ± 5.70	35.43–63.42	28.2 ± 2.45	24.0–31.2	23.06 ± 1.33	20.7–24.93
Adipose tissue (% body mass)	48.54 ± 3.65	40.43–57.23	32.5 ± 3.4	26.0–37.0	-	-
Weight loss (kg)	-	-	32.4 ± 8.4	23.0–42.0	-	-
Glucose (mmol/L)	5.91 ± 1.37	3.67–9.85	4.82 ± 0.55	4.12–5.66	5.2 ± 0.3	4.8–5.4
HbA1c (%)	5.79 ± 0.51	5.06–7.20	5.1 ± 0.47	4.59–5.65	4.9 ± 0.28	4.6–5.3
Total cholesterol (mmol/L)	4.96 ± 0.91	3.13–7.87	4.76 ± 0.66	3.92–5.9	4.31 ± 0.20	4.24–4.78
LDL cholesterol (mmol/L)	3.04 ± 0.97	1.04–5.65	2.99 ± 0.35	2.54–3.58	2.82 ± 0.11	2.74–2.90
HDL cholesterol (mmol/L)	1.21 ± 0.25	0.59–1.78	1.38 ± 0.27	1.08–1.68	1.47 ± 0.26	1.24–1.76
Triglycerides (mmol/L)	1.43 ± 0.72	0.52–3.23	1.40 ± 0.38	1.02–1.85	1.32 ± 0.2	1.1–1.46
CRP (mg/L)	10.65 ± 5.29	1.21–21.61	4.51 ± 2.45	1.81–7.21	3.24 ± 1.72	0.7–5.20
Comorbidities	*N*	(%)	*N*	(%)	*N*	(%)
Hypertension	31	50.0%	5	33.3%	none	none
Type 2 diabetes mellitus/prediabetes *	23	37.0%	3	20.0%	none	none
Hyperlipidemia	34	55.0%	6	40.0%	none	none

BMI—body mass index calculated as weight (kg) divided by height squared (m^2); CRP—C-reactive protein; HbA1c—glycated hemoglobin, HDL—high-density lipoproteins; LDL—low-density lipoproteins; N—number of subjects; * impaired fasting glucose and/or impaired glucose tolerance.

2.2. The Advanced Glycosylation End-Product Receptor (AGER) Gene and Long Non-Coding RNA AGER-1 Expression in Adipose Tissue of Obese Individuals before and after Bariatric Surgery and Normal Weight Subjects

AGER expression at the mRNA level was higher in the subcutaneous adipose tissue of obese patients (SAT-O) compared to the SAT of normal weight individuals (SAT-N, $p = 0.04$). Surgically induced weight loss was associated with a significant decrease in *AGER* mRNA levels in SAT (SAT-PO) ($p < 0.001$). Interestingly, there were substantially higher *AGER* mRNA levels in SAT-O compared to the visceral adipose tissue in obese participants (VAT-O), whereas in normal weight women, no significant differences in *AGER* expression were found between SAT and VAT (Figure 1a). When the obese study participants were stratified according to diabetic status, we found that what distinguished women with T2DM from

normoglycemic women was the higher expression of *AGER* at the mRNA level in VAT ($p = 0.018$, Figure 1b).

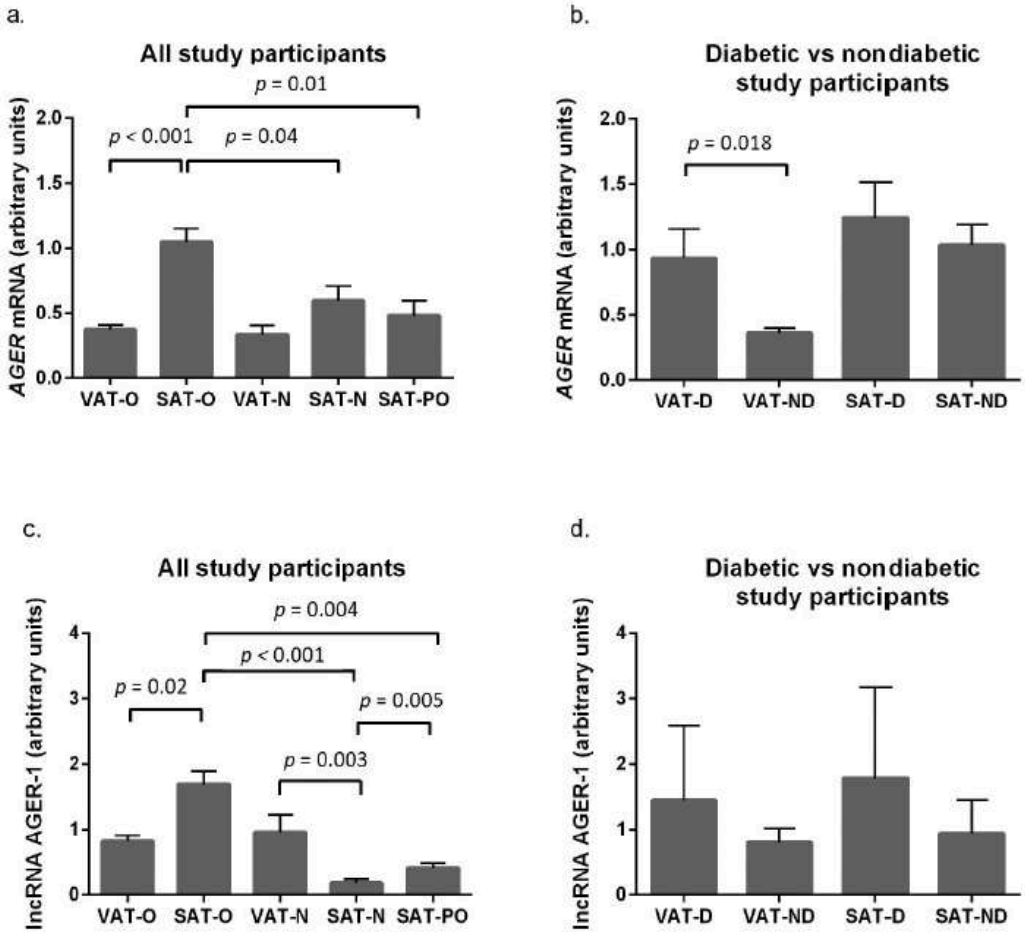

Figure 1. *AEGR* mRNA (**a**,**b**) and lncAGER-1 (**c**,**d**) levels in visceral (VAT) and subcutaneous (SAT) adipose tissue of obese subjects before (O) and after surgical weight loss (PO), in normal weight subjects (N) and diabetic (D) and non-diabetic (ND) obese study participants. Results are presented as median with interquartile range.

The expression profile of lncAGER-1 in the studied tissues resembled that of *AGER* in many respects (Figure 1c). lncAGER-1 levels were significantly higher in the SAT of obese women compared to normal weight women ($p < 0.001$), and weight loss was associated with a marked decrease in lncAGER-1 levels ($p = 0.004$), but not to the level observed in the SAT of normal weight women ($p = 0.005$). There were differences in lncAGER-1 levels (Figure 1c) between adipose depots depending on the presence of obesity: in obese women, lncAGER-1 expression was higher in SAT ($p = 0.02$), whereas in normal weight women, it was higher in VAT ($p = 0.003$). Stratification of obese patients according to glycemic status revealed a similar trend in the expression profile of lncAGER-1 to *AGER* between the tissues examined, but the differences were not significant (Figure 1d).

Subsequent analysis (Figure 2) revealed a significant positive correlation between *AGER* mRNA and lncAGER-1 levels in the VAT of the obese diabetic subjects ($p = 0.04$, $r_s = 0.487$, Figure 2b).

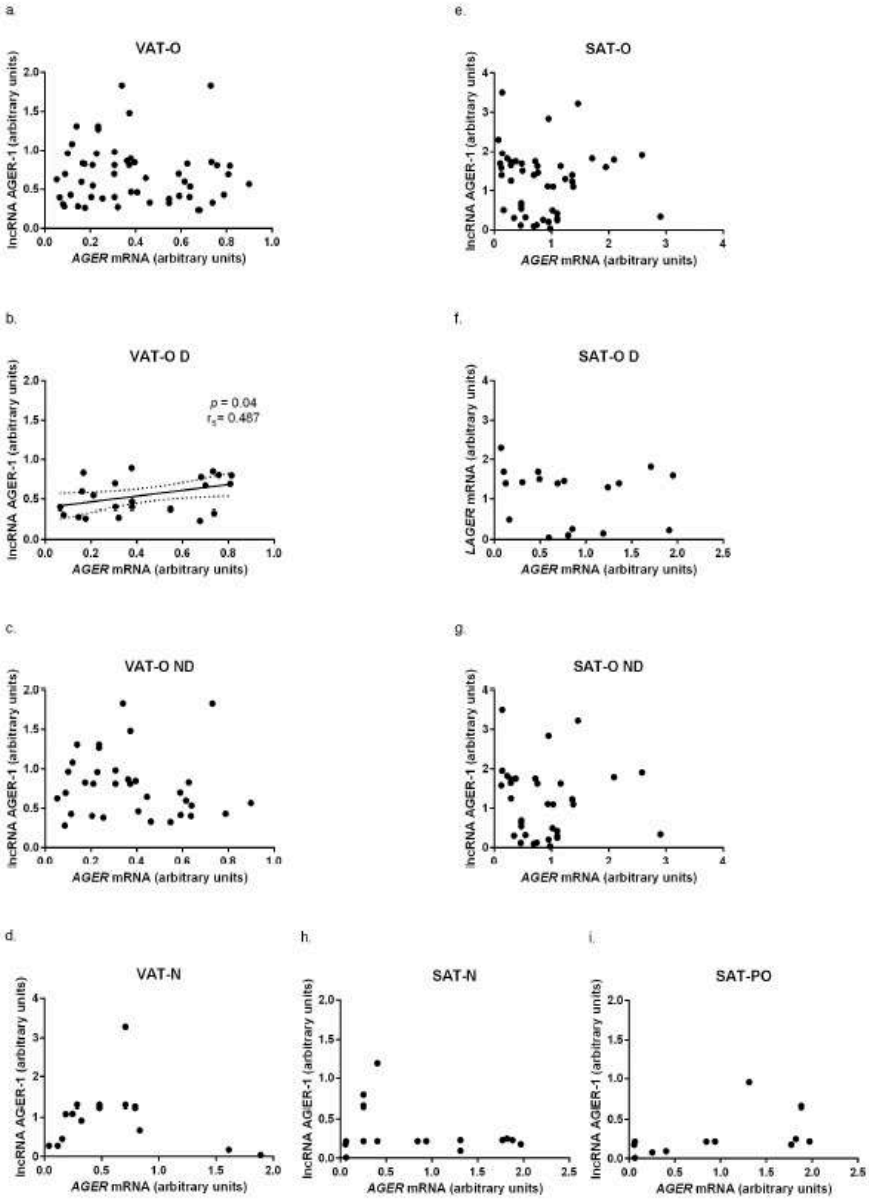

Figure 2. Correlations between AGER mRNA levels and AGER-1 lncRNA levels in visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) of all obese (O), obese diabetic (D), obese non-diabetic (ND), normal weight (N), and post-bariatric surgery (PO) study participants. Black dots represent particular participants. Lines represent linear regression analysis with 95% CI interval. (**a**–**i**) refer to the phenomena observed in the different fat deposits in the different study groups.

2.3. Expression of Pro-Inflammatory Adipokines in Adipose Tissue of Obese Subjects, Stratified by Diabetic Status

The finding that *AGER* mRNA levels differ between the tissues studied, and are higher in obese individuals (particularly those with T2DM), prompted us to investigate whether these differences affect the local inflammatory milieu. To this end, we examined the expression of genes that encode selected pro-inflammatory adipokines, namely interleukin 1 (*IL1B*), interleukin 6 (*IL6*), interleukin 8 (*IL8*), and resistin (*RETN*), at mRNA and protein levels, and correlated their concentrations with *AGER* mRNA levels. Due to the paucity

of biological material, protein measurements were not performed in the PO group (18 to 24 months post-bariatric surgery). In our previous studies on a smaller group of patients, we found that the concentration of these adipokines in adipose tissue is significantly altered in the course of obesity [19,20]. Similarly, in the group of women studied in this work, we found that obesity was associated with an increase in the expression of genes encoding pro-inflammatory adipokines at both the mRNA and protein levels (Supplementary Figures S1 and S2).

The study group was then stratified according to glycemic status, and adipokine mRNA levels in adipose tissue were compared between the diabetic and non-diabetic study participants (Figure 3).

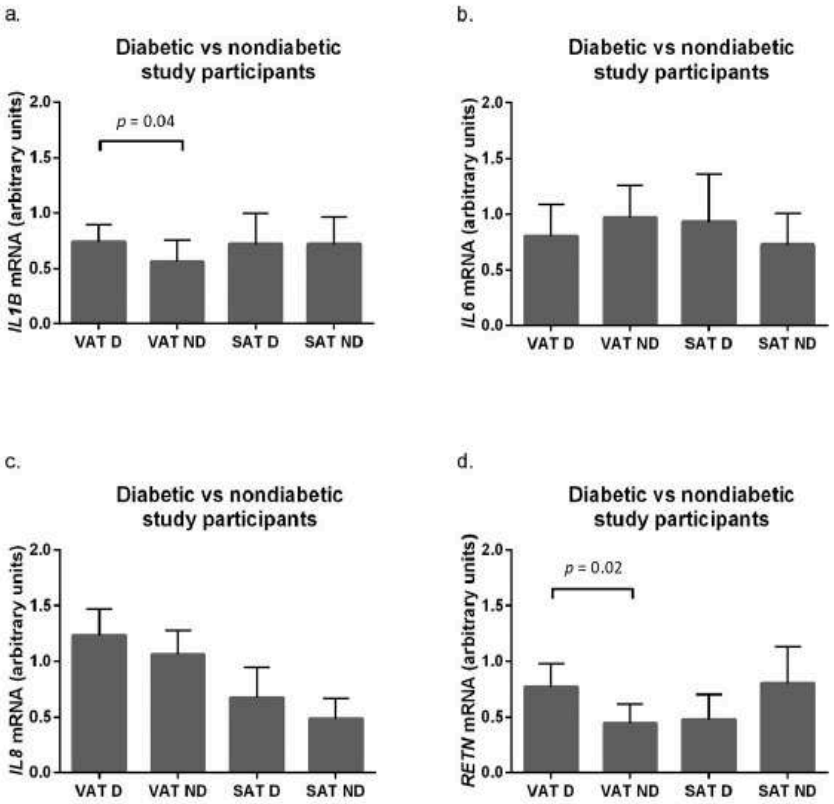

Figure 3. mRNA levels of genes encoding (**a**) interleukin 1b (*IL1B*), (**b**) interleukin 6 (*IL6*), (**c**) interleukin 8 (*IL8*), and (**d**) resistin (*RETN*) in visceral (VAT) and subcutaneous (SAT) adipose tissue of diabetic (D) and non-diabetic (ND) obese subjects. Results are presented as median and interquartile range.

This stratification revealed that the mRNA levels for *IL1B* ($p = 0.04$) and resistin ($p = 0.02$) were significantly higher in the VAT of the diabetic, obese study participants (VAT-D) compared to the non-diabetic participants (VAT-ND). However, glycemic status did not affect adipokine protein levels in a given adipose tissue depot (Supplementary Figure S3). To verify whether the observed differences in adipokine mRNA levels between diabetic and non-diabetic subjects could be related to *AGER* expression, correlation analyses were performed. Significant positive correlations were found between *AGER* mRNA levels and *IL1B* ($p = 0.008$, $r_s = 0.525$) and *RETN* ($p = 0.004$, $r_s = 0.60$) mRNA levels in visceral adipose tissue of obese diabetic participants (Figure 4).

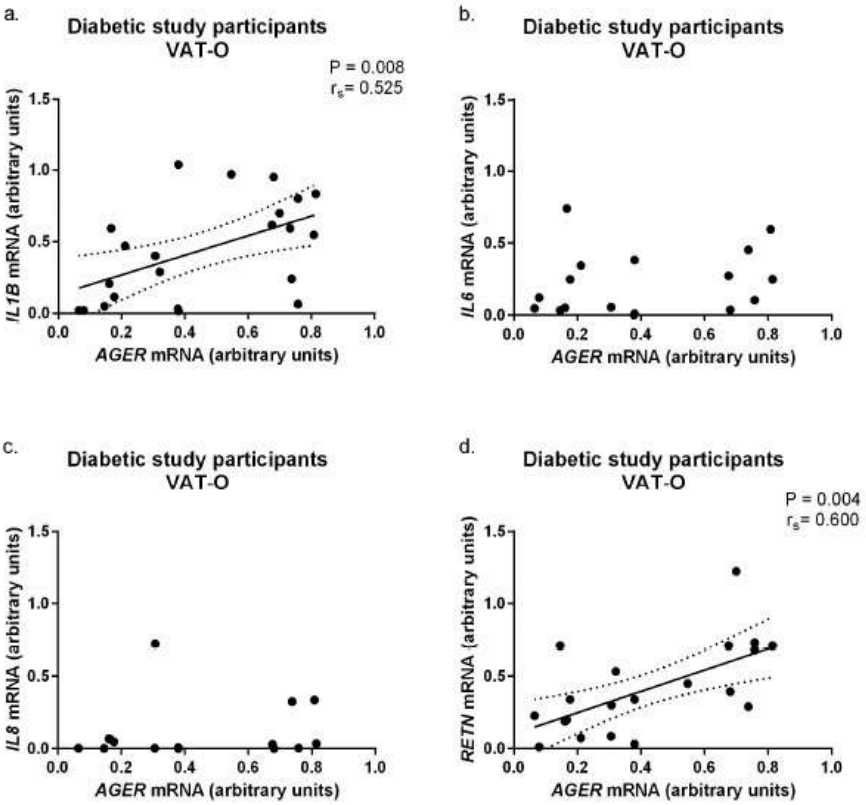

Figure 4. Correlations between AGER mRNA concentrations and mRNA levels of interleukin 1b (IL1B, (**a**)), interleukin 6 (IL6, (**b**)), interleukin 8 (IL8, (**c**)), and resistin (RETN, (**d**)) in the visceral adipose tissue of obese diabetic study participants (VAT-O). Black dots represent particular participants. Lines represent linear regression analysis with 95% CI interval.

3. Discussion

Obesity-related adipose tissue dysfunction appears to play a critical role in the pathogenesis of metabolic complications, including insulin resistance and T2DM [1,4]. One manifestation of adipose tissue dysfunction is chronic inflammation, resulting from impaired adipocyte energy homeostasis that involves the extracellular matrix [4,5]. The key pathway in the development of metabolic inflammation and insulin resistance is the receptor for advanced glycation end-products' (known as AGER or RAGE) signaling. Therefore, in this work, we investigated whether human obesity and T2DM are associated with altered expression of the AGER gene and its regulator lncAGER-1 in adipose tissue, and, if so, whether these changes affect the local inflammatory milieu. We found that obesity in women is associated with increased expression of *AGER* in subcutaneous adipose tissue, whereas AGER-mediated development of metabolic inflammation—which predisposes to increased insulin resistance and T2DM—preferentially affects the visceral depot.

Our finding regarding the elevated *AGER* expression in adipose tissue in obesity is consistent with the results of previous reports. Gaens et al. conducted a study on subcutaneous tissues—obtained from 10 patients with grade I/II obesity (mean BMI 34.2 ± 4.0 kg/m^2) and from 9 normal weight controls—and found that *AGER* expression in both mRNA and protein levels is higher in the course of obesity [21]. Visceral adipose tissue was not analyzed in that study. However, when comparing tissues from different depots of obese patients, the authors found a significant increase in *AGER* mRNA levels in VAT compared to SAT. In our study, which focused only on women with obesity, we observed the opposite trend. As adipose tissue is a highly heterogeneous organ, its depots are biologically

different, which is reflected in differences in the expression profiles of various genes, and translates into differences in lipolytic and inflammatory activity or insulin sensitivity [2]. In obesity, the functional differences between the different adipose tissue depots might be both accentuated and abolished [19,22], and these processes can be mediated by epigenetic mechanisms that buffer the effects of the environment [23]. There are many potential reasons for the discrepancy between our findings and the Gaens et al. study, such as differences in gender and metabolic status (in the study by Gaens et al., 30% of the participants were men and most of the patients had impaired glucose tolerance or T2DM) [21]. The latter aspect is important since, after stratifying the obese study participants according to glycemic status, we observed that VAT from diabetic subjects had higher levels of *AGER* mRNA than VAT of normoglycemic individuals. A similar association between T2DM and higher concentrations of *AGER* mRNA was previously observed in pericardial adipocytes [24].

Given the role of AGER in regulating the inflammatory response, we reasoned that the association between higher expression of this receptor in adipose tissue and T2DM might not be coincidental. Chronic low-grade inflammation is a primary event of obesity-related insulin resistance [25]. An essential role in inflammatory signaling in human adiposity is played by pro-inflammatory adipokines, including IL-1β, IL-6, IL-8, and resistin [19,20,26–29]. We demonstrated here, and before [19], that in the obese study participants, levels of mRNAs encoding these molecules were significantly increased in SAT. This reflects findings from other studies, wherein SAT, not VAT, was found to be a leader of cytokine synthesis [29–31]. The debate over which depot of adipose tissue plays the dominant role in the development of obesity-related inflammation has been ongoing for years, with several papers in the literature pointing to VAT as the main source of pro-inflammatory mediators [32–34]. Our observations suggest that patients' glycemic status is crucial in this case. In our study, we observed higher *IL1B* and *RETN* mRNA in VAT of patients with obesity plus T2DM, rather than in obese patients with a euglycemic state. In preclinical studies, IL-1β has been identified as a key mediator of macrophage-induced insulin resistance in human adipocytes, and its high levels in VAT predispose obese rodents to the development of diabetes [35,36]. In addition, this cytokine orchestrates the mobilization of other inflammatory mediators, e.g., IL-6 contributing to insulin resistance and dyslipidemia [37]. In turn, resistin can be either the sender or recipient of pro-inflammatory processes by engaging the 5 AMP-activated protein kinase (AMPK) and AMPK-independent suppressor of cytokine signaling-3 (SOCS-3) pathways [38–40]. Subsequently, high levels of resistin correlate with insulin resistance, glucose intolerance, and endothelial dysfunction marker concentration [41]. In conclusion, when assessing the relationship between *AGER* expression and adipokine-encoding genes, it is crucial to define glycemic status, as different correlations can be expected in normoglycemic individuals and others with T2DM [42].

Our results suggest that AGER might be the chief of tri-directional communication between obesity, T2DM, and inflammation. In addition to inducing insulin resistance in adipose tissue, there is evidence that AGER ligands can induce pancreatic islet inflammation and beta cell apoptosis [43,44]. This raises the question of what mechanisms regulate *AGER* expression in obesity.

Previous studies proposed that lncRNAs, arising from noncoding transcripts, might contribute to the pathogenesis of metabolic disturbances, as well as cancer, neurodegenerative, and immune system diseases [45,46]. LncRNAs have a variety of functions; some (despite their name) encode peptides, but primarily, they exert post-translational effects on gene expression levels (both up-and down-regulated) and compete for the binding of specific miRNAs with mRNAs [47]. Here, we reported that lncAGER1, a functionally proven *AGER* regulator, may modulate its expression profile in adipose tissue. What's more, obesity promoted an increase in lncAGER-1 level in SAT, whereas in normal weight subjects, higher expression of this transcript was found in VAT. Interestingly, tandem obesity and diabetes-induced increased levels of lncAGER-1 in VAT were positively correlated with *AGER* expression. Our findings indirectly suggest that lncAGER-1 may induce in-

flammation mediated by *AGER*, therefore predisposing individuals to increased insulin resistance and T2DM. Functional studies have proven that lncAGER-1 acts as a miRNA sponge and indirectly modulates the expression of *AGER* in lung or colorectal cancer [48,49]. However, this is the first report to suggest lncAGER-1 as a contributor to obesity-related insulin resistance and T2DM. In summary, our study reveals the possible role of lncRNA in the upregulation of *AGER* expression in the adipose tissue and therefore, indirectly, in the development of metabolic inflammation in the course of obesity; however, these phenomena seem to be depot-specific.

The main limitation of this study is its descriptive nature, which implies the need for further functional studies to confirm our findings. When describing the correlations between RNA and lncRNA levels, we can only surmise that they reflect tissue regulatory processes. Although the role of lncAGER-1 in regulation of *AGER* expression has been confirmed in functional studies in other tissues and organs, our results require in vitro verification in human adipocyte cell lines. Another limitation is the relatively small number of control tissues obtained from the normal weight individuals and patients who underwent bariatric surgery. On the other hand, the main strengths of the current study are its homogeneous, well-characterized population, with 62 participants in the study group, and its innovative nature, which can be considered a novel attempt to elucidate the mechanisms linking obesity and type 2 diabetes mellitus.

4. Materials and Methods

4.1. Tissue and Blood Sample Collection

Visceral (VAT) and subcutaneous (SAT) adipose tissue samples were collected from patients with obesity during bariatric surgery. SAT samples were obtained from the lower abdomen during abdominoplasty in previously obese study participants who were 18 to 24 months post-bariatric surgery. In the control group, VAT and SAT pairs were obtained at the time of elective cholecystectomy in a manner analogous to the study group. All adipose tissue samples were immediately frozen at −80 °C and then homogenized in liquid nitrogen. Before surgery, all participants underwent a medical examination and provided 15 mL of venous blood. All sera and adipose tissue samples were stored at −80 °C until molecular and biochemical measurements were performed.

This study was approved by the Bioethics Committee of the Medical University of Warsaw and the Centre for Postgraduate Medical Education in Warsaw (decision no. KB 147/2009 issued on 28 July 2009, KB 91/A/2010 issued on 19 July 2010, KB 117/A/2011 issued on 14 November 2011, and KB 38/A/2022 issued on 16 May 2022) and written informed consent was obtained from all participants.

4.2. Isolation of Total RNA, Reverse Transcription, and Real-Time PCR

Total RNA was isolated and reverse-transcribed to cDNA using previously described methods [19]. Real-time PCR was performed in triplicate using a LightCycler 480 Instrument II (Roche, Mannheim, Germany), as previously described [50]. The specific primers used to analyze lncAGER-1 and gene expression at the mRNA level in adipose tissue are listed in Table 2.

Table 2. Primers used for the analysis of lncRNA and gene expression at the mRNA level in adipose tissue.

Gene/lncRNA	Description		Primers	Ref.
AGER	advanced glycosylation end-product receptor	F	5′ TGTGCTGATCCTCCCTGAGA 3′	[51]
		R	5′ CGAGGAGGGGCCAACTGCA 3′	
lncAGER-1	long non-coding RNA AGER-1	F	5′ AACCAGGAGGAAGAGGAGGA 3′	[48]
		R	5′ TTGGCAAGG TGGGGTTATAC 3′	
IL1B	interleukin 1β	F	5′ CACCAAGCTTTTTTGCTGTGAGT3′	[19]
		R	5′ GCACGATGCACCTGTACGAT 3′	

Table 2. *Cont.*

Gene/lncRNA	Description		Primers	Ref.
IL6	interleukin 6	F	5′ CCTTCGGTCCAGTTGCCTTC 3′	[19]
		R	5′ GTGGGGCGGCTACATCTTTG 3′	
IL8	interleukin 8	F	5′ CACCGGAAGAACCATCTCACT 3′	[19]
		R	5′ TCAGCCCTCTTCAAAAACTTCTCC 3′	
RETN	resistin	F	5′ GCTGTTGGTGTCTAGCAAGAC 3′	[20]
		R	5′ CATCATCATCATCATCTCCAG 3′	
ACTB	β-actin	F	5′ CAGCCTGGATAGCAACGTAC 3′	[19,20,51]
		R	5′ TTCTACAATGAGCTGCGTGTG 3′	

F—forward, R—reverse.

4.3. Isolation of a Protein Fraction from Adipose Tissue and Measurement of Cytokine Concentrations

Isolation of the adipose tissue protein fraction was performed as previously described [19,20]. An ELISA-based chemiluminescent Q-plex Custom array (Quansys Bioscience, West Logan, UT, USA) was used to measure interleukin (IL) 1β, 6, 8, and resistin concentrations in adipose tissue protein extracts. Luminescence was assessed using a Molecular Imager Versa Doc™ MP 5000 system (Bio-Rad, Hercules, CA, USA), according to the manufacturer's guidelines. Results were analyzed using Q-View software version 2.17 (Quansys Bioscience, West Logan, UT, USA). Measurements of interleukins and resistin concentrations in adipose tissue were normalized to total protein concentrations in protein extracts. Mean total protein concentrations in VAT and SAT extracts from obese and normal weight participants were not significantly different ($p > 0.05$).

4.4. Statistical Analysis

The normality of distribution and homogeneity of variance of the studied parameters were checked using the Shapiro–Wilk and Levene tests, respectively. Differences in mRNA and protein levels in the tissues studied were calculated using Student's t/Mann–Whitney U tests. The Bonferroni adjustment for multiple comparisons was not applied [52,53]. The Spearman correlation test was used for correlations between quantitative values. All statistical analyses were performed using the Statistica software package v.10 (StatSoft, Tulsa, OK, USA) and GraphPad Prism software v.7 (GraphPad Software, San Diego, CA, USA).

5. Conclusions

In conclusion, our data suggest that obesity in women is associated with increased expression of *AGER* in SAT. However, AGER-mediated development of metabolic inflammation—which predisposes to increased insulin resistance and type 2 diabetes mellitus—preferentially affects VAT. Given the descriptive nature of our study, these data need to be verified in vitro.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms242417447/s1.

Author Contributions: Conceptualization, A.K.; methodology, K.G. and A.K.; investigation, K.G., K.K., M.W., M.I.J., Z.B., W.L., M.J., A.B., P.J., W.T., B.N. and A.K.; data acquisition, M.W., M.I.J., W.L., M.J., A.B., P.J., W.T., B.N. and A.K.; data curation, K.G. and A.K.; writing—original draft preparation, K.G. and A.K.; writing—review and editing, M.P.-K. and A.K.; supervision, A.K., K.C. and M.P.-K.; project administration, A.K.; funding acquisition, A.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Science Centre Poland, grant 2018/31/B/NZ5/01556.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics of the Medical University of Warsaw and the Centre for Postgraduate Medical Education in Warsaw (decision no. KB 147/2009 issued on 28 July

2009, KB 91/A/2010 issued on 19 July 2010, KB 117/A/2011 issued on 14 November 2011, and KB 38/A/2022 issued on 16 May 2022).

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

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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7.3 Advanced glycation end-product receptor gene (RAGE) polymorphisms in patients with acute coronary syndrome – a case- control study in the Polish population

Gutowska *et al. BMC Medical Genomics* (2025) 18:143
https://doi.org/10.1186/s12920-025-02215-3

BMC Medical Genomics

RESEARCH Open Access

Advanced glycation end-product receptor gene (RAGE) polymorphisms in patients with acute coronary syndrome – a case-control study in the Polish population

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Abstract

Background The development of coronary artery disease (CAD) is the result of complex interactions between environmental and genetic factors. While the former is well known, the genetic factors that predispose individuals to the development of CAD are still under investigation. The aim of our study was to investigate whether single nucleotide polymorphisms (SNPs) in the gene encoding the receptor for advanced glycation end products (*RAGE*), specifically rs2070600 G/A and rs184003 G/T, may determine predisposition to acute coronary syndrome (ACS) and severity of coronary artery disease (CAD) in the Polish population.

Methods Two *RAGE* SNPs were genotyped in 336 patients with a history of acute coronary syndrome (ACS): 175 < 50 years, 161 ≥ 50 years, and 160 ethnically, age- and sex-matched controls via the restriction fragment length polymorphism method. Allele frequencies were compared between groups via the chi^2 test on a 2 × 2 contingency table. Genotype distribution was analyzed assuming three modes of inheritance: dominant, codominant, or recessive. The values of the variables between the study groups were compared using Student's t-test or the Mann-Whitney *U* test, as appropriate.

Results For the rs184003 G/T polymorphism, the frequency of genotypes containing the T allele (GT + TT) was significantly greater in patients with a history of ACS than in healthy age- and sex-matched controls (28.87% vs. 11.25%, $p < 0.0001$, OR = 3.2 [95% CI: 1.86–5.52]). Moreover, individuals possessing this allele had lower high-density lipoprotein (HDL) cholesterol levels (mean 1.02 mmol/l vs. 1.14 mmol/l, $p = 0.01$) and higher median troponin I concentrations at the time of ACS (32.2 ng/ml vs. 24.4 ng/ml, $p = 0.04$). These genotypes were also significantly less common in patients with ACS before the age of 50 than in those diagnosed later (20.0% vs. 38.5%, $p = 0.0002$, OR = 0.4 [95%CI: 0.25–0.65]). In the case of rs2070600 G/A polymorphism, the genotype and allele frequencies were not significantly different between the study groups and subgroups.

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Conclusions Our findings suggest that the rs184003 SNP in the *RAGE* gene may play a role in determining genetic susceptibility to CAD and ACS in the Polish population. However, they do not support the hypothesis that the studied SNPs impact the incidence of ACS at a young age.

Keywords Acute coronary syndrome (ACS), Coronary artery disease (CAD), Genetic predisposition, Receptor for advanced glycation end products (RAGE), Single nucleotide polymorphisms (SNP)

Background

Persistent accumulation of vessel-occluding plaques in the intimal layer of medium- and large-sized arteries initiates damage to the endothelial cells responsible for the regulation of vascular integrity and barrier function [1, 2]. Under proinflammatory conditions, disrupted homeostasis promotes the restriction of blood flow, vascular stenosis, and cell hypoxia, eventually leading to atherosclerosis, which is one of the major causes of morbidity and mortality from cardiovascular disease [3, 4]. Accumulating evidence has shown that atherosclerosis is a complex disease involving multiple determinants, including genetic and modifiable environmental factors, as well as the impact of comorbidities, such as hypertension, diabetes mellitus, and dyslipidemia [5, 6]. However, the list of factors influencing the pathogenesis of this disease is still incomplete and warrants further study.

An increasing number of studies have suggested that advanced glycation end products (AGEs) contribute to diabetes-related complications [7]. AGEs are a heterogeneous group of macromolecular derivatives formed by an irreversible Maillard reaction that interact with the multiligand receptor for AGEs (RAGE), which is expressed in numerous tissues and cells, including vascular smooth cells [8, 9]. The binding of AGEs to RAGE leads to the activation of the inflammatory response through the activation of nuclear factor-β, oxidative stress, and proinflammatory cytokines [10, 11]. Both inflammatory and oxidative stress pathways play a role in atherosclerosis, which is initiated not only by hypertension or obesity but also by the deposition of excess sugars that promote the formation of AGEs [12]. Thus, the expression of RAGE on cells involved in plaque formation (e.g., endothelial cells, smooth muscle cells, and neutrophils) has been strongly demonstrated, particularly in diabetic patients [13]. However, subsequent studies have shown that RAGE also triggers thrombotic, profibrotic, and inflammatory responses in euglycemic patients [14]. Interestingly, the metalloproteinase-mediated proteolytic cleavage of RAGE results in the generation of a soluble isoform (sRAGE), and in addition, the direct alternative splicing of RAGE results in the generation of endogenous secretory RAGE (esRAGE), both of which interfere with membrane-bound RAGE [15]. Previous studies have shown that sRAGE has an anti-inflammatory and atheroprotective effect and that its high level slows the progression of intima-media thickness (IMT). It also reduces the risk of major coronary events and mortality [16]. Nevertheless, some studies have questioned the atheroprotective effect of sRAGE and associated it with the incidence of fatal and nonfatal cardiovascular disease [17, 18], whereas another study denied any relationship between sRAGE and carotid IMT and plaque [19]. These findings emphasize the important role of RAGE in atherosclerosis and related complications, but its causal role remains unclear.

Coronary artery disease (CAD) is a manifestation of atherosclerosis in the heart. Its genetic background is suggested by a familial and ethnic predisposition to the disease as well as a sex-related incidence. Both genome-wide association studies (GWASs) and candidate gene studies have identified a number of loci and single genes that may determine genetic predisposition to coronary atherosclerosis in a population-specific manner [20]. In our work, we hypothesized that the associations of sRAGE and/or esRAGE with the risk of coronary atherosclerosis may be determined by genetic variants in the *RAGE* gene. The aim of our study was therefore to assess the distribution of genotypes and alleles of two single nucleotide polymorphisms (SNPs) in *RAGE*, among Polish patients with acute coronary syndrome (ACS) stratified by age, and in healthy, age- and sex-matched controls.

Materials and methods

Study design

A case-control study was conducted, involving Polish patients with ACS and age- and sex-matched healthy control subjects.

Study participants

The study population included individuals whose basic clinical characteristics were described previously [21]. The study group consisted of 175 unrelated patients (139 men and 36 women, 82.9% vs. 17.2%) aged less than 50 years (26–49 years, mean 43.5 years) who were admitted to the Department of Cardiology Grochowski Hospital in Warsaw for a first episode of acute coronary syndrome. ACS diagnosis was established on the basis of clinical symptoms (stenocardial pain), electrocardiography findings (non-ST-segment elevation myocardial infarction and ST-segment elevation myocardial infarction), and elevated troponin levels. Two control groups were included in the study. The first group comprised

160 ethnically matched unrelated healthy subjects aged 30–49 years (mean age 42 years), 105 men (65.6%) and 55 women (34.4%) with no history of CAD or diabetes who were recruited from healthy blood donors in collaboration with the regional blood center. The second control group consisted of 161 unrelated patients aged over 50 years (50–92 years, mean 65 years), 130 men (64.3%) and 41 women (35.7%) who were hospitalized in the Department of Cardiology Grochowski Hospital in Warsaw for a first episode of ACS (meeting the above criteria). All patients with ACS had percutaneous coronary intervention performed. Coronary angiography was recorded in digital form and assessed for ongoing study by an independent invasive cardiologist blinded to the patient history and ECG and echocardiographic data. CAD extent was assessed using SYNTAX and EXTENT scores [22, 23]. The baseline clinical characteristics of both ACS groups are summarized in Supplementary Table 1.

Data on comorbidities, including current treatment, were collected via patient questionnaires (Supplementary File 1) and physical examinations on admission. Hypertension was assessed on the basis of history and treatment but also on the basis of the mean value of two measurements of systolic (SBP) and diastolic (DBP) blood pressure performed at 5-minute intervals. Hypertension was defined as values ≥ 140 mmHg SBP and/or ≥ 90 mmHg DBP according to the European Society of Hypertension, and European Society of Cardiology guidelines [24]. Diabetes was assessed by history and treatment or by a fasting plasma glucose level ≥ 126 mg/dl (7.0 mmol/l) or ≥ 200 mg/dl (11.1 mmol/l) in an oral glucose tolerance test or an HbA1c value ≥ 6.5% according to the European Association for Study of Diabetes and American Diabetes Association guidelines [25]. Data on depression and smoking status, including duration and intensity (number of cigarettes per day), were collected during a patient interview. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Blood samples were taken on admission (to determine admission glucose) and the following morning (to determine other parameters). Biochemical analyses, including glucose, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and plasma triglyceride (TG) levels, were performed on fasting blood samples (except for glucose at enrollment) via standard enzymatic methods and COBAS INTEGRA 800 equipment (Roche Diagnostics GmbH).

The study adhered to the tenets of the Declaration of Helsinki, the research program was approved by the local ethical committees, and written informed consent was obtained from all the participants.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood mononuclear cells via the salting-out method [26]. Single nucleotide polymorphisms in *RAGE* (GenBank NC_000006.12:32183665) were generated via polymerase chain reaction (PCR) amplification followed by digestion with restriction enzymes (restriction fragment length polymorphism – RFLP – method). Briefly, fragments of *RAGE* were amplified via PCR. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, annealing at 62 °C for 30 s, extension at 72 °C for 30 s, and a final step at 72 °C for 5 min. Each 12.5 µl reaction contained 50 ng of DNA, 2.0 mM $MgCl_2$, 10 pmol of each primer, 0.25 mM deoxynucleoside triphosphate and 1 unit of Taq polymerase (Invitrogen Carlsbad, USA) in the corresponding buffer. A total of 2.5 µl of the PCR product was digested with 1 unit of the proper restriction enzyme (Fermentas, Waltham, Massachusetts, USA) at 37 °C for 3 h. The reaction conditions (including primer sequences, annealing temperatures, and restriction enzymes) are listed in Table 1. The obtained restriction fragments were visualized on a 3% agarose gel (Merck, Darmstadt, Germany). To confirm the accuracy of the method employed, randomly selected samples from 10% of subjects were analyzed via direct sequencing by the external service (Laboratory of DNA sequencing and synthesis, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw Poland).

Statistical analysis

Allele frequencies were compared between groups via the chi^2 test on a 2 × 2 contingency table via the Statistica software package (StatSoft Inc., Tulsa, OK, USA). Genotype distribution was analyzed assuming three modes of inheritance: dominant, codominant, or recessive. For each model, the p-value for association was calculated. The appropriateness of the model was also assessed via the chi^2 test. These calculations were performed via the

Table 1 Genotyping conditions

SNP	primers	annealing	product	restriction enzyme	alleles
rs184003 G/T (1704G/T)	F: 5'GCCCTCTCCTCAAATCCACT3' R: 5'TGGTCCTGAGGCCCTATCT3'	62°C	275 bp	BfaI	T 156, 119 bp G 156, 77, 42 bp
rs2070600 G/A	F: 5'GAAGGTCCTGTCTCCCCAG3' R: 5'AATGAGGCCAGTGGAAGTCA3'	62°C	272 bp	AluI	G 272 bp A 213, 59 bp

SNP Single-nucleotide polymorphism, *F* Forward, *R* Reverse, *BP* Base pairs

Web-Assotest program (available at http://www.ekstroem.com/assotest/assotest.html). p values less than 0.05 were considered significant. Conservative Bonferroni correction for multiple testing was applied. The values of the variables between the study groups were compared via Student's t-test or the Mann−Whitney *U* test when appropriate. Assessment of normality of the distribution was performed with the Kolmogorov–Smirnov test.

Results

As the distributions of the genotypes and alleles of the studied polymorphisms did not differ between the female and male study participants, all the analyses were performed together without stratification by sex. For both the polymorphisms studied and for all the groups and subgroups tested, the distributions of genotypes and alleles were consistent with Hardy-Weinberg equilibrium ($p > 0.05$).

The genotype and allele distributions of RAGE polymorphisms differ between ACS patients and age- and sex-matched controls

We started our analysis by comparing the frequencies of the different genotypes and alleles of the investigated polymorphisms between the study groups (Table 2).

For the rs184003 G/T polymorphism, the frequency of genotypes containing the T allele (GT + TT) was significantly greater in patients with a history of ACS than in healthy age- and sex-matched controls (28.87% vs. 11.25%, $p < 0.0001$, OR = 3.2 [95% CI: 1.86–5.52]). Consistently, allele T was more common in ACS patients than in the healthy age- and sex-matched group (15.33% vs. 5.94%, $p < 0.0001$, OR = 2.86 [95%CI: 1.72–4.47]).

The situation was different for the rs2070600 G/A polymorphism: genotype and allele frequencies were not significantly different between the study groups (after Bonferroni correction).

Genotype and allele distributions of RAGE polymorphisms differ between patients with ACS before and after age 50

In subsequent analyses, we wanted to test whether the presence of any of the polymorphic variants studied could predispose the participants in our study to ACS at a younger age. To do this, we compared the frequencies of individual genotypes and alleles in patients diagnosed with ACS before and after the age of 50 and related them to the frequencies observed in the healthy age- and sex-matched group.

For the rs184003 G/T polymorphism, the frequency of genotypes differed between patients diagnosed with ACS before and after the age of 50. Genotypes containing the polymorphic T allele (GT and TT) were significantly less common in patients with ACS before the age of 50 than in those diagnosed later (20.0% vs. 38.5%, $p = 0.0002$, OR = 0.4 [95%CI: 0.25–0.65]). The frequency of genotypes containing the polymorphic T allele was greater in the group of patients diagnosed with ACS before the age of 50 years than in the group of healthy controls (20% vs. 11.25%), but this difference was not statistically significant after Bonferroni correction ($p > 0.05$). In the group of patients with ACS diagnosed after the age of 50, the frequency of genotypes containing the polymorphic T allele was significantly greater than that in healthy controls (38.5% vs. 11.25%, $p < 0.0001$, OR = 0.24 [95%CI: 0.09–0.75]).

In the case of the rs2070600 G/A polymorphism, we observed that although the frequency of genotypes did not differ between all ACS patients and controls, genotypes containing the A allele (GA + AA) were less common in patients who had ACS before the age of 50 (14.29% vs. 24.85%), but this difference was not statistically significant after correction.

Distribution of RAGE genotypes and severity of coronary artery disease

In this study, we explored whether *RAGE* genotype distribution differs in patients with different severity of coronary artery stenosis, too. The dataset included measures of CAD severity, specifically the degree of stenosis in individual coronary arteries, along with the mean and median stenosis values.

Among younger patients, the median degree of stenosis (defined as average across vessels) was 25.75%. In older patients, the median degree of stenosis was 49.27%. These observations may suggest a potential trend in which certain genotypes may be associated with a more advanced form of CAD.

However, we observed no significant correlation between the degree of coronary artery stenosis and the genotypes of the RAGE polymorphisms studied in the examined groups of CAD patients. In patients diagnosed with ACS under the age of 50, the median degree of coronary artery stenosis was 22.83% in carriers of the rs184003 T polymorphism (GT and TT), compared to 26.35% in those with the GG genotype. For participants who experienced ACS at 50 years of age or older, the median degree of coronary artery stenosis was 47.15% for GT and TT genotype carriers, versus 50.25% for GG genotype carriers. For the rs2070600 SNP, the median degree of coronary artery stenosis was 27.30% in younger patients with genotypes containing the A allele (GA + AA), compared to 25.25% in GG genotype carriers. In older participants, these values were 48.15% and 52.55%, respectively.

Table 2 Distribution of genotypes and alleles of *RAGE* polymorphisms in the study groups

	ACS < 50		Healthy controls		p* OR [95% CI]	ACS ≥ 50		p** OR [95% CI]	All ACS		p*** OR [95% CI]
rs184003	%	%	*N*	%	%	*N*	*N*	%	%	*N*	%	
Genotypes	175		160			161				336		
GG	140	80.0	142	88.75		99		61.50		239	71.13	
GT	33	18.85	17	10.65		58		36.02		91	27.08	
TT	2	1.15	1	0.60		4		2.48		6	1.79	
GT + TT vs. GG					p = 0.027^ 1.97 [1.3–3.65]			p = 0.0002 0.4 [0.25–0.65]			p < 0.0001 3.2 [1.86–5.52]
GG vs. TT					p = 0.499			p = 0.405			p = 0.268
GT + GG vs. TT					p = 0.611			p = 0.350			p = 0.271
Alleles											
G	313	89.43	301	94.06	p = 0.03^	256		79.50	p < 0.0001	569	84.67	p < 0.0001
T	37	10.57	19	5.94	1.87 [1.08–3.32]	66		20.50	0.24 [0.09–0.75]	103	15.33	2.86 [1.72–4.71]
rs2070600	N	%	N	%		N		%		N	%	
Genotypes	175		160			161				336		
GG	150	85.71	123	76.87		121		75.15		271	80.65	
GA	24	13.71	32	20.0		35		21.74		59	17.56	
AA	1	0.58	5	3.13		5		3.11		6	1.79	
GA + AA vs. GG					p = 0.052			p = 0.081			p = 0.334
GG vs. AA					p = 0.097			p = 0.095			p = 0.244
GA + GG vs. AA					p = 0.087			p = 0.089			p = 0.357
Alleles											
G	324	92.57	278	86.87	p = 0.023^	277		86.02	p = 0.02^	601	89.43	p = 0.140
A	26	7.43	42	13.13	0.53 [0.31–0.89]	45		13.98	0.49 [0.29–0.82]	71	10.56	

ACS Acute coronary syndrome, *CI* Confidence, *N* Number, *OR* Odds ratio, *SNP* Single-nucleotide polymorphism

^ *p*-values non-significant after Bonferroni correction

*calculations performed for ACS < 50 vs. Healthy controls

**calculations performed for ACS < 50 vs. ACS ≥ 50

***calculations performed for All ACS vs. Healthy controls

Patients with the polymorphic rs184003 T allele have different clinical characteristics from GG homozygotes

To test whether the polymorphisms studied can modulate the clinical course of ACS, we examined the distribution of biochemical and clinical parameters between study participants, stratified by the presence of polymorphic alleles of the genetic variants studied.

We observed that patients carrying the T allele of the rs184003 polymorphism (GT + TT genotypes) had slightly lower HDL cholesterol levels (mean 1.02 mmol/l vs. 1.14 mmol/l, $p = 0.01$) than those homozygous for the G allele. These patients also had significantly higher median troponin I levels at the time of ACS (32.2 ng/ml vs. 24.4 ng/ml, $p = 0.04$) than did the carriers of the GG genotype. However, we did not observe statistically significant differences in total cholesterol, LDL cholesterol, triglycerides, glucose on admission, fasting glucose, or renal function parameters between study participants carrying different variants of the rs184003 G/T polymorphism.

For the rs2070600 G/A polymorphism, we found no statistically significant differences in biochemical parameters between patient groups stratified according to the presence of the polymorphic A allele (AA + GA vs. GG).

We also tested whether the genotype frequencies of the polymorphisms studied differed among patients with diabetes, a family history of cardiovascular disease, and those who had suffered an ST-elevation myocardial infarction, but we detected no statistically significant differences, probably due to the limited number of study participants that negatively impacted the power of the performed analysis.

Discussion

The development of CAD is the result of complex interactions between environmental and genetic factors. While the former is well known and includes, among others, older age, smoking, and comorbidities such as diabetes, hypercholesterolemia, or poorly controlled blood pressure, the genetic factors that predispose individuals to the development of CAD are still under investigation [20]. The aim of our case-control study was to investigate whether single nucleotide substitution polymorphisms (rs184003 G/T and rs2070600 G/A) in the *RAGE* gene may determine predisposition to CAD in the Polish population.

The choice of candidate gene and polymorphisms studied was not accidental. As mentioned above, RAGE has a well-established role in the pathogenesis of CAD [11], while its pharmacological blockage or genetic deletion results in significant suppression of atherosclerosis progression [27]. The *RAGE* gene is located on chromosome 6p21.3 (at a locus involved in inflammatory and immune responses and tissue compatibility complex III) and is overexpressed in atherosclerotic plaques [28]. It has also been identified as a likely candidate gene associated with vascular and neurological complications such as atherosclerosis, CAD, ischemic stroke, and Alzheimer's disease [29]. More than 50 polymorphic variants have been identified in the *RAGE* region, and association studies have linked them to the risk of a range of diseases, including cardiovascular, metabolic, and cancer [30]. Of these, the rs2070600 G/A SNP in exon 3 resulting in a missense mutation and glycine (G)-to-serine (S) substitution at position 82 of the amino acid chain is of special interest since it is located in the putative site of ligand binding, which could influence the AGE/RAGE interaction. The polymorphic A variant has potentially increased ligand binding affinity, which may predispose patients to or aggravate atherosclerosis [31]. In addition, the rs2070600 SNP has been reported to decrease the proteolysis of RAGE, thus affecting serum levels of sRAGE. Carriers of the GG genotype have been reported to have higher plasma levels of sRAGE than GA heterozygotes and AA homozygotes [32]. The rs2070600 SNP was subsequently associated with increased stimulation of RAGE by its ligands and the potential of a proinflammatory pathway [33]. However, data from studies on the association of this polymorphism with the risk of CAD in humans are inconclusive. Notably, the effect of the rs2070600 SNP on the development of coronary atherosclerosis may be population-specific [34]. In our study, the observed frequency of the polymorphic A allele in the healthy control group (13.98%) was similar to that described in other Caucasian populations [34]. In contrast, in the CAD groups, the prevalence of the A allele and GA/AA genotypes was lower than that in healthy controls, especially in those who had ACS at the age of 50 years or older. This finding would be interesting because a lower frequency of the polymorphic rs2070600 variant should theoretically result in less inflammation induced by the AGE-RAGE pathway and lower levels of sRAGE, which protects against the development of atherosclerosis. However, the difference in the frequency of polymorphic alleles and genotypes observed between the study groups did not reach statistical significance.

The second SNP studied in the *RAGE* is rs184003, a G/T substitution in introns 7/8 (known as 1704G/T). Much less is known about the biological significance of this SNP and its association with human disease. Since introns contain many functional elements, including intron-splicing enhancers and silencers that regulate alternative splicing, trans-splicing elements, and other regulatory elements, intron mutations can have potential consequences similar to those in the coding parts of genes. Polymorphisms in introns may also confer disease susceptibility or otherwise modulate the genotype-phenotype relationship [35]. The rs184003 polymorphic

allele T has subsequently been found to be associated with a number of diseases and conditions, including insulin resistance and diabetes, high blood pressure, and different types of cancer [29]. The pathophysiological relevance of this polymorphism is not fully understood, but in a study of patients with gastric cancer, the presence of the rs184003 T allele was associated with higher sRAGE levels as compared with the wild-type homozygotes [36]. In turn in patients with schizophrenia, rs184003 was found to be in linkage disequilibrium with two other SNPs, rs2071288 and rs17846798, creating a specific haplotype whose carriers had lower serum esRAGE concentrations. esRAGE is an isoform of RAGE that lacks transmembrane and signaling domains and is thought to act as a decoy receptor, binding AGEs and reducing AGE-RAGE interactions and the activity of related intracellular signaling pathways [37]. This concept appears to be consistent with observational data suggesting that individuals with the T allele have significantly lower plasma levels of antioxidants, such as total carotenoids, lutein, lycopene, and tocopherol, than individuals with the 1704G allele. This suggests a potential role for the G1704T polymorphism in oxidative stress [38].

In our study, the observed frequency of this allele in the healthy age- and sex-matched group reflected that reported in other European Caucasian populations (4.9–6.8%) [29, 39]. However, in patients with CAD, the polymorphic genotypes TT and GT were found significantly more frequently. A similar trend indicating a link between the T allele with CAD risk was observed in a study of the Chinese population [40]. In this study, the frequency of the T allele in patients with CAD was significantly higher compared to healthy controls (20.62% vs. 17.59%), while the presence of the polymorphic genotypes (GT + TT) was associated with a risk of coronary artery atherosclerosis (OR = 2.39). However, it should be noted that, although this study was much larger than ours, it included patients with CAD (more than 50% stenosis in at least one of the three major coronary arteries or major branches), but not with a history of ACS. To our knowledge, our study is the first to describe a potential association between the rs184003 polymorphism and the occurrence of ACS in European Caucasian populations.

From a pathophysiological point of view, the higher prevalence of a genetic variant potentially associated with increased inflammation and oxidative stress in a group of patients with symptomatic CAD seems reasonable. Moreover, we observed that carriers of the polymorphic rs184003 T allele had significantly higher mean troponin I levels at the time of ACS than did those homozygous for the wild-type G allele and tended toward lower HDL-C concentrations. However, in the studied population, the distribution of the investigated genotypes in the subgroups stratified by the myocardial infarction type or severity of coronary artery stenosis did not differ significantly.

This research highlights a potential link between CAD severity and genetics. Our initial findings suggest that certain genotypes may affect the degree of artery stenosis, whereas previous studies have focused mostly on genetic contributions to the early onset of myocardial infarction [41, 42]. This finding is consistent with other studies that revealed genetic factors that influence the development of atherosclerosis without reference to acute coronary events [43]. However, our results should be interpreted cautiously, as the observed differences were not statistically significant.

To identify factors predisposing patients to the development of CAD and the incidence of ACS at a young age, we compared the frequencies of the genotypes studied in patients stratified by age. However, in our study, the frequency of polymorphic genotypes was greater in individuals diagnosed with ACS in their 50 s than in those in younger age groups. This finding was surprising since one would expect the pathogenic allele to be more common in younger patients with ACS, i.e., those younger than 50 years of age. These results therefore suggest a potential impact of rs184003 on the prevalence of CAD and ACS in the Polish population; however, this polymorphism does not appear to increase the risk of ACS at a young age.

We are aware of the potential limitations of this study. The most important limitation is the relatively small size of the study groups, which obviously affects, especially in the subgroup analysis, the power of the results obtained. On the other hand, the careful selection of patients ensures that they are well characterized and that we have complete clinical data that can be used for future meta-analyses. In addition, by analyzing only two of the more than fifty SNPs in the *RAGE* gene, we may have missed other potentially pathogenic variants. Furthermore, our lack of ability to measure sRAGE/esRAGE levels in the patient's serum prevents us from assessing the extent to which the studied *RAGE* gene variants influence receptor concentrations in the bloodstream, and consequently the risk of ACS.

When discussing the potential limitations of our study, we must also acknowledge that when referring to the control group as 'healthy, age- and gender-matched controls', we cannot guarantee that these individuals did not have hidden coronary artery disease (CAD). According to applicable Polish legislation, only individuals who are healthy and show no signs or symptoms of disease (including chronic conditions that increase the risk of atherosclerosis) are permitted to donate blood. Additionally, only non-smokers were eligible to participate in this study. However, these criteria could not guarantee that the control group did not have latent coronary artery

disease. Moreover, we did not collect information on donors' family history of coronary artery disease.

Among the factors that may have influenced the results of this study, it should be noted that the 'healthy control group' was selected as a reference point for patients who had experienced ACS before the age of 50. Consequently, the entire study group of patients with a history of ACS (including individuals both under and over 50 years of age) was significantly older than the 'healthy control group'.

Finally, the definition of ACS in young patients has not been precisely determined. In the literature, this term is used in studies describing individuals who experienced ACS at ages below 45, 30, and even 20 years. To ensure a similar number of participants in the groups being compared in our study, we set this threshold at under 50 years. Setting a different threshold (e.g. at age 45 or 55) would undoubtedly affect the results. All these above-mentioned factors could have influenced the results of our study.

Conclusions

In conclusion, we found a higher frequency of the T allele of the *RAGE* rs184003 polymorphism among Polish patients with ACS, while the distribution of the rs2071288 SNP alleles and genotypes did not differ in patients with ACS and in the healthy age- and sex-matched group. However, our findings do not suggest a link between the studied SNPs and the occurrence of ACS at a young age. Further, well-designed studies with large sample sizes are needed to validate the present results.

Abbreviations

ACS	Acute coronary syndrome
AGEs	Advanced glycation end products
BMI	Body mass index
CAD	Coronary artery disease
DBP	Diastolic blood pressure
esRAGE	Endogenous secretory receptor for advanced glycation end products
GWAS	Genome-wide association studies
HDL	High-density lipoprotein cholesterol
IMT	Intima-media thickness
LDL	Low-density lipoprotein cholesterol
OR	Odds ratio
PCR	Polymerase chain reaction
RAGE	Receptor for advanced glycation end products
RAGE	The gene for the receptor for advanced glycation end products
RFLP	Restriction fragment length polymorphism
SBP	Systolic blood pressure
SNP	Single nucleotide polymorphism
sRAGE	Soluble isoform of the receptor for advanced glycation end products
TG	Triglycerides

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12920-025-02215-3.

Supplementary material 1.

Supplementary material 2.

Acknowledgements
None.

Authors' contributions
All the authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by KG, MA, JP and AK. The first draft of the manuscript was written by KG and AK, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

Funding
This work was supported by the National Science Centre Poland, grant 2018/31/B/NZ5/01556.

Data availability
The datasets generated and/or analyzed during the current study are available in the repository available at: https://figshare.com/articles/dataset/RAGE_rs184003_rs2070600_genotyping_xlsx/28887671?file=54028739.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Center of Postgraduate Medical Education Warsaw, Poland (decision 161/2015). Informed consent was obtained from all individual participants included in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 24 April 2025 / Accepted: 18 August 2025
Published online: 30 September 2025

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8. Podsumowanie i wnioski

Celem niniejszej pracy doktorskiej było zbadanie roli szlaku receptora końcowych produktów zaawansowanej glikacji (RAGE) w rozwoju związanych z otyłością powikłań kardiometabolicznych. W pracy przedstawiono aktualny stan wiedzy dotyczący roli szlaku RAGE w rozwoju otyłości i jej powikłań oraz przeprowadzono własne badania na materiale biologicznym pochodzącym od pacjentów z otyłością, cukrzycą typu 2 oraz wywiadem ostrych zespołów wieńcowych. Ponadto wskazano na możliwość wykorzystania strategii terapeutycznych modulujących szlak RAGE w leczeniu otyłości i jej powikłań.

W **pracy nr 1** (*Receptor for the Advanced Glycation End Products (RAGE) Pathway in Adipose Tissue Metabolism*) podsumowano dotychczasowy stan wiedzy na temat roli szlaku RAGE w powstawaniu dysfunkcji tkanki tłuszczowej i zwiększonego ryzyka rozwoju towarzyszących jej powikłań metabolicznych. Opisano proces formowania AGEs oraz szlak sygnałowy RAGE, a także związek aktywacji tego szlaku z rozwojem zapalenia metabolicznego. Przedstawiono wyniki badań wskazujące, że wywołany aktywacją szlaku RAGE stan zapalny pełni kluczową rolę w rozwoju insulinooporności i cukrzycy typu 2 oraz wpływa na funkcję endokrynną tkanki tłuszczowej poprzez zaburzenie procesu sekrecji adipokin. Fakt ten sprawia, że modulowanie funkcji RAGE może stanowić cenną strategię terapeutyczną w prewencji i leczeniu związanych z otyłością powikłań. Tym niemniej większość przeprowadzonych prac miała charakter przedkliniczny (badania *in vitro* i na modelach zwierzęcych), a badania prowadzone u ludzi obejmowały małe grupy uczestników. W związku z tym, aby rozważyć ingerencję w szlak RAGE jako strategię terapeutyczną, konieczne jest przeprowadzenie prac na większych grupach badanych, aby potwierdzić rolę szlaku RAGE w rozwoju otyłości i jej powikłań u człowieka.

Biorąc pod uwagę fakt, że aktywacja szlaku RAGE bierze udział w rozwoju zapalenia metabolicznego, w **pracy nr 2** (*AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus*) postanowiono ocenić czy w przebiegu otyłości i cukrzycy typu 2 dochodzi do zmian ekspresji genu kodującego RAGE (*AGER*) w tkance tłuszczowej oraz czy zmiany te wpływają na lokalny poziom ekspresji genów kodujących adipokiny i cytokiny prozapalne (rozwój dysfunkcji tkanki tłuszczowej). Dodatkowo, ze względu na opisywaną w literaturze rolę długiego niekodującego RNA AGER-1 (lncAGER-1) w regulacji ekspresji *AGER* w innych

tkankach, zbadano, czy ten mechanizm epigenetyczny może być zaangażowany w związane z otyłością i cukrzycą zmiany w aktywności transkrypcyjnej *AGER*. Badając tkanki tłuszczowe pochodzące od 62 kobiet chorujących na otyłość, 15 kobiet po chirurgicznym leczeniu otyłości i 20 kobiet o prawidłowej masie ciała, wykazano, że w przebiegu otyłości dochodzi do wzrostu stężenia mRNA dla *AGER* w podskórnej tkance tłuszczowej, a spowodowana operacją bariatryczną redukcja masy ciała prowadzi do obniżenia tego stężenia, do wartości porównywalnych do obserwowanych w tkance tłuszczowej osób o prawidłowej masie ciała. Z kolei, po podziale badanej grupy kobiet z otyłością w zależności od współwystępowania cukrzycy, zaobserwowano, że poziom mRNA dla *AGER* był istotnie wyższy w trzewnej tkance tłuszczowej u pacjentek z cukrzycą typu 2 i korelował ze zwiększonym stężeniem lncAGER-1 i mRNA genów kodujących rezystynę i interleukinę 1b. Uzyskane wyniki sugerują, że otyłość u kobiet wiąże się ze zwiększoną ekspresją *AGER* w podskórnej tkance tłuszczowej i zmiany te mogą ustąpić wraz z redukcją masy ciała. Jednak rozwój związanego z aktywacją RAGE zapalenia metabolicznego, predysponującego do insulinooporności i cukrzycy typu 2 – dotyczy przed wszystkim tkanki tłuszczowej trzewnej. Biorąc pod uwagę opisowy charakter prowadzonych prac obserwacje te wymagają potwierdzenia w badaniach funkcjonalnych.

Mając na uwadze dane literaturowe wskazujące na udział RAGE w rozwoju miażdżycy i choroby niedokrwiennej serca, w **pracy nr 3** (*Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population*) zbadano związek dwóch polimorfizmów *AGER* (rs184003 G/T, rs2070600 G/A) z predyspozycją do rozwoju choroby niedokrwiennej serca. Badaniem objęto 336 mężczyzn z wywiadem ostrego zespołu wieńcowego (ACS) i 160 zdrowych mężczyzn bez wywiadu choroby niedokrwiennej serca i innych chorób przewlekłych. U chorych po ACS zaobserwowano istotnie wyższą częstość genotypów zawierających polimorficzny allel T (GT+TT) polimorfizmu rs184003 w genie kodującym RAGE w porównaniu do grupy kontrolnej. Ponadto wykazano, że obecność allelu T koreluje z niższym stężeniem cholesterolu HDL (*ang. high density lipoprotein*) i wyższym stężeniem troponiny I w czasie wystąpienia ACS. Nie stwierdzono natomiast związku między badanymi polimorfizmami a ryzykiem wczesnego wystąpienia ACS (< 50 roku życia) w badanej grupie. Uzyskane wyniki wskazują na potencjalną rolę polimorfizmu rs184003 *AGER* w kształtowaniu predyspozycji genetycznej do wystąpienia miażdżycy tętnic wieńcowych i ACS w polskiej populacji.

9. Wnioski

Wyniki uzyskane z niniejszej pracy doktorskiej pozwalają na sformułowanie następujących wniosków:

1. W przebiegu otyłości u człowieka dochodzi do wzrostu ekspresji genu kodującego receptor końcowych produktów zaawansowanej glikacji (*AGER*) w tkance tłuszczowej podskórnej, a redukcja masy ciała wiąże się z obniżeniem stężenia mRNA tego genu do wartości porównywalnych do obserwowanych w tkance tłuszczowej osób o prawidłowej masie ciała.

2. U chorych z otyłością powikłaną cukrzycą typu 2 dochodzi do wzrostu ekspresji *AGER* w trzewnej tkance tłuszczowej, co koreluje ze zwiększonym lokalnym stężeniem prozapalnych adipokin.

3. W regulacji ekspresji *AGER* w tkance tłuszczowej może pośredniczyć długie niekodujące RNA AGER1 (lncAGER-1), choć obserwacja ta wymaga potwierdzenia w badaniach funkcjonalnych.

4. Obecność allelu T polimorfizmu rs184003 w genie kodującym receptor końcowych produktów zaawansowanej glikacji (*AGER*) może predysponować do rozwoju miażdżycy naczyń wieńcowych i jej powikłania jakim jest ostry zespół wieńcowy (ACS) w polskiej populacji.

5. Badane polimorfizmy *AGER* (rs184003 G/T, rs2070600 G/A) w polskiej populacji nie wydają się predysponować do wystąpienia ACS w młodym wieku (< 50 rż).

Podsumowując, przedstawione wyniki prac oryginalnych składające się na niniejszą rozprawę doktorską wskazują na potencjalną rolę szlaku receptora końcowych produktów zaawansowanej glikacji (RAGE) w patogenezie związanych z otyłością powikłań metabolicznych (insulinooporności i cukrzycy typu 2) oraz miażdżycy tętnic wieńcowych u człowieka. Mogą one stanowić podstawę do dalszych badań funkcjonalnych, które być może odpowiedzą na pytanie dotyczące zasadności zastosowania strategii terapeutycznych ingerujących w szlak RAGE w leczeniu otyłości i jej powikłań.

10. Piśmiennictwo

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11. Opinia Komisji Bioetycznej lub Etycznej

UCHWAŁA KOMISJI BIOETYCZNEJ
PRZY CENTRUM MEDYCZNEGO KSZTAŁCENIA PODYPLOMOWEGO
Nr 94/2025
z dnia 08 października 2025r.

Komisja Bioetyczna przy Centrum Medycznego Kształcenia Podyplomowego, powołana przez Dyrektora Centrum Medycznego Kształcenia Podyplomowego Zarządzeniem Nr 56/2023 z dnia 4 maja 2023 r. w sprawie powołania Komisji Bioetycznej przy Centrum Medycznym Kształcenia Podyplomowego, z późn. zm.,

zapoznała się z wnioskiem o wyrażenie opinii o projekcie eksperymentu medycznego pt.: **„Badanie związku pomiędzy polimorfizmami genu kodującego receptor dla końcowych produktów glikacji (advanced glication end-products AGE-s) i wystąpieniem ostrego zespołu wieńcowego”**

zgłoszonym przez: **dr hab. n. med. Michał Ambroziak, prof. CMKP**
Kierujący eksperymentem medycznym

miejsce prowadzenia badania:
Klinika Kardiologii CMKP
Szpital Grochowski im. dr med. Rafała Masztaka Sp. o.o.
04-073 Warszawa, ul. Grenadierów 51/59

Po zapoznaniu się z całością dokumentacji, Komisja Bioetyczna, działając na podstawie art. 29 ust. 2 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz. U z 2021r. poz. 790, z późn. zm.) w zw. a art. 37a ust.1 ustawy z dnia 6 września 2001 r. Prawo Farmaceutyczne (t.j. Dz.U. z 2021 r. poz. 1997, z późn.zm.) oraz rozporządzenie Ministra Zdrowia z dnia 26 stycznia 2023r. w sprawie komisji bioetycznej i Odwoławczej Komisji Bioetycznej (Dz. U. z 2023r. poz. 218 z późn. zm.).

w składzie:
1) dr n. med. Tomasz Bużański - Przewodniczący Komisji Bioetycznej
2) ks. prof. dr hab. Jan Krokos - Zastępca Przewodniczącego Komisji Bioetycznej
3) prof. dr hab. n. med. Urszula Fiszer
4) dr hab. n. med. Alina Kuryłowicz, prof. CMKP
5) dr hab. n. med. Adam J. Sybilski, prof. CMKP
6) dr hab. n. med. Marcin Tyrakowski, prof. CMKP
7) dr n. med. Małgorzata Bińkowska
8) dr n. med. Agnieszka Jasik
9) dr n. farm. Marek Wleklik
10) dr n. med. Tomasz Imiela – przedstawiciel Okręgowej Rady Lekarskiej w Warszawie
11) mgr piel. Izabela Stępień
12) mec. Katarzyna Kaczorowska
13) Piotr Fonrobert – przedstawiciel fundacji Polskiej Koalicji Pacjentów Onkologicznych.

- wyraziła pozytywną opinię* w zakresie rozpoczęcia eksperymentu medycznego.

- ~~wyraziła negatywną opinię* i odrzuciła wniosek z powodu~~:

Przy obecności 7 członków Komisji, w głosowaniu tajnym, oddano głosów: „za” 7, „przeciw” 0.

dr hab. n. med. Alina Kuryłowicz nie brała udziału w podejmowaniu uchwały.

- przedstawiła rekomendację ~~o konieczności~~/braku konieczności* ubezpieczenia odpowiedzialności cywilnej ww. eksperymentu medycznego.

1

POSIEDZENIE KOMISJI BIOETYCZNEJ

PRZY CENTRUM MEDYCZNEGO KSZTAŁCENIA PODYPLOMOWEGO

08 października 2025 r. – lista obecności -

1. dr n. med. Tomasz Bużański Przewodniczący Komisji Bioetycznej	Signed by / Podpisano przez: Tomasz Bużański Date / Data: 2025-10-08 20:31
2. ks. prof. dr hab. Jan Krokos Zastępca Przewodniczącego Komisji Bioetycznej	Signed by / Podpisano przez: Jan Krokos Date / Data: 2025-10-08 08:24
3. prof. dr hab. n. med. Urszula Fiszer	
4. dr hab. n. med. Alina Kuryłowicz, prof. CMKP	Podpisano przez/ Signed by: ALINA KURYŁOWICZ Data/ Date: 08.10.2025 11:17 mSzafir
5. dr hab. n. med. Adam J. Sybilski, prof. CMKP	
6. prof. dr hab. n. med. Marcin Tyrakowski	
7. dr n. med. Małgorzata Bińkowska	
8. dr n. med. Agnieszka Jasik	Signed by / Podpisano przez: Agnieszka Jasik Date / Data: 2025-10-08 10:05
9. dr n. farm. Marek Wleklik	Signed by / Podpisano przez: Marek Wleklik Date / Data: 2025-10-08 10:34
10. dr n. med. Tomasz Imiela - przedstawiciel Okręgowej Rady Lekarskiej w Warszawie	
11. mgr Izabela Stępień	Signed by / Podpisano przez: Izabela Stępień Date / Data: 2025-10-09 17:13
12. mec. Katarzyna Kaczorowska	Signed by / Podpisano przez: Katarzyna Anna Kaczorowska Date / Data: 2025-10-08 10:49
13. Piotr Fonrobert - przedstawiciel Fundacji Polskiej Koalicji Pacjentów Onkologicznych	Signed by / Podpisano przez: Piotr Fonrobert Date / Data: 2025-10-08 08:38

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

Warszawa, 14.10.2025
(miejscowość, data)

Wiesław Tarnowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Artur Binda
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....

(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Paweł Jaworski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

P. Jaworski

.....
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Michał Wąsowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badaniai wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Monika Puzianowska-Kuźnicka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*
oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
weryfikacja i redakcja tekstu i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Monika Puzianowska-Kuźnicka
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
weryfikacja i redakcja tekstu i wynosi 5%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....

(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Marta Izabela Jonas
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przeprowadzenie doświadczeń i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Alina Kuryłowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: przygotowanie koncepcji pracy, opracowanie metod, weryfikacja i redakcja tekstu, korespondencja z recenzentami, pozyskanie funduszy na publikację artykułu i wynosi 25%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Alina Kuryłowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.
Receptor for the Advanced Glycation End Products (RAGE) Pathway in Adipose Tissue Metabolism.
oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami i wynosi 35%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (50%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Alina Kuryłowicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus*
oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przygotowanie koncepcji pracy, opracowanie metod, weryfikacja i redakcja tekstu, korespondencja z recenzentami, pozyskanie funduszy na publikację artykułu i wynosi 20%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Zbigniew Bartoszewicz
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus*.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
przeprowadzenie doświadczeń i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 15.10.2025
(miejscowość, data)

Wojciech Lisik
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

PODPIS ZAUFANY
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Dokument podpisany elektronicznie podpisem zaufanym

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Jakub Podraza
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzenie doświadczeń do powstania pracy i wynosi 10%

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....

(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Bartłomiej Noszczyk
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.* Autorstwa: Gutowska, K., Koźniewski, K., Wąsowski, M., Jonas, M. I., Bartoszewicz, Z., Lisik, W., Jonas, M., Binda, A., Jaworski, P., Tarnowski, W., Noszczyk, B., Puzianowska-Kuźnicka, M., Czajkowski, K., & Kuryłowicz, A, opublikowanej w *International journal of molecular sciences*, *24*(24), 17447.

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

Kierownik Kliniki Chirurgii Plastycznej
Centrum Medycznego Kształcenia Podyplomowego
prof. dr hab. med. Bartłomiej Noszczyk
(podpis oświadczającego)

Warszawa, 14.10.2025
(miejscowość, data)

Andrzej Budaj
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
weryfikacja i redakcja tekstu i wynosi 5%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Maurycy Jonas
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
rekrutacja pacjentów do badania i wynosi 3%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

Maurycy Jonas
.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Krzysztof Koźniewski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *AGER-1 Long Non-Coding RNA Levels Correlate with the Expression of the Advanced Glycosylation End-Product Receptor, a Regulator of the Inflammatory Response in Visceral Adipose Tissue of Women with Obesity and Type 2 Diabetes Mellitus*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

przeprowadzenie doświadczeń do powstania pracy i wynosi 10%

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (35%).

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

Krzysztof Koźniewski

.....

(podpis oświadczającego)

Warszawa, 15.10.2025
(miejscowość, data)

Michał Ambroziak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population.*

oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

rekrutacja pacjentów, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentamii wynosi 20%

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:

(imię i nazwisko kandydata do stopnia)

przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)[3]

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

dr hab. n. med. Michał Ambroziak
specjalista chorób wewnętrznych
KARDIOLOG

Warszawa, 04.10.2025
(miejscowość, data)

Krzysztof Czajkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: weryfikacja i redakcja tekstu i wynosi 5%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Krzysztof Czajkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: weryfikacja i redakcja tekstu i wynosi 5%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)

Warszawa, 04.10.2025
(miejscowość, data)

Krzysztof Czajkowski
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. *Advanced glycation end-product receptor gene (RAGE) polymorphism in patients with acute coronary syndrome – a case-control study in the Polish population* oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi: weryfikacja i redakcja tekstu i wynosi 5%.

Wkład Klaudii Gutowskiej w powstawanie publikacji obejmował:
(imię i nazwisko kandydata do stopnia)
przeprowadzenie doświadczeń, przygotowanie koncepcji artykułu i pierwotnej wersji tekstu, korespondencja z recenzentami (30%).
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek/mgr Klaudii Gutowskiej

.....
(podpis oświadczającego)