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Tkanka tłuszczowa a zawartość wybranych związków lipidowych i lipofilnych w osoczu krwi osób dorosłych

Adipose tissue and the content of selected lipid and lipophilic compounds in the blood plasma of adults

Rozprawa doktorska na stopień doktora
w dziedzinie nauk medycznych i nauk o zdrowiu
w dyscyplinie nauki o zdrowiu
przedkładana Radzie Dyscypliny Nauk o Zdrowiu
Warszawskiego Uniwersytetu Medycznego

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Warszawa, 2025 rok

Słowa kluczowe: tokoferole; tokotrienole; stan odżywienia; profil lipidowy; stan zapalny; osoby dorosłe; ocena spożycia

Key words: tocopherols; tocotrienols; nutritional status; lipids profile; inflammation; adults; dietary assessment

Pragnę złożyć serdeczne podziękowania Pani Promotor,
Dr hab. Magdalenie Górnickiej, prof. SGGW,
za nieocenione wsparcie, cenne wskazówki i bezcenną pomoc podczas realizacji tej pracy. Jestem ogromnie wdzięczny za cierpliwość, życzliwość i nieustanną gotowość do dzielenia się swoją wiedzą i doświadczeniem, a także za spokój, optymizm i przyjacielskie nastawienie, które sprawiały, że droga do doktoratu była fascynującą i rozwijającą przygodą. Dziękuję, że zawsze mogłem liczyć na wsparcie – zarówno merytoryczne, jak i wynikające z autentycznej troski – pełne zrozumienia, wsparcia, życzliwości i empatii.

Serdecznie dziękuję ***Współautorom*** artykułów naukowych, za okazaną pomoc, cenne wskazówki i wsparcie przy tworzeniu tej pracy. Jestem wdzięczny za Wasze zaangażowanie i cenne uwagi, które miały ogromny wpływ na jej ostateczny kształt. Dziękuję za otwartość na dyskusje i inspirujące pomysły.

Serdecznie dziękuję całemu ***Zespołowi Katedry Żywienia Człowieka Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie,***
za wsparcie na każdym etapie prowadzonych badań. Jestem wdzięczny za życzliwość, inspirujące rozmowy oraz otwartość, z jaką zostałem przyjęty do grona akademickiego. Dziękuję za pomoc, cenne wskazówki i serdeczną atmosferę, które sprawiły, że czas spędzony na pracy badawczej był nie tylko wyzwaniem naukowym, ale także niezwykle wartościowym doświadczeniem. Dziękuję za każdy gest wsparcia, motywację i stworzenie przestrzeni do rozwoju oraz wymiany myśli.

Serdecznie dziękuję Pani Dyrektor Instytutu Nauk o Żywieniu Człowieka Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie, ***prof. dr hab. Krystynie Gutkowskiej***, za wsparcie, motywację oraz życzliwość, które towarzyszyły mi na każdym etapie pracy. Jestem niezwykle wdzięczny za stworzenie inspirującego środowiska naukowego, otwartość na dyskusję oraz cenne wskazówki, które pozwoliły mi rozwijać się zarówno jako badacz, jak i dydaktyk. Dziękuję za zaufanie, możliwość realizacji moich badań w tak sprzyjających warunkach oraz za wszelką okazaną mi pomoc i serdeczność.

Dziękuję ***mojej Mamie i osobom najbliższym***, za niesłabnącą wiarę w moje powodzenie, wspieranie mnie oraz wyrozumiałość. Jestem wdzięczny za każdą rozmowę, każde pokrzepiające słowo i nieustające wsparcie, które pozwalało mi dążyć do celu z determinacją i spokojem.

Wykaz publikacji stanowiących rozprawę doktorską

Niniejsza rozprawa doktorska powstała na podstawie cyklu 3 opublikowanych, tematycznie powiązanych artykułów naukowych:

1. **Szewczyk, K**., Chojnacka, A., i Górnicka, M. (2021). *Tocopherols and Tocotrienols—Bioactive Dietary Compounds; What Is Certain, What Is Doubt?* International Journal of Molecular Sciences, 22, 1–17.

   Impact Factor = 6,2 MNiSW= 140 pkt.

   *Cytowania = 155

2. **Szewczyk, K**., i Górnicka, M. (2023). *Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults*. Nutrients, 15, 1–18.

   Impact Factor = 4,8 MNiSW= 140 pkt.

   *Cytowania = 4

3. **Szewczyk, K**., Bryś, J., Brzezińska, R., i Górnicka, M. (2025*). Nutritional Status of Vitamin E and Its Association with Metabolic Health in Adults.* Nutrients, 17(3), 408.

   Impact Factor = 4,8 MNiSW= 140 pkt.

   *Cytowania = 0

Sumaryczny Impact Factor = 15,8 **MNiSW = 420 pkt.**

*Cytowania wg bazy Scopus

Spis treści

Wykaz stosowanych skrótów

AI	(ang. Adequate Intake) – spożycie wystarczające
BIA	(ang. Bio–electrical Impedance Analysis) – analiza impedancji bioelektrycznej
BMI	(ang. Body Mass Index) – indeks masy ciała
BW	(ang. Body Weight) – masa ciała
CAWI	(ang. Computer-Assisted Web Interview) – wywiad internetowy wspomagany komputerowo
CI	(ang. Confidence Interval) – przedział ufności
CRP	(ang. C-reactive protein) – białko C-reaktywne
D5D	(ang. Δ5-desaturase) – Δ5-desaturaza
D6D	(ang. Δ6-desaturase) – Δ6-desaturaza
DGLA	(ang. dihomo-γ-linolenic acid) – kwas dihomo-γ-linolenowy
EFSA	(ang. European Food Safety Authority) – Europejski Urząd ds. Bezpieczeństwa Żywności
FFQ	(ang. Food Frequency Questionaire), kwestionariusz częstotliwości spożycia
FM	(ang. Fat Mass) – masa tkanki tłuszczowej
GC-FID	(ang. Gas Chromatography with Flame Ionization Detector) – chromatografia gazowa z detektorem płomieniowo jonizacyjnym
H	(ang. Height) – wysokość ciała
HC	(ang. Hip Circumference) – obwód bioder
HDL	(ang. High Density Lipoprotein) – lipoproteina o wysokiej gęstości
HPLC-DAD	(ang. High Performance Liquid Chromatography with diode-array detection) – wysokosprawna chromatografia cieczowa z detektorem diodowym
ISAK	(ang. The International Society for the Advancement of Kinanthropometry) – Międzynarodowe Towarzystwo Rozwoju Kinanthropometrii
LDL	(ang. Low Density Lipoprotein) – lipoproteiny o niskiej gęstości
LOA	(ang. Limits of agreements) – zakres zgodności
MUFA	(ang. Monounsaturated Fatty Acids) – jednonienasycone kwasy tłuszczowe
n-3	(ang. omega−3 fatty acids) – kwasy tłuszczowe omega 3
n-6	(ang. omega−6 fatty acids) – kwasy tłuszczowe omega 6
NF-κB	(ang. nuclear factor kappa-light-chain-enhancer of activated B cells) – jądrowy czynnik transkrypcyjny
NIZP-PZH-PIB	(ang. National Institute of Human Health - National Institute of Hygiene – National Institute of Research) - Narodowy Instytut Zdrowia Publicznego – Państwowy Zakład Higieny – Państwowy Instytut Badawczy
PUFA	(ang. Polyunsaturated Fatty Acids) – wielonienasycone kwasy tłuszczowe
SCD16	(ang. stearoyl-CoA desaturase-16) – stearoyl-CoA desaturaza-16
SCD18	(ang stearoyl-CoA desaturase-18) – stearoyl-CoA desaturaza-18
SD	(ang. standard deviation) – odchylenie standardowe
SFA	(ang. Saturated Fatty Acids) – nasycone kwasy tłuszczowe
T	(ang. Tocopherol) – tokoferol
T3	(ang. Tocotrienol) – tokotrienol
T3s	(ang. Tocotrienols) – tokotrienole
TC	(ang. Total Cholesterol) – cholesterol całkowity
TG	(ang. Triglycerides) – triacyloglicerole
Ts	(ang. Tocopherols) – tokoferole
UL	(ang. Upper Level Intake) – górny tolerowany poziom spożycia
USDA	(ang. U.S. Department of Agriculture) – Departament Rolnictwa Stanów Zjednoczonych
VAT	(ang. Visceral Adipose Tissue) – trzewna tkanka tłuszczowa

VitE	(ang. Vitamin E) – witamina E
WC	(ang. Waist Circumference) – obwód talii
WHO	(ang. World Health Organization) – Światowa Organizacja Zdrowia
WHR	(ang. Waist-Hip Ratio) – wskaźnik talia – biodro
WHtR	(ang. Waist-to-Height Ratio) – wskaźnik talia – wysokość ciała
α-TTP	(ang. α-tocopherol transfer protein) – białko przenoszące α-tokoferol

Streszczenie w języku polskim

Otyłość, definiowana jako nadmierne gromadzenie tkanki tłuszczowej, jest globalnym problemem zdrowotnym, prowadzącym do licznych zaburzeń metabolicznych, takich jak insulinooporność, cukrzyca typu 2, nadciśnienie tętnicze czy choroby układu krążenia. Szczególnie niebezpieczna jest trzewna tkanka tłuszczowa (VAT), powiązana z przewlekłym stanem zapalnym o niskim nasileniu, który sprzyja dysfunkcji metabolicznej i chorobom sercowo-naczyniowym. Podwyższone stężenia wolnych kwasów tłuszczowych w osoczu dodatkowo nasilają stres oksydacyjny i zaburzenia wrażliwości na insulinę. Istotną rolę w łagodzeniu tych procesów odgrywają przeciwutleniacze, które poprzez redukcję stresu oksydacyjnego i obniżanie stanów zapalnych mogą zmniejszać ryzyko powikłań zdrowotnych związanych z otyłością, co podkreśla znaczenie diety bogatej w polifenole, karotenoidy oraz witaminy A i E.

Witamina E, obejmująca cztery tokoferole (α-, β-, γ-, δ-) i cztery tokotrienole (α-, β-, γ-, δ-) to naturalny składnik żywności, występujący głównie w olejach roślinnych, niektórych nasionach roślin oleistych i orzechach. Głównymi formami występującymi w diecie człowieka są α- i γ-tokoferole, ze względu na ich najwyższą zawartość w dostępnych produktach spożywczych. Niemniej jednak α-tokoferol jest uznany za najbardziej aktywną biologicznie formę witaminy E, która występuje w osoczu i innych tkankach organizmu w najwyższym stężeniu. Ponadto formy α- zarówno tokoferoli, jak i tokotrienoli są uważane za najbardziej aktywne metabolicznie. Niniejsza praca koncentruje się na analizie bioaktywnej roli poszczególnych izoform witaminy E występujących w żywności oraz zbadaniu i opisaniu powiązań pomiędzy stężeniem wybranych tokoferoli i tokotrienoli w osoczu a zdrowiem metabolicznym osób dorosłych z prawidłową i nadmierną zawartością tkanki tłuszczowej. Jako kryteria zdrowia metabolicznego przyjęto zawartość tkanki tłuszczowej, profil lipidów ustrojowych, takich jak cholesterol i jego frakcje, profil wolnych kwasów tłuszczowych we krwi, aktywność wybranych enzymów związanych z metabolizmem lipidów oraz stężenie białka C-reaktywnego (CRP) jako markera stanu zapalnego.

Celem głównym badania, stanowiącego podstawę empiryczną niniejszej pracy, była ocena związku między tkanką tłuszczową a stężeniem lipidów ustrojowych takich

jak cholesterol i jego frakcje, profilem kwasów tłuszczowych, a także stężeniem związków lipofilnych (α- i γ-tokochromanoli) w osoczu krwi osób dorosłych.

Badanie, składające się z badania obserwacyjnego i ankietowego, zostało zrealizowane w latach 2020-2024. W pierwszym badaniu dokonano oceny składu ciała, zawartości związków lipidowych tj. cholesterolu i jego frakcji, wolnych kwasów tłuszczowych oraz stężenia białka CRP i witaminy E w grupie 127 osób dorosłych. Drugie badanie obejmowało ocenę spożycia poszczególnych form witaminy E w diecie na podstawie opracowanego półilościowego kwestionariusza częstotliwości spożycia produktów będących źródłem witaminy E oraz kalkulatora do oceny ich zawartości.

W pierwszym badaniu wykazano, że wyższa zawartość tkanki tłuszczowej była główną determinantą niższego stężenia α- i γ-tokochromanoli w osoczu. Ponadto niższe stężenie cholesterolu całkowitego i jego frakcji determinowało niższe stężenie witaminy E, przy czym najsilniejsze zależności stwierdzono dla frakcji lipoprotein o wysokiej gęstości (HDL). Niedostateczny stan odżywienia witaminą E, mierzony stężeniem α-tokoferolu w osoczu, stwierdzono u 27% osób, przy czym u 30% z nadmierną, a jedynie u 12% osób z prawidłową zawartością tkanki tłuszczowej. Tokoferole stanowiły 70,8% sumy α- i γ-tokochromanoli, podczas gdy tokotrienole stanowiły 29,2%.

Nadmierny, w stosunku do wartości rekomendowanych, poziom tkanki tłuszczowej był też związany z wyższymi wartościami białka CRP. Z kolei α- i γ- tokochromanole wykazały istotnie ujemne korelacje z CRP i potwierdziły potencjalną przeciwzapalną rolę witaminy E.

Osoby z otyłością wykazywały zwiększoną aktywność Δ6-desaturazy (D6D) oraz stearoyl-CoA desaturaz (SCD16 i SCD18). Wzrost aktywności D6D może nasilać przewlekły stan zapalny, a podwyższona aktywność SCD wpływa na metabolizm lipidów i potencjalnie modyfikuje biodostępność witaminy E. Ujemna korelacja między izoformami witaminy E a aktywnością SCD nie została jednak potwierdzona w analizie wieloczynnikowej, co wymaga dalszych badań.

Analiza otrzymanych wyników wykazała, że stężenie α- i γ-tokochromanoli u osób dorosłych z nadmierną zawartością tkanki tłuszczowej może być niewystarczające w stosunku do proponowanych wartości referencyjnych i powiązane z gorszym zdrowiem metabolicznym. Biorąc pod uwagę, że ryzyko chorób metabolicznych wzrasta wraz z wiekiem i otyłością, ale wiąże się również z uwarunkowaną biologicznie zmianą składu ciała w kierunku zwiększenia zawartości

tkanki tłuszczowej, wartości stężenia α-tokoferolu we krwi na poziomie określanym jako prozdrowotny (>30 μmol/l) mogłyby być korzystne dla zdrowia.

W drugim badaniu wykazano, że spożycie z dietą tokochromanoli wyniosło średnio 11,3 mg równoważnika α-tokoferolu/osobę/dzień. Uwzględniając wartość normy wystarczającego spożycia (AI) wg norm żywienia dla populacji polskiej, stwierdzono, że jedynie u 57% osób badanych spożycie witaminy E z dietą było na poziomie wystarczającego spożycia. Dominującymi formami tokoferoli w diecie były α- i γ-, a głównymi źródłami α-tokoferolu były migdały i nasiona słonecznika, podczas gdy chipsy, krakersy, nachosy i olej rzepakowy były głównymi źródłami γ-tokoferolu. Wśród tokotrienoli dominowała forma β-. Ich głównymi źródłami w diecie były chleb pełnoziarnisty, makaron pełnoziarnisty, brązowy ryż i płatki kukurydziane. Tokoferole stanowiły 94,3% całkowitej ilości spożywanej witaminy E, podczas gdy tokotrienole tylko 5,7%.

W kontekście uzyskanych wyników, stwierdzono, że nadmierna zawartość tkanki tłuszczowej może być związana z niższym stężeniem związków lipofilnych, w tym witaminy E we krwi, co może osłabiać ochronę antyoksydacyjną organizmu. Zwiększone spożycie tej witaminy, zwłaszcza u mężczyzn i osób po 40 roku życia może stanowić kluczowy element profilaktyki zdrowia i dietoterapii chorób kardiometabolicznych przy koniecznej optymalizacji struktury diety pod kątem doboru źródeł poszczególnych form witaminy E.

Streszczenie w języku angielskim

Title: Adipose tissue and the content of selected lipid and lipophilic compounds in the blood plasma of adults

Obesity, defined as excessive accumulation of adipose tissue, is a global health issue leading to numerous metabolic disorders, such as insulin resistance, type 2 diabetes, hypertension, and cardiovascular diseases. Visceral adipose tissue (VAT) is particularly hazardous, as it is associated with a chronic low-grade inflammatory state that promotes metabolic dysfunction and cardiovascular diseases. Elevated plasma concentrations of free fatty acids further exacerbate oxidative stress and impair insulin sensitivity. Antioxidants play a crucial role in mitigating these processes by reducing oxidative stress and lowering inflammation, which may decrease the risk of obesity-related health complications. This highlights the importance of a diet rich in polyphenols, carotenoids, and vitamins A and E.

Vitamin E comprises four tocopherols (α-, β-, γ-, δ-) and four tocotrienols (α-, β-, γ-, δ-), which are naturally present in foods, particularly in vegetable oils, certain oilseeds, and nuts. The primary forms of vitamin E found in the human diet are α- and γ-tocopherols due to their high content in available food products. However, α-tocopherol is recognized as the most biologically active form of vitamin E, exhibiting the highest concentrations in plasma and other tissues. Moreover, the α-forms of both tocopherols and tocotrienols are considered the most metabolically active. This study focuses on analyzing the bioactive role of different isoforms of vitamin E found in food and investigating the associations between plasma concentrations of selected tocopherols and tocotrienols and the metabolic health of adults with normal and excessive adipose tissue content. The criteria for metabolic health assessment included adipose tissue content, lipid profile (cholesterol and its fractions), plasma free fatty acid profile, the activity of selected lipid metabolism-related enzymes, and C-reactive protein (CRP) concentration as an inflammatory marker.

The primary aim of the study, forming the empirical basis of this work, was to assess the relationship between adipose tissue content and the concentration of body lipids such as cholesterol and its fractions, free fatty acid profiles, and the plasma levels of lipophilic compounds (α- and γ-tocochromanols) in adults.

The study, consisting of an observational study and a survey, was conducted in 2020-2024. In the first study, body composition, lipid compounds (cholesterol and its

fractions, free fatty acids), CRP levels, and vitamin E concentrations were assessed in a group of 127 adults. The second study involved developing a semi-quantitative food frequency questionnaire (FFQ) for vitamin E-rich products and a dietary assessment calculator to estimate the intake of different vitamin E isoforms, followed by an evaluation of their consumption in a group of 447 adults.

The first study revealed that higher adipose tissue content was the primary determinant of lower plasma α- and γ-tocochromanol concentrations. Furthermore, lower total cholesterol and its fractions were associated with reduced vitamin E levels, with the strongest correlations observed for high-density lipoprotein (HDL) cholesterol. Inadequate vitamin E status, measured by plasma α-tocopherol concentration, was observed in 27% of participants, including 30% of individuals with excessive adipose tissue and only 12% of those with normal adipose tissue content. Tocopherols accounted for 70.8% of the total α- and γ-tocochromanols, while tocotrienols constituted 29.2%.

Excess adipose tissue beyond recommended levels was also associated with higher CRP concentrations. In contrast, α- and γ-tocochromanols exhibited significant negative correlations with CRP, supporting the potential anti-inflammatory role of vitamin E. Individuals with obesity demonstrated increased activity of Δ6-desaturase (D6D) and stearoyl-CoA desaturases (SCD16 and SCD18). Elevated D6D activity may exacerbate chronic inflammation, while increased SCD activity affects lipid metabolism and potentially modifies vitamin E bioavailability. However, multivariate analysis did not confirm the negative correlation between vitamin E isoforms and SCD activity, necessitating further research.

Analysis of the obtained results indicated that plasma α- and γ-tocochromanol concentrations in adults with excessive adipose tissue might be insufficient relative to proposed reference values and associated with poorer metabolic health. Given that the risk of metabolic diseases increases with age and obesity and is also influenced by biologically driven changes in body composition favoring increased fat mass, maintaining plasma α-tocopherol concentrations at a health-promoting level (>30 μmol/L) could be beneficial for overall health.

In the second study, dietary tocopherol and tocotrienol intake average 11.3 mg α-tocopherol equivalents per person per day. Considering the adequate intake (AI) value established in the Polish dietary guidelines, only 57% of participants met the recommended intake level for vitamin E. The dominant dietary tocopherol forms were

α- and γ-tocopherols, with almonds and sunflower seeds being the primary sources of α-tocopherol, whereas chips, crackers, nachos, and rapeseed oil were the main sources of γ-tocopherol. Among tocotrienols, the β-form was predominant, with whole-grain bread, whole-grain pasta, brown rice, and cornflakes serving as the principal dietary sources. Tocopherols accounted for 94.3% of total vitamin E intake, while tocotrienols constituted only 5.7%.

In light of the findings, excessive adipose tissue content may be associated with lower plasma levels of lipophilic compounds, including vitamin E, potentially weakening the body's antioxidant defense. Increased vitamin E intake may support metabolic health, particularly among individuals with excessive adipose tissue. Therefore, selecting appropriate dietary sources of vitamin E, which is predominantly in plant oils.

1. Wstęp

Otyłość, definiowana jako nadmierne gromadzenie tkanki tłuszczowej, stała się jednym z najpoważniejszych globalnych problemów zdrowotnych XXI wieku. Zgodnie z danymi Światowej Organizacji Zdrowia (WHO), od 1975 roku wskaźnik występowania otyłości na świecie wzrósł trzykrotnie (WHO, 2024). W 2022 roku 2,5 miliarda dorosłych osób miało nadwagę, a w tej grupie 890 milionów osób cierpiało na otyłość. Problem ten dotyka w coraz większym stopniu zarówno kraje rozwinięte, jak i rozwijające się. W Polsce w 1996 roku odsetek osób z nadmierną masą ciała wynosił 28%, natomiast w 2022 roku wzrósł do 58% (Eurostat, 2024). Jeśli aktualne trendy utrzymają się, Lobstein i wsp. (2023) prognozuje, że w roku 2035 ponad 35% dorosłych mężczyzn i ponad 25% dorosłych kobiet w Polsce będzie otyłych, a na świecie będzie już 4 mld osób z otyłością lub z nadwagą (ponad 50% populacji), w tym 1,9 mld osób z otyłością (Pawlewicz, 2024). Otyłość znacząco zwiększa ryzyko występowania wielu chorób przewlekłych, w tym sercowo-naczyniowych, cukrzycy typu 2, nowotworów, miażdżycy, insulinooporności, dyslipidemii i nadciśnienia. Stanowi także obciążenie dla zdrowia psychicznego, prowadząc do depresji, lęku oraz pogorszenia jakości życia. Problem otyłości wiąże się również z obciążeniem ekonomicznym, wpływając na spadek produktywności oraz trudności społeczne osób z otyłością (Schrover i wsp., 2016).

Kluczowym elementem w zrozumieniu problemu otyłości jest rola tkanki tłuszczowej. Jest ona najważniejszym rezerwuarem energii w organizmie. Spełnia liczne funkcje — magazynuje kwasy tłuszczowe, które mogą być zużyte na cele energetyczne, bierze udział w regulacji przemian energetycznych organizmu, jest niezbędna dla prawidłowej homeostazy glukozy i regulacji wrażliwości na insulinę, ma również wpływ na regulację sytości (Avelino i wsp., 2024; Liu i wsp., 2024). W stanie resorpcyjnym komórki tkanki tłuszczowej — adipocyty, pobierają z krwi duże ilości glukozy i kwasów tłuszczowych, a następnie z tych substratów syntetyzują triacyloglicerole. Gdy spada podaż substratów energetycznych, np. w okresie głodu, zasoby zmagazynowane w tkance tłuszczowej są uwalniane do krwi w postaci kwasów tłuszczowych, które następnie mogą być wykorzystywane przez inne tkanki (Wen i wsp., 2022). Ponadto tkanka tłuszczowa magazynuje związki, jak np. witaminy rozpuszczalne w tłuszczach i karotenoidy (Kohlmeier i Kohlmeier, 1995) oraz pełni rolę narządu endokrynnego. Adipocyty, główne komórki tkanki tłuszczowej, wytwarzają ponad 50 hormonów, cytokin, chemokin oraz cząsteczek sygnałowych, zwanych łącznie

adipokinami. Do najważniejszych adipokin należą leptyna, adiponektyna, wiskatyna, apelina, waspina, hepcydyna, chemeryna oraz omentyna, które są wydzielane z tkanki tłuszczowej i przekazują informacje do innych metabolicznie aktywnych narządów i modulują szereg szlaków metabolicznych (Luo i Liu, 2016). Budowa i aktywność tkanki tłuszczowej wpływają na procesy metaboliczne, co czyni ją centralnym punktem w badaniach nad otyłością i jej skutkami zdrowotnymi. W odpowiedzi na zmiany bilansu energetycznego tkanka tłuszczowa jest dynamicznie przebudowywana, co wpływa na liczbę i wielkość adipocytów oraz funkcjonowanie adipokin, oddziałując na funkcjonowanie narządów oraz całego organizmu. Dlatego dysfunkcja tkanki tłuszczowej odgrywa ważną rolę w rozwoju otyłości i związanych z nią zaburzeń (Luo i Liu, 2016; Murawska-Ciałowicz, 2017).

Rozmieszczenie tkanki tłuszczowej w organizmie i jej aktywność mają szeroki wpływ na profil lipidowy oraz występowanie ogólnoustrojowego stanu zapalnego o niskim nasileniu, prowadzącego do kaskady reakcji, zwiększając ryzyko występowania chorób (Hill i wsp., 2018). Nadmierna zawartość tkanki tłuszczowej jest ściśle związana z podwyższonym poziomem kwasów tłuszczowych w osoczu, które odgrywają znaczącą rolę w rozwoju zaburzeń metabolicznych. Podwyższony poziom kwasów tłuszczowych jest ściśle związany ze zmienioną wrażliwością na insulinę, przyczyniając się do insulinooporności tkanek obwodowych: mięśni, wątroby i komórek śródbłonka, zwiększając w ten sposób ryzyko cukrzycy typu 2, nadciśnienia, dyslipidemii i bezalkoholowej stłuszczeniowej choroby wątroby (Chait i den Hartigh, 2020 ; Kojta i wsp., 2020 ; Maurer i Clayton, 2022 ; Morais i wsp., 2022; Elkanawati i wsp., 2024). U osób otyłych wskaźnik rotacji kwasów tłuszczowych jest wyższy, co wskazuje na ich zwiększoną aktywność metaboliczną (Badoud i wsp., 2017). Podwyższone stężenie kwasów tłuszczowych prowadzi również do dysfunkcji śródbłonka, zwiększając ryzyko miażdżycy i chorób sercowo-naczyniowych (Yamagata, 2023).

Poza kwasami tłuszczowymi w osoczu krwi, kluczową rolę w modulacji ryzyka chorób kardiometabolicznych, odgrywają proporcje lipidów ustrojowych takich jak cholesterol całkowity, lipoproteiny o dużej i małej gęstości (HDL i LDL), triacyloglicerole (TG) oraz lipofilne przeciwutleniacze, w tym witamina E, które odgrywa potencjalną rolę terapeutyczną w redukcji stresy oksydacyjnego i stanu zapalnego (Sołtysik i wsp., 2022; Florkowski i wsp., 2024).

Witamina E (tokochromanole) to rozpuszczalne w lipidach przeciwutleniacze syntetyzowane przez rośliny i mikroorganizmy fotosyntetyczne. Obejmuje 8 izoform: α-, β-, γ- i δ-tokoferole oraz α-, β-, γ- i δ-tokotrienole, spośród których α-tokoferol jest najbardziej aktywny biologicznie. Tokoferole i tokotrienole różnią się strukturą łańcucha bocznego i liczbą podstawnika metylowego. Naturalny α-tokoferol (RRR-α-tokoferol) jest najlepiej przyswajalny przez organizm ludzki dzięki wysokiemu powinowactwu do białka transportującego α-tokoferol (α-TTP). Ma on najwyższą aktywność biologiczną spośród pozostałych form tokochromanoli. Tylko forma α-tokoferol jest uznawana za składnik odżywczy niezbędny dla człowieka. Natomiast dla pozostałych izoform witaminy E wskazywane są podobne funkcje przeciwutleniające czy przeciwzapalne (Birringer i wsp., 2018).

Badania wskazują, że różne formy witaminy E, takie jak α-tokoferol i γ-tokoferol, mogą zmniejszać stres oksydacyjny i stan zapalny poprzez zmniejszanie ekspresji cytokin prozapalnych oraz ochronę lipidów ustrojowych przed peroksydacją (Asbaghi i wsp., 2020). Tokotrienole, zwłaszcza γ-tokotrienol wykazują właściwości przeciwzapalne poprzez modulowanie aktywności jądrowego czynnika transkrypcyjnego NF-κB i redukcję markerów zapalnych (Pang i Chin, 2019). Formy α- i γ- tokotrienoli hamują infiltrację makrofagów zmniejszając stan zapalny. Ponadto, modulują uwalnianie cytokin zapalnych i szlaków sygnałowych, poprawiając zdrowie metaboliczne i potencjalnie zmniejszając ryzyko powikłań związanych z otyłością (Zhao i wsp., 2016). Badania sugerują, że osoby z nadmierną zawartością tkanki tłuszczowej mogą mieć niższe stężenie związków lipofilnych o działaniu przeciwutleniającym w osoczu krwi jak np. α-tokoferol (Mah i wsp., 2015; Traber i wsp., 2017). Z drugiej strony, tokoferole i tokotrienole mogą zapobiegać uszkodzeniom spowodowanym przez stan zapalny i reaktywne formy tlenu, zmniejszając w ten sposób negatywne skutki otyłości. Jednocześnie nadmierna zawartość tkanki tłuszczowej może generować w organizmie zwiększone zapotrzebowanie na przeciwutleniacze, co może powodować ich większe wykorzystanie, prowadząc do ich zmniejszonego stężenia we krwi (Koprivica i Miljković, 2024).

Podsumowując, niniejsze badania zostały przeprowadzone w celu opisania powiązań między zawartością tkanki tłuszczowej w organizmie a profilem wybranych tokoferoli i tokotrienoli, lipidów osocza takich jak cholesterol, jego frakcje, wolne

kwasy tłuszczowe oraz występującym ogólnoustrojowym stanem zapalnym o niskim nasileniu u osób z nadmierną masą ciała.

Wykazanie związku między stężeniem wybranych izoform witaminy E a zdrowiem metabolicznym (rozumianym jako prawidłowa zawartość tkanki tłuszczowej, prawidłowy profil lipidowy, wyższa aktywność enzymów zaangażowanych w metabolizm lipidów - D5D i niższa D6D, SCD16 i SCD18 – oraz niski poziom stanu zapalnego, mierzony białkiem CRP) może mieć istotne znaczenie dla opracowania skuteczniejszych strategii dietetycznych oraz interwencyjnych w celu ograniczenia skutków nadmiernej masy ciała i poprawy ogólnego stanu zdrowia populacji. Wyniki przeprowadzonych badań dostarczają również cennych informacji na temat roli poszczególnych form witaminy E w modulacji procesów zapalnych i metabolicznych, co stanowi istotny krok w kierunku spersonalizowanego podejścia do terapii choroby otyłościowej. Ważny aspekt aplikacyjny pracy stanowi opracowanie narzędzi do dokładniejszej analizy spożycia tokoferoli i tokotrienoli, co może przyczynić się do lepszego zrozumienia zależności między dietą a poziomem witaminy E w organizmie.

2. Cel i zakres pracy oraz hipotezy badawcze

Cel główny:

Ocena związku między nadmierną zawartością tkanki tłuszczowej a stężeniem lipidów ustrojowych takich jak cholesterol i jego frakcje, profil kwasów tłuszczowych, oszacowana aktywność enzymów związanych z metabolizmem lipidów, a także stężeniem związków lipofilnych (α- i γ-tokochromanoli) w osoczu krwi osób dorosłych.

Cele szczegółowe:

(1) analiza związku między zawartością tkanki tłuszczowej a stężeniem α- i γ-tokochromanoli we krwi;

(2) ocena stanu odżywienia witaminą E u osób dorosłych z prawidłową i nadmierną zawartością tkanki tłuszczowej;

(3) ocena powiązań zdrowia metabolicznego ze stanem odżywienia witaminą E u osób dorosłych z nadmierną zawartością tkanki tłuszczowej;

(4) ocena spożycia tokoferoli i tokotrienoli oraz ich źródeł w grupie osób dorosłych.

Zakres pracy

Działania badawcze i uzyskane wyniki podzielono na dwie części, przypisując im odpowiadające publikacje (Tabela 1).

Tabela 1. Badania zrealizowane w ramach pracy doktorskiej.

BADANIE 1. – analiza piśmiennictwa na temat biologicznego działania witaminy E na zdrowie człowieka; ocena składu ciała, zawartości związków lipidowych tj. cholesterolu i jego frakcji, wolnych kwasów tłuszczowych, białka CRP i stanu odżywienia witaminą E.	
Publikacje 1. i 2.	*1. Tocopherols and Tocotrienols—Bioactive Dietary Compounds; What Is Certain, What Is Doubt?* *2.Nutritional Status of Vitamin E and Its Association with Metabolic Health in Adults.*
BADANIE 2. – charakterystyka źródeł pokarmowych witaminy E, opracowanie kwestionariusza i kalkulatora do oceny spożycia z dietą tokoferoli i tokotrienoli u osób dorosłych; ocena spożycia tych związków z dietą wśród osób dorosłych.	
Publikacja 3.	*3. Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults.*

Zakres pracy obejmował dwa prowadzone równolegle badania:

BADANIE I – celem była ocena składu ciała, zawartości związków lipidowych tj. cholesterolu i jego frakcji, wolnych kwasów tłuszczowych, białka CRP i witaminy E w grupie osób dorosłych i obejmowało następujące etapy:

1. Uzyskanie zgody Komisji Etyki Badań Naukowych z Udziałem Ludzi przy Wydziale Nauk o Żywieniu Człowieka i Konsumpcji SGGW w Warszawie (Uchwała nr 05p/2019);

2. Rekrutacja osób do udziału w badaniu, pozyskanie zgód na włączenie do badania oraz kwalifikacja na podstawie kryteriów włączenia i wyłączenia;

3. Przeprowadzenie pomiarów antropometrycznych (masa ciała, wysokość ciała, obwód talii, obwód bioder) oraz analizy składu ciała z wykorzystaniem metody bioimpedancji elektrycznej;

4. Zebranie materiału biologicznego do dalszych analiz;

5. Przeprowadzenie analiz biochemicznych krwi, obejmujących:

- Oznaczanie stężenia wybranych tokoferoli i tokotrienoli z wykorzystaniem metody HPLC-DAD;
- Oznaczanie profilu kwasów tłuszczowych z wykorzystaniem metody GC-FID; oraz oszacowanie aktywności enzymów zaangażowanych w metabolizm lipidów;
- Oznaczanie zawartości cholesterolu i jego frakcji oraz stężenia białka CRP.

6. Analizę statystyczną uzyskanych wyników w celu określenia zależności pomiędzy zawartością tkanki tłuszczowej, profilu lipidowego, profilu wolnych kwasów tłuszczowych a stężeniem badanych izoform witaminy E.

BADANIE II – celem była ocena spożycia witaminy E i jej izoform w grupie osób dorosłych i obejmowało następujące etapy:

1. Zebranie danych o zawartości poszczególnych izoform witaminy E w żywności;
2. Opracowanie bazy danych produktów i kalkulatora Vit_E.CAL do obliczania zawartości poszczególnych form witaminy E w spożywanej żywności w programie MS Excel;
3. Opracowanie kwestionariusza częstotliwości spożycia produktów będących głównym źródłem witaminy E w polskiej diecie (VitE-FFQ) kompatybilnego z powstałym kalkulatorem Vit_E.CAL.;
4. Przeprowadzenie badania pilotażowego;
5. Zebranie danych o spożyciu z wykorzystaniem VitE-FFQ oraz 1-dniowego zapisu diety w grupie 447 osób dorosłych;

6. Oszacowanie spożycia witaminy E i jej poszczególnych form przy użyciu Vit_E.CAL.;
7. Ocenę adekwatności spożycia witaminy E w grupie badanej w odniesieniu do poziomu normy wystarczającego spożycia (AI);
8. Analizę statystyczną uzyskanych wyników w celu określenia różnic w adekwatności spożycia witaminy E w zależności od płci, wieku i BMI oraz porównania zgodności danych o jej spożyciu zebranych za pomocą dwóch metod.

Hipotezy badawcze

Przyjęto **hipotezę główną** w brzmieniu: zawartość tkanki tłuszczowej może wpływać na stężenie związków lipidowych i lipofilnych we krwi, a tym samym na stan zapalny w organizmie. Status ten może być uwarunkowany składem diety i związany z czynnikami socjodemograficznymi. Poza tym sformułowano następujące hipotezy badawcze:

H1: Nadmierna zawartość tkanki tłuszczowej i otyłość centralna są powiązane z niższymi stężeniami α-tokoferolu, γ-tokoferolu, α-tokotrienolu i γ-tokotrienolu oraz nieprawidłowym profilem związków lipidowych w osoczu krwi, a w konsekwencji z gorszym zdrowiem metabolicznym;

H2: Nadmierna zawartość tkanki tłuszczowej jest związany z wyższym stanem zapalnym, mierzonym stężeniem białka CRP, a wyższe stężenie izoform witaminy E w osoczu związane jest z jego obniżeniem;

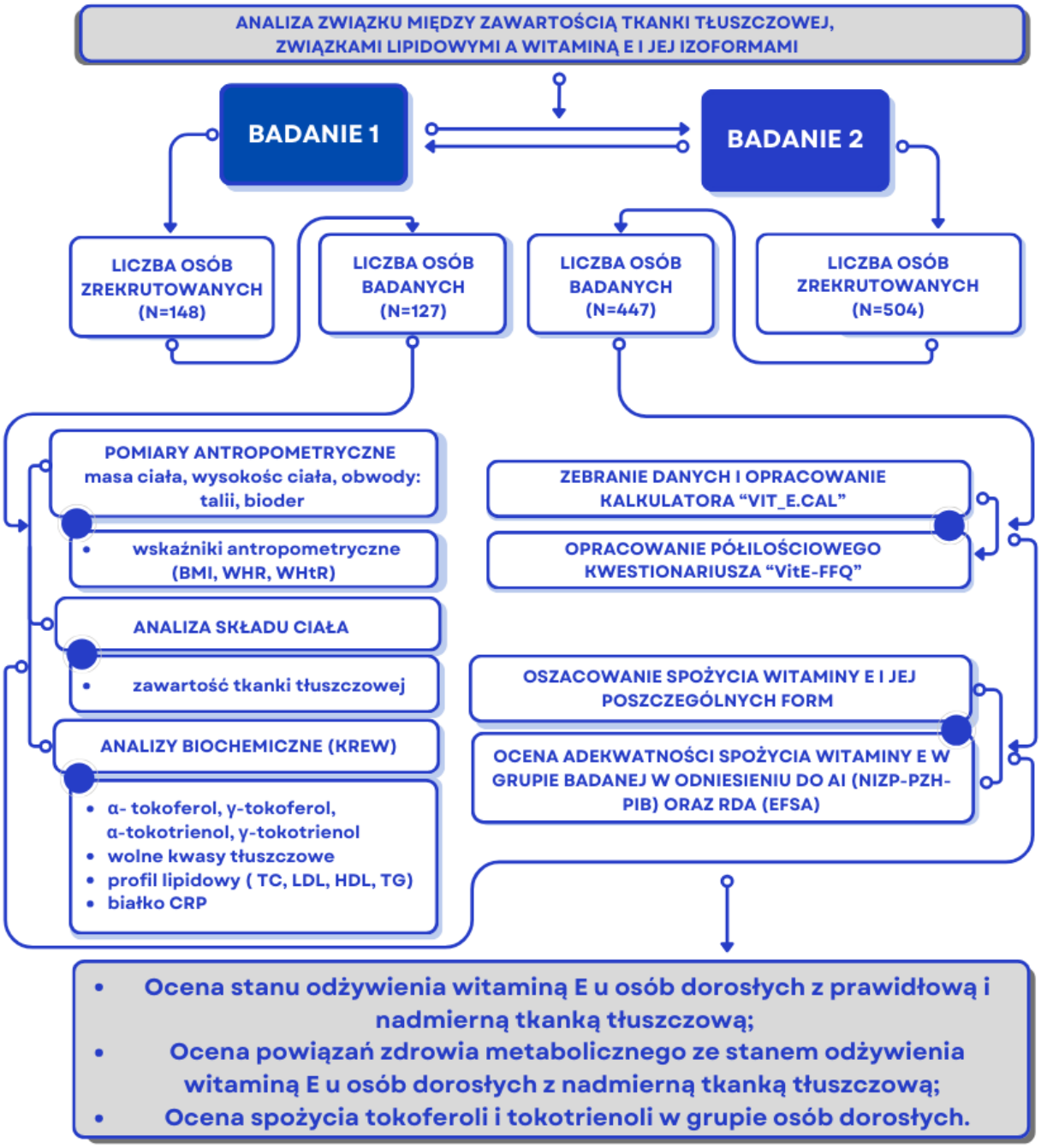
H3: Niedostateczne spożycie witaminy E częściej występuje u mężczyzn, osób powyżej 40 roku życia oraz osób z nadmierną masą ciała.

3. Materiał i metody badawcze

W poniższym rozdziale opisano ogólny zakres przeprowadzonych badań, stanowiących empiryczną podstawę dysertacji. Bardziej szczegółowy opis badań znajduje się w dołączonych do rozprawy artykułach naukowych, stanowiących powiązany tematycznie cykl poświęcony określeniu związku między tkanką tłuszczową a stężeniem lipidów ustrojowych takich jak cholesterol i jego frakcje, profilem kwasów tłuszczowych, a także stężeniem związków lipofilnych (α- i γ-tokochromanoli)

w osoczu krwi osób dorosłych (Szewczyk i wsp., 2021; Szewczyk i Górnicka, 2023; Szewczyk i wsp., 2025).

Badanie, które stanowi podstawę niniejszej rozprawy zostało przeprowadzone w latach 2020-2024 po uzyskaniu zgody Komisji Etyki Badań Naukowych z Udziałem Ludzi przy Instytucie Nauk o Żywieniu Człowieka SGGW (nr uchwały 05p/2019). Przebieg badania przedstawia Rycina 1.



Rycina 1. Schemat badań.

3.1. Badanie 1

Grupa badana

Rekrutację uczestników badania przeprowadzono w okresie październik 2021 – październik 2022. Chęć udziału w badaniu zgłosiło 148 osób. Po zastosowaniu kryteriów włączenia i wyłączenia do udziału w badaniu włączono 127 osób zgłaszających chęć udziału w badaniu (szczegółową charakterystykę przedstawiono w Artykule 2).

Pomiary antropometryczne

Parametry antropometryczne ,takie jak wysokość ciała (H), masa ciała (BW), obwód talii (WC) i obwód bioder (HC) mierzono przy użyciu profesjonalnego sprzętu z zachowaniem standardowych procedur zgodnie z wytycznymi Międzynarodowego Towarzystwa Rozwoju Kinantropometrii (ISAK) dotyczącymi Międzynarodowych Standardów Oceny Antropometrycznej (Esparza-Ros i wsp., 2019). Masę ciała mierzono przy użyciu elektronicznej wagi cyfrowej z dokładnością do 0,1 kg (SECA 799, Hamburg, Niemcy). Wysokość ciała mierzono za pomocą stadiometru (SECA 220, Hamburg, Niemcy) z dokładnością do 0,1 cm. Obwód talii (WC) mierzono za pomocą taśmy odpornej na rozciąganie, która zapewnia stałe napięcie 100 g (SECA 201, Hamburg, Niemcy) w połowie odległości między grzebieniem biodrowym a brzegiem żebrowym (dolnym żebrem) na przedniej linii pachowej w spoczynkowej pozycji wydechowej. Obwód bioder (HC) mierzono w miejscu największej wypukłości mięśni pośladkowych. Wszystkie pomiary zostały wykonane dwukrotnie w lekkim ubraniu i bez butów, a obliczone wartości średnie zostały wykorzystane do dalszych analiz (Esparza-Ros i wsp., 2019; Górnicka i wsp., 2022).

Na podstawie wyników pomiarów antropometrycznych obliczono wskaźniki antropometryczne, takie jak wskaźnik masy ciała (BMI), wskaźnik talia-biodra (WHR) oraz wskaźnik talia-wzrost (WHtR), przyjmując następujące kryteria:

- Wskaźnik BMI (WHO, 2010), wartości poniżej 18,5 kg/m² klasyfikowano jako niedowagę, zakres od 18,5 do 24,99 kg/m² jako prawidłową masę ciała, wartości między 25,0 a 29,99 kg/m² jako nadwagę, natomiast BMI wynoszące 30,0 kg/m² lub więcej wskazuje na otyłość.
- Wskaźnik WHR wyliczano jako stosunek obwodu talii do obwodu bioder (WHR=WC/HC), przyjmując za wartość progową wskazującą na otyłość

brzuszną (androidalną), wiążącą się ze zwiększonym ryzykiem metabolicznym, przyjęto wartość powyżej 0,85 u kobiet oraz powyżej 0,90 u mężczyzn (WHO, 2011).

- Wskaźnik WHtR, obliczany jako stosunek obwodu talii do wysokości ciała (WHtR=WC/H), uznawany jest za bardziej czuły wskaźnik niż BMI w ocenie otyłości brzusznej i ryzyka kardiometabolicznego. Wartość WHtR wynosząca 0,5 lub więcej jest uznawana za wskazujący na zwiększone ryzyko zdrowotne niezależnie od płci (Nevill i wsp., 2023).
- Zgodnie z kryteriami diagnostycznymi WHO, prawidłowe wartości obwodu talii przyjęto jako <80 cm dla kobiet i <94 cm dla mężczyzn (WHO, 2011).

Skład ciała, w tym zawartość tkanki tłuszczowej, oceniano przy użyciu bioimpedancji elektrycznej z wykorzystaniem wieloczęstotliwościowego ośmiopunktowego analizatora Tanita (Tanita BC-418 MA, Tanita Co., Tokio, Japonia). Pomiary wykonywano w standaryzowanych warunkach zgodnie z protokołem producenta. Zgodnie z procedurą uczestników badania poinformowano o konieczności bycia na czczo lub co najmniej 4 godziny po posiłku, unikanie intensywnej aktywności fizycznej przez co najmniej 12 godzin przed badaniem, niepicie alkoholu przez 24 godziny i kofeiny przez 4 godziny przed badaniem oraz oddanie moczu przed analizą BIA (Holmes i Racette, 2021).

Klasyfikacja osób do grup badanych

Na podstawie zawartości tkanki tłuszczowej, ocenianej metodą bioimpedancji elektrycznej, osoby badane podzielono na dwie grupy, przyjmując jako punkt odcięcia wskazujący na nadmierną jej zawartość, wartości powyżej 30% masy ciała dla kobiet oraz 25% masy ciała dla mężczyzn (Severeyn i wsp., 2020). Osoby z nadmierną zawartością tkanki tłuszczowej stanowiły grupę badaną, natomiast osoby z prawidłową – grupę kontrolną.

Badania biochemiczne

W ocenie poziomu badanych związków materiałem badawczym była krew żylna, pobierana przez wykwalifikowany personel zgodnie ze standardową procedurą w warunkach laboratoryjnych. Krew pobierana była od pacjentów po 12-godzinnym poście, po nocnym odpoczynku, z żyły obwodowej w dole stawu łokciowego. Po pobraniu krwi próbki odwirowano w wirówce obrotowej przez 10 minut

w temperaturze +4 °C, 8000 obr./min. Osocze znad osadu przeniesiono do plastikowych probówek. Wszystkie pobrane próbki osocza chroniono przed światłem i przechowywano zamrożone w temperaturze -80 °C do czasu analiz.

✓ *Oznaczenie profilu lipidowego i białka CRP*

Ocena poziomu stężenia cholesterolu całkowitego, cholesterolu LDL, cholesterolu HDL, triacylogliceroli, białka CRP jako markera stanu zapalnego wykonana została we współpracy z laboratorium zewnętrznym, zgodnie z procedurą obowiązującą w danej placówce. Na podstawie wartości referencyjnych stosowanych w laboratorium przyjęto następujące punkty odcięcia profilu lipidowego, które zinterpretowano jako prawidłowe poziomy:

- Cholesterol całkowity (TC) < 190 mg/dl;
- Lipoproteiny o dużej gęstości (HDL) > 50 mg/dl dla kobiet i 40 mg/dl dla mężczyzn;
- Lipoproteiny o małej gęstości (LDL) < 115 mg/dl;
- Triacyloglicerole (TG) < 150 mg/dl.

✓ *Oznaczenie stężenia wybranych tokoferoli i tokotrienoli*

Do oznaczeń i dalszych analiz wybrano 4 tokochromanole, tj. α-tokoferol, γ-tokoferol, α-tokotrienol oraz γ-tokotrienol. Jak wskazują badania, zwyczajowa dieta dostarcza głównie form α- i γ- witaminy E. Do oznaczenia poszczególnych izoform wykorzystano metodę wysokosprawnej chromatografii cieczowej (HPLC) połączonej z detektorem DAD, wykorzystującą oprogramowanie chromatograficzne ClarityChrom. Z każdej próbki osocza przygotowano dwie próbki analityczne, do opracowania wyników wykorzystano średnią z dwóch pomiarów. Na podstawie stężenia α-tokoferolu w osoczu stan odżywienia oceniono jako (1) niski dla wartości poniżej 12 μmol/l, (2) adekwatny dla zakresu od 12 do 30 μmol/l i (3) prozdrowotny dla wartości powyżej 30 μmol/l (Péter i wsp., 2015).

✓ *Oznaczenie profilu kwasów tłuszczowych*

Próbki do analiz przygotowano w oparciu o zmodyfikowane metody (Sholola i Cooperstone, 2022; Su i wsp., 2002). Identyfikację kwasów tłuszczowych przeprowadzono na podstawie wartości czasu retencji w porównaniu ze standardem (Supelco 37 Component FAME Mix). Wyniki profilu kwasów tłuszczowych wyrażono jako względne procenty każdego kwasu tłuszczowego (obliczono % powierzchni pików

chromatograficznych dla kwasów tłuszczowych). Każda próbka została przeanalizowana dwa razy oddzielnie, a do opracowania wyników wykorzystano średnią z dwóch pomiarów.

Na podstawie uzyskanych wyników oszacowano aktywność enzymów zaangażowanych w metabolizm lipidów. Oceny dokonano na podstawie obliczenia stosunków względnej zawartości produktów do ich odpowiednich prekursorów dla każdego enzymu. Aktywność stearoilo-CoA desaturazy (SCD) określano na podstawie stosunku kwasu palmitoleinowego do palmitynowego (16:1n-7/16:0) dla SCD-16 oraz stosunku kwasu oleinowego do stearynowego (18:1n-9/18:0) dla SCD-18. W analogiczny sposób aktywność Δ6-desaturazy (D6D) wyznaczano na podstawie stosunku kwasu dihomo-γ-linolenowego do kwasu linolowego (20:3n-6/18:2n-6), natomiast aktywność Δ5-desaturazy (D5D) oceniano poprzez stosunek kwasu arachidonowego do kwasu dihomo-γ-linolenowego (20:4n-6/20:3n-6). Dodatkowo stosunek kwasu stearynowego do kwasu palmitynowego (18:0/16:0) wykorzystano do oszacowania aktywności elongazy (Domínguez-López i wsp., 2022).

Analiza statystyczna

Dane przedstawiono jako procenty dla zmiennych kategorycznych oraz jako średnie z odchyleniami standardowymi (SD) dla zmiennych ciągłych. Normalność rozkładu danych oceniono przed analizami statystycznymi za pomocą testu Shapiro–Wilka. Różnice między grupami oceniano przy użyciu testu Chi^2 Pearsona dla zmiennych jakościowych oraz testu Kruskala-Wallisa z analizą post-hoc dla porównań obejmujących więcej niż dwie grupy lub testu U Manna-Whitneya dla porównań dwóch grup w przypadku zmiennych ciągłych. Do zbadania zależności między izoformami witaminy E a czynnikami takimi jak zmienne antropometryczne, aktywności enzymów, profile kwasów tłuszczowych i parametry lipidowe zastosowano korelację rang Spearmana. Istotne korelacje poddano dalszej analizie za pomocą regresji jednowariantowej i wielowariantowej, aby zidentyfikować kluczowe predyktory α-tokoferolu i innych izoform witaminy E. Przeprowadzono również regresję wielokrotną metodą krokową. Modele były adjustowane względem wieku, płci i statusu palenia papierosów. Analizy przeprowadzono na nieprzekształconych zmiennych, stratyfikując je na całą grupę oraz osobno dla kobiet i mężczyzn. Poziom istotności

ustalono na $p \leq 0,05$. Analizy statystyczne wykonano przy użyciu oprogramowania STATISTICA w wersji 13.0 (StatSoft Inc., Tulsa, OK, USA; StatSoft, Kraków, Polska).

3.2. Badanie 2

Grupa badana

W II badaniu wzięło udział 504 uczestników. Finalnie uwzględniono wypełnione kwestionariusze od 447 respondentów; 57 kwestionariuszy odrzucono ze względu na niespełnienie kryteriów i brak danych dotyczących spożycia (szczegółową charakterystykę przedstawiono w Artykule 3).

Kwestionariusz VitE-FFQ i kalkulator Vit_E.CAL do oceny spożycia tokoferoli i tokotrienoli

Na potrzeby niniejszej pracy został opracowany półilościowy kwestionariusz częstotliwości spożycia żywności – VitE-FFQ. Kwestionariusz obejmuje 8 grup produktów spożywczych, takich jak warzywa, owoce i przetwory owocowe, rośliny strączkowe i produkty strączkowe, orzechy i nasiona oleiste, tłuszcze, zboża, ryby i przetwory rybne oraz przekąski i „inne". Kwestionariusz VitE-FFQ składa się z listy 67 produktów spożywczych będących źródłami tych związków i ich typowej wielkości porcji według „Albumu fotografii produktów i potraw" (Szponar i wsp., 2000). Produkty spożywcze wybrano na podstawie zawartości witaminy E oznaczonej jako równoważnik α-tokoferolu według „Tabele składu i wartości odżywczej żywności" (Kunachowicz i wsp., 2020). Z uwagi na fakt, że polskie „Tabele składu i wartości odżywczej żywności" (Kunachowicz i wsp., 2020) podają jedynie całkowitą zawartość witaminy E wyrażoną jako równoważnik α-tokoferolu, do obliczenia poszczególnych tokoferoli i tokotrienoli wykorzystano bazę danych Departamentu Rolnictwa Stanów Zjednoczonych (USDA) (U.S. Department of Agriculture). W opracowanym kwestionariuszu VitE-FFQ poproszono respondentów o wskazanie zwyczajowej liczby porcji wymienionych produktów spożywanych średnio w ciągu typowego tygodnia przez okres 6 miesięcy poprzedzających wypełnienie kwestionariusza. Ponadto w informacji dla badanych wskazano, że respondent powinien przypomnieć sobie wszystkie składniki potraw, takie jak użyte tłuszcze lub dodane orzechy lub nasiona oleiste. Kalkulator do obliczania zawartości tokoferoli i tokotrienoli Vit_E.CAL opracowano w programie MS Excel.

Kalkulator Vit_E.CAL pozwala obliczyć dzienne spożycie poszczególnych izoform witaminy E, jak również ich sumę wyrażoną jako mg równoważnika α-tokoferolu w oparciu o równanie: Wit. E [mg równoważnika α-tokoferolu] = mg α-tokoferolu + 0,4 × mg β-tokoferolu + 0,1 × mg γ-tokoferolu + 0,01 × δ-tokoferolu + 0,3 × α-tokotrienolu + 0,05 × β-tokotrienolu + 0,01 × γ-tokotrienolu.

Ocena spożycia tokoferoli i tokotrienoli w wybranej grupie badanej

Badanie prowadzono przez okres 12 miesięcy: od września 2021 r. do września 2022 r. w grupie dorosłych Polaków metodą doboru losowego. W tym okresie uczestnicy zostali poproszeni o wypełnienie autorskiego kwestionariusza VitE-FFQ oraz 1-dniowego zapisu diety, bezpośrednio po wypełnieniu FFQ. Dodatkowo zapytano o podstawowe dane socjodemograficzne, takie jak wiek, wykształcenie, miejsce zamieszkania oraz dane antropometryczne, takie jak aktualna wysokość i masa ciała. Na podstawie informacji o aktualnej wysokości i masie ciała obliczono wskaźnik BMI. Do zebrania wszystkich danych wykorzystano technikę wywiadu internetowego wspomaganego komputerowo (CAWI).

Wyniki uzyskane z VitE-FFQ porównano dla każdego respondenta do wartości normy wystarczającego spożycia (AI) dla równoważnika α-tokoferolu: 8 mg/dzień dla kobiet oraz 10 mg/dzień dla mężczyzn (Jarosz i wsp., 2020) oraz do wartości AI rekomendowanej przez Europejski Urząd ds. Bezpieczeństwa Żywności (EFSA, 2015) dla α-tokoferolu: 11 mg/dzień dla kobiet oraz 13 mg/dzień dla mężczyzn. Zgodnie z procedurą oceny adekwatności spożycia w grupie (Jarosz i wsp., 2020), jako dostateczne spożycie przyjęto wartości równe lub powyżej wskazanych norm i obliczono odsetek osób, u których spożycie było dostateczne.

Analiza statystyczna

Ocenę przydatności narzędzia przeprowadzono poprzez porównanie wyników VitE-FFQ z 1-dniowym zapisem diety. Analiza statystyczna obejmowała:

1. Porównanie spożycia tokoferoli i tokotrienoli, obliczone dwiema metodami (1-dniowy całodzienny zapis żywieniowy oraz autorski kwestionariusz VitE-FFQ): Normalność rozkładu oceniono testem Shapiro-Wilka, następnie zastosowano test U Manna-Whitneya.
2. Analizę korelacji wyników: po weryfikacji normalności rozkładu (test Shapiro-Wilka) dla danych nieparametrycznych użyto korelacji rang Spearmana.

3. Ocenę zgodności metod za pomocą wykresów Blanda-Altmana: wartości granic zgodności (LOA) obliczono jako sumę średniej bezwzględnej różnicy spożycia i ±1,96 odchylenia standardowego tej różnicy. Wskaźnik Blanda-Altmana (%) określono jako odsetek osób poza LOA. Dobrą powtarzalność potwierdzało 95% różnic mieszczących się w ±2 SD, co odpowiada wskaźnikowi Blanda-Altmana ≤5%.
4. Porównanie grup: dla zmiennych kategorycznych zastosowano test Chi-kwadrat Pearsona.

Przyjęto poziom istotności $p \leq 0,05$. Analizy przeprowadzono przy użyciu oprogramowania STATISTICA w wersji 13.0 (StatSoft Inc., Tulsa, OK, USA; StatSoft, Kraków, Polska).

4. Syntetyczne omówienie wyników badań

Artykuł: Szewczyk, K., Bryś, J., Brzezińska, R., i Górnicka, M. (2025). Nutritional Status of Vitamin E and Its Association with Metabolic Health in Adults. Nutrients, 17(3), 408.

Osoby z prawidłowym poziomem tkanki tłuszczowej wykazywały istotnie wyższe stężenia izoform witaminy E niż osoby z jej nadmierną zawartością (odpowiednio: 32,9 *vs.* 19,6 µmol/l dla α-tokoferolu, 12,6 *vs.* 8,69 µmol/l dla γ-tokoferolu, 7,79 *vs.* 4,41 µmol/l dla α-tokotrienolu, 10,7 *vs.* 7,47 µmol/l dla γ-tokotrienolu oraz 64,0 *vs.* 40,2 µmol/l dla sumy tokoferoli i tokotrienoli; $p < 0,001$). Poziom α-tokoferolu przekraczający 30 µmol/l, uznawany za korzystny dla zdrowia, odnotowano u 50% osób z prawidłową ilością tkanki tłuszczowej i jedynie u 12% osób z jej nadmierną zawartością. Ponadto, niski stan odżywienia witaminą E występował częściej u osób z nadmierną zawartością tkanki tłuszczowej (30%) niż u badanych z jej prawidłowym poziomem (12%).

Zaobserwowano istotne różnice we wszystkich parametrach antropometrycznych między badanymi grupami. W grupie osób z prawidłową masą ciała mężczyźni, w porównaniu do kobiet, mieli wyższą masę ciała (82,9 kg *vs.* 61,2 kg), większy obwód bioder (100,4 cm *vs.* 74,5 cm) i talii (91,9 cm *vs.* 74,5 cm), a także wyższe wartości BMI (25,7 *vs.* 22,0), WHR (0,91 *vs.* 0,78) i WHtR (0,51 *vs.* 0,45). Jednocześnie ich procentowy udział tkanki tłuszczowej był niższy (18,6% *vs.* 21,4%). Kobiety częściej miały prawidłowe BMI (91% *vs.* 46% u mężczyzn), ale także częściej

wykazywały androidalny typ rozmieszczenia tkanki tłuszczowej według WHR (47% *vs.* 4%). W grupie osób z nadmierną zawartością tkanki tłuszczowej mężczyźni byli wyżsi (181,3 cm *vs.* 163,7 cm), mieli większą masę ciała (113,0 kg *vs.* 90,5 kg), obwód talii (118,7 cm *vs.* 105,6 cm) i wyższy WHR (1,08 *vs.* 0,93) niż kobiety. Z kolei kobiety miały większy obwód bioder (114,1 cm *vs.* 110,3 cm) i wyższy procentowy udział tkanki tłuszczowej (42,6% *vs.* 30,8%). W tej grupie nadwagę lub otyłość na podstawie BMI stwierdzono u wszystkich mężczyzn (100%) oraz 84% kobiet. Zaobserwowane różnice były istotne statystycznie ($p < 0,001$).

Stężenie α-tokoferolu korelowało ujemnie z WHtR ($r = -0,394$), masą tkanki tłuszczowej (FM; $r = -0,387$), obwodem talii (WC; $r = -0,382$), procentowym udziałem tkanki tłuszczowej (BF; $r = -0,359$), wskaźnikiem masy ciała (BMI; $r = -0,346$), masą ciała (BW; $r = -0,340$), obwodem bioder (HC; $r = -0,334$) oraz wskaźnikiem WHR ($r = -0,277$). Podobne zależności zaobserwowano również dla pozostałych analizowanych izoform witaminy E (α- i γ-) oraz ich sumarycznej wartości.

Osoby z nadmierną zawartością tkanki tłuszczowej wykazywały niekorzystny profil lipidowy. W porównaniu do grupy kontrolnej, miały istotnie niższe stężenie HDL (46,2 ± 11,0 mg/dl u kobiet i 38,6 ± 12,6 mg/dl u mężczyzn *vs.* 71,2 ± 10,1 mg/dl u kobiet i 54,8 ± 14,5 mg/dl u mężczyzn; $p < 0,001$). Częściej w tej grupie występował niski poziom HDL (<50 mg/dl u kobiet i <40 mg/dl u mężczyzn; 65% kobiet i 67% mężczyzn *vs.* 3% kobiet i 15% mężczyzn). Stężenie LDL było istotnie niższe w grupie osób z nadmierną zawartością tkanki tłuszczowej (107,4 ± 31,0 mg/dl u mężczyzn i 113,1 ± 30,8 mg/dl u kobiet *vs.* 132,2 ± 35,2 mg/dl u mężczyzn i 121,5 ± 28,5 mg/dl u kobiet; $p < 0,001$). Stężenie triacylogliceroli (TG) było istotnie wyższe u osób z nadmierną zawartością tkanki tłuszczowej (168,5 ± 55,0 mg/dl u kobiet i 197,4 ± 91,2 mg/dl u mężczyzn *vs.* 77,7 ± 35,0 mg/dl u kobiet i 118,0 ± 81,1 mg/dl u mężczyzn; $p < 0,001$). Osoby z nadmierną zawartością tkanki tłuszczowej wykazywały odmienny profil kwasów tłuszczowych w osoczu w porównaniu do osób z prawidłową jej zawartością, a także istotnie wyższy udział jednonienasyconych kwasów tłuszczowych (MUFA) – 30,5% u kobiet i 33,8% u mężczyzn *vs.* odpowiednio 22,7% i 25,8%. Jednocześnie charakteryzowały się niższym udziałem wielonienasyconych kwasów tłuszczowych (PUFA) – 32,2% u kobiet i 31,1% u mężczyzn *vs.* odpowiednio 37,6% i 37,0%. Zaobserwowano także wyższy udział kwasów tłuszczowych z rodziny n-3 (5,18% u kobiet i 4,46% u mężczyzn *vs.* 4,01% i 3,71%) oraz niższy udział kwasów n-6 (27,1% u kobiet i 26,7% u mężczyzn *vs.* 33,6% i 33,3%).

Suma tokoferoli i tokotrienoli ujemnie korelowała z poziomem jednonienasyconych kwasów tłuszczowych (MUFA; r = -0,339), natomiast dodatnio z poziomem wielonienasyconych kwasów tłuszczowych (PUFA; r = 0,242). Zaobserwowano dodatnią korelację między stężeniem α-tokoferolu (α-T) a HDL (r = 0,487) oraz LDL (r = 0,255), natomiast ujemną korelację z triacyloglicerolami (TG; r = -0,295). Podobne zależności dotyczyły również innych izoform witaminy E (α- i γ-) oraz ich sumarycznej wartości. Jednak w przypadku cholesterolu całkowitego i LDL zależności te nie były istotne dla pozostałych form witaminy E. Wszystkie izoformy witaminy E we krwi istotnie i silnie korelowały ze stężeniem cholesterolu HDL. Analiza oszacowanej aktywności enzymów związanych z metabolizmem lipidów wykazała, że osoby z nadmierną zawartością tkanki tłuszczowej miały istotnie wyższą aktywność D6D, SCD16 i SCD18 ($p<0,001$) oraz niższą aktywność D5D ($p=0,007$). Zaobserwowano ujemne korelacje między SCD16 z izoformami witaminy E (α-T: r = -0,265; α-T3: r = -0,247; γ-T: r = -0,194; suma Ts i T3s: r = -0,235) oraz podobne, lecz słabsze korelacje dla SCD18 (α-T: r = -0,237; α-T3: r = -0,197; γ-T: r = -0,182; suma Ts i T3s: r = -0,236). Nie stwierdzono istotnych zależności między witaminą E a aktywnością pozostałych enzymów.

Jednowymiarowa regresja liniowa wykazała, że istotnymi predyktorami wyższego stężenia izoform witaminy E były wyższe wartości (stężenia) HDL, niższe: MUFA, CRP, WC, WHtR, FM i BMI. Silniejsze powiązania stwierdzono dla form α-tokochromanoli.

Wyniki wieloczynnikowej analizy regresji liniowej wykazały, że najistotniejszymi predyktorami dla α-tokoferolu były udział kwasów MUFA (β = 0,476, 95% CI: −0,933; −0,019, p = 0,041) oraz stężenie HDL (β = 0,371, 95% CI: 0,071; 0,671, p = 0,016). Model ten wyjaśnił 29% zmienności stężenia α-tokoferolu we krwi. Najistotniejszym predyktorem dla α-tokotrienolu było stężenie HDL (β = 0,347, 95% CI: 0,011; 0,684, p = 0,043). Model ten wyjaśnił 22% zmienności koncentracji α-T3 we krwi. Dla γ-T oraz γ-T3, stężenie HDL było istotnym predyktorem (β = 0,330, 95% CI: 0,014; 0,646, p = 0,041 i β = 0,364, 95% CI: 0,023; 0,705, p = 0,036); jednakże ogólne modele nie były statystycznie istotne (R^2 = 0,017, p = 0,118 i R^2 = 0,172, p = 0,105).

Wyniki analizy wielokrotnej regresji liniowej wykazały statystycznie istotną, ujemną korelację między zawartością tkanki tłuszczowej a α-tokoferolem (β = -0,201, CI: -0,389; -0,012, p = 0,037) oraz pozytywną, istotną statystycznie korelację

z cholesterolem całkowitym (β = 0,234, CI: 0,073; 0,394, p = 0,005) i HDL (β = 0,230, CI: 0,038; 0,421, p = 0,019). Po uwzględnieniu wieku, płci i palenia papierosów, potwierdzono, że wyższe stężenie α-tokoferolu związane było z wyższym stężeniem HDL (β = 0,389, CI: 0,038; 0,421, p < 0,001) i LDL (β = 0,231, CI: 0,068; 0,395, p = 0,006). Biorąc pod uwagę płeć, w grupie kobiet wyższe wartości stężenia α-tokoferolu związane były z wyższym stężeniem HDL (β = 0,443, CI: 0,203; 0,684, p < 0,001), a u mężczyzn z LDL (β = 0,429, CI: 0,148; 0,800, p = 0,004).

Analiza wyników stężenia białka C-reaktywnego (CRP), markera stanu zapalnego, wykazała istotne różnice między grupami o różnej zawartości tkanki tłuszczowej. W grupie osób z prawidłową zawartością tkanki tłuszczowej średnie stężenie CRP wyniosło 0,42 ± 1,7 mg/dl, w grupie z nadmierną zawartością tkanki tłuszczowej było istotnie wyższe i wyniosło 2,39 ± 4,2 mg/dl (p < 0,001). Analiza korelacji wykazała istotne, ujemne zależności między stężeniem białka C-reaktywnego (CRP) a poziomami badanych izoform witaminy E. Najsilniejsze istotne korelacje zaobserwowano dla α-tokoferolu (r = -0,464) oraz α-tokotrienolu (r = -0,453). Umiarkowane, ujemne korelacje stwierdzono również dla γ-tokotrienolu (r = -0,355) oraz sumy tokoferoli i tokotrienoli (r = -0,454). Słabszą, ale nadal istotną, ujemną korelację zaobserwowano dla γ-tokoferolu (γ-T, r = -0,270).

Artykuł: Szewczyk, K., i Górnicka, M. (2023). Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults. Nutrients, 15, 1–18.

Średnie dzienne spożycie witaminy E wyrażonej jako równoważnik α-tokoferolu, wyniosło w badanej grupie 11,3 mg, a spożycie samego α-tokoferolu 12,0 mg/dobę. Dominującymi formami witaminy E były α- i γ-tokoferol, stanowiące odpowiednio 55% i 38% całkowitej zawartości tokoferoli. Główne źródła α-tokoferolu w diecie to migdały i nasiona słonecznika, natomiast γ-tokoferol pochodził głównie z chipsów, krakersów, nachosów i oleju rzepakowego. Spośród tokotrienoli największy udział miała forma β- (49%), a następnie α- i γ- (po 24%). Ich główne źródła to pieczywo pełnoziarniste, makaron razowy, brązowy ryż i płatki kukurydziane. Tokoferole stanowiły 94,3% całkowitej ilości witaminy E w diecie, a tokotrienole jedynie 5,7%.

Mężczyźni częściej charakteryzowali się niższym spożyciem równoważnika α-tokoferolu w porównaniu do kobiet (56% vs. 40%; p = 0,002), jak i α-tokoferolu (68% vs. 55%; p = 0,015). Ponadto deklarowane spożycie u osób powyżej 40. roku życia rzadziej realizowało wartości normy EFSA na spożycie α-tokoferolu niż u osób młodszych (18 - 40 lat) (29% vs. 44% p = 0,043), podczas gdy w odniesieniu do normy AI (Jarosz i wsp., 2020) dla równoważnika α-tokoferolu różnice te nie były istotne statystycznie. Nie wykazano istotnych różnic w adekwatności spożycia witaminy E czy α-tokoferolu w zależności od masy ciała. Mimo to, można zauważyć tendencję, że aż 63% osób, zarówno w grupie osób z nadwagą jak i w grupie z otyłością, spożywało niedostateczną ilość α-tokoferolu.

5. Podsumowanie i weryfikacja hipotez badawczych

Celem badania była ocena związku między nadmierną zawartością tkanki tłuszczowej a stężeniem związków lipidowych i lipofilnych w osoczu krwi osób dorosłych. W szczególności, analizowano związek między zawartością tkanki tłuszczowej a stężeniem witaminy E, oceniano stan odżywienia witaminą E u osób z prawidłową i nadmierną zawartością tkanki tłuszczowej, a także oceniano powiązania zdrowia metabolicznego ze stanem odżywienia witaminą E badanych osób. Dodatkowo, opracowano stosowne narzędzia oraz oceniano adekwatność spożycia tokoferoli i tokotrienoli w badanej grupie osób dorosłych.

Przeprowadzone badania pozwoliły zrealizować cel główny oraz cele szczegółowe, jak również zweryfikować przyjęte hipotezy badawcze.

Pozytywnie zweryfikowano pierwszą hipotezę badawczą, zakładającą, że: *nadmierna zawartość tkanki tłuszczowej i otyłość centralna są powiązane z niższymi stężeniami α-tokoferolu, γ-tokoferolu, α-tokotrienolu i γ-tokotrienolu oraz nieprawidłowym profilem związków lipidowych w osoczu krwi, a w konsekwencji z gorszym zdrowiem metabolicznym.*

Wyniki badania potwierdziły, że osoby z nadmierną zawartością tkanki tłuszczowej charakteryzowały się niekorzystnym profilem lipidowym, odmiennym profilem kwasów tłuszczowych oraz zmienioną aktywnością enzymów metabolizmu lipidów, co wskazuje na złożony związek między tkanką tłuszczową a gospodarką lipidową. Niskie stężenie α-tokoferolu w osoczu korelowało z wyższą zawartością tkanki tłuszczowej oraz stężeniem cholesterolu całkowitego i HDL. Sugeruje to, że nadmierna zawartość tkanki tłuszczowej i niższe stężenie izoform witaminy E może być związane

z gorszym zdrowiem metabolicznym. Osoby z nadmierną zawartością tkanki tłuszczowej wykazywały istotnie niższe stężenia wszystkich analizowanych izoform witaminy E, co wskazuje na potencjalny wpływ tkanki tłuszczowej na metabolizm i dystrybucję tych związków w organizmie człowieka. Stan odżywienia witaminą E był ściśle powiązany z profilem lipidowym. Osoby z otyłością charakteryzowały się wyższym udziałem kwasów tłuszczowych MUFA i n-3, podczas gdy osoby z prawidłową zawartością tkanki tłuszczowej w organizmie wykazywały wyższe stężenia wielonienasyconych kwasów tłuszczowych (PUFA), szczególnie kwasów tłuszczowych n-6. Z uwagi, iż kwasy tłuszczowe służą jako potencjalne markery spożycia tłuszczu w diecie, obserwowane różnice w profilach kwasów tłuszczowych przy różnych poziomach tkanki tłuszczowej mogą wskazywać na odrębne wzory dietetyczne lub reakcje metaboliczne na spożycie tłuszczu. Wyższy udział wielonienasyconych kwasów tłuszczowych (PUFA) i kwasów n-6 u osób o prawidłowej zawartości tkanki tłuszczowej może wskazywać na wyższe spożycie produktów będących ich źródłem. Osoby z nadmierną zawartością tkanki tłuszczowej wykazywały podwyższoną aktywność niektórych enzymów zaangażowanych w metabolizm kwasów tłuszczowych tj. Δ6-desaturazy, stearoyl-CoA desaturazy-16 i stearoyl-CoA desaturazy-18. Zwiększona aktywność D6D może nasilać prozapalną syntezę eikozanoidów, wywołujących stan zapalny, a SCD katalizuje przekształcanie SFA w MUFA, takie jak kwas oleinowy, które są kluczowymi składnikami triacylogliceroli, fosfolipidów i estrów cholesterolu, które między innymi transportują i magazynują witaminę E (Czumaj i Śledziński, 2020; Paton i Ntambi, 2009). W związku z tym podwyższona aktywność stearoyl-CoA desaturazy (SCD) może wpływać na modylację profilu lipidowego i może to być związane z obniżoną dostępnością i wchłanianiem witaminy E (Ntambi, 2013). Jednak podwyższona aktywność SCD jest często związana z gromadzeniem się lipidów w tkankach, co może zmieniać lokalne stężenia witaminy E i osłabiać jej rolę ochronną. Taka akumulacja lipidów jest związana z insulinoopornością i zaburzeniami metabolicznymi, ponieważ MUFA przyczyniają się do syntezy triacylogliceroli, co potencjalnie prowadzi do odkładania się lipidów w wątrobie i tkankach obwodowych. Z kolei D5D przekształca kwas dihomo-γ-linolenowy (DGLA) w przeciwzapalne PUFA, co sugeruje, że wyższa aktywność D5D może wspierać prawidłowy profil lipidowy i zdrowie metaboliczne (Galli, 2024).

Istotne ujemne korelacje między wskaźnikami otyłości (WHtR, FM, WC, BF, BMI, WHR) a stężeniem badanych izoform witaminy E potwierdzają związek między

nadmierną zawartością tkanki tłuszczowej i otyłością centralną mierzoną obwodem talii, wskaźnikami WHR i WHtR a niższym stężeniem związków lipofilnych, w tym przypadku α- i γ- tokochromanoli.

Na podstawie przedstawionych wyników można stwierdzić, że hipoteza 1 została potwierdzona. Nadmierna zawartość tkanki tłuszczowej oraz otyłość centralna wiążą się z obniżonymi stężeniami α-tokoferolu, γ-tokoferolu, α-tokotrienolu i γ-tokotrienolu, a także z nieprawidłowym profilem lipidowym osocza krwi, co przyczynia się do pogorszenia zdrowia metabolicznego.

Mimo, iż zaobserwowano ujemną korelację między stężeniem badanych izoform witaminy E, a markerem stanu zapalnego - białkiem CRP, dokładniejsze analizy statystyczne nie potwierdziły tego związku. Uzyskane wyniki sugerują, że niższe stężenie α- i γ- tokochromanoli może być związane z gorszym zdrowiem metabolicznym, jednak konieczne są dalsze badania do pełnego zrozumienia tych zależności.

W związku z powyższym należy stwierdzić, że uzyskane wyniki tylko częściowo potwierdzają hipotezę 2. Wykazano, że nadmierna zawartość tkanki tłuszczowej była związana z wyższym stanem zapalnym, mierzonym stężeniem białka CRP, natomiast potrzebne są dalsze badania nad oceną zależności pomiędzy stężeniem tokochromanoli i ich wpływem na stan zapalny.

Dominującymi formami witaminy E w diecie były α- i γ-tokoferol oraz β-, α- i γ-tokotrienol. Analiza spożycia poszczególnych izoform witaminy E wykazała, że mężczyźni oraz osoby powyżej 40. roku życia charakteryzowały się częściej niedostatecznym ich spożyciem. Wyniki nie wykazały istotnych zależności między nadmierną masą ciała a spożyciem witaminy E.

Uzyskane wyniki tylko częściowo potwierdzają hipotezę 3. Na podstawie uzyskanych wyników stwierdzono, ze niedostateczne spożycie witaminy E częściej występowało u mężczyzn i osób powyżej 40 roku życia, natomiast potrzebne są dalsze badania nad oceną zależności pomiędzy stężeniem tokochromanoli a zawartością tkanki tłuszczowej i masy ciała u osób dorosłych.

6. Stwierdzenia i wnioski

Badania przeprowadzone w ramach niniejszej pracy pozwoliły na realizację celu i częściowo pozytywną weryfikację postawionych hipotez, a także skłaniają do sformułowania następujących stwierdzeń:

1. Nadmierna zawartość tkanki tłuszczowej w organizmie determinuje niekorzystny profil związków lipidowych (niskie stężenie lipoprotein o wysokiej gęstości HDL, podwyższone stężenie triacylogliceroli, jak również zaburzony profil kwasów tłuszczowych) i niższe stężenie lipofilnych (α- i γ- tokochromanoli w osoczu krwi). Ponadto zwiększona jest szacowana aktywność enzymów zaangażowanych w przekształcania kwasów tłuszczowych w kierunku produktów pro-zapalnych.
2. Nadmierna tkanka tłuszczowa i niższe stężenia α- i γ- tokochromanoli są związane z nasileniem stanu zapalnego, przy czym te zależności wymagają dalszych badań.
3. Jedynie 52% osób badanych realizowało spożycie witaminy E na poziomie normy AI. Niedostateczne spożycie częściej stwierdzano w grupie mężczyzn i osób powyżej 40 roku życia. Dominującymi formami witaminy E w diecie badanych osób były α- i γ-tokoferole oraz α-, β- i γ-tokotrienole.

W kontekście uzyskanych wyników badań i weryfikacji hipotez badawczych uzasadniony jest **wniosek,** że *nadmierna zawartość tkanki tłuszczowej w organizmie może wpływać na niższe stężenie poszczególnych izoform witaminy E we krwi, a tym samym na niski stan odżywienia witaminą E i niższą obronę antyoksydacyjną organizmu*. Podobnie słusznym wydaje się **wniosek,** że *zwiększenie spożycia witaminy E, zwłaszcza u mężczyzn i osób po 40 roku życia może stanowić kluczowy element profilaktyki zdrowia i dietoterapii chorób kardiometabolicznych przy koniecznej optymalizacji struktury diety pod kątem doboru źródeł poszczególnych form witaminy E*.

W kontekście uzyskanych wyników badań można też sformułować **wniosek o charakterze metodycznym**, wskazujący na to, że *opracowany kwestionariusz i kalkulator do oceny spożycia witaminy E i poszczególnych jej form może być wykorzystany w dalszych badaniach do oceny zależności między ich spożyciem a stężeniem we krwi w kontekście innych niezakaźnych chorób przewlekłych.*

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8. Kopie opublikowanych prac wraz z oświadczeniami współautorów

International Journal of
Molecular Sciences

MDPI

Review

Tocopherols and Tocotrienols—Bioactive Dietary Compounds; What Is Certain, What Is Doubt?

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Abstract: Tocopherols and tocotrienols are natural compounds of plant origin, available in the nature. They are supplied in various amounts in a diet, mainly from vegetable oils, some oilseeds, and nuts. The main forms in the diet are α- and γ-tocopherol, due to the highest content in food products. Nevertheless, α-tocopherol is the main form of vitamin E with the highest tissue concentration. The α- forms of both tocopherols and tocotrienols are considered as the most metabolically active. Currently, research results indicate also a greater antioxidant potential of tocotrienols than tocopherols. Moreover, the biological role of vitamin E metabolites have received increasing interest. The aim of this review is to update the knowledge of tocopherol and tocotrienol bioactivity, with a particular focus on their bioavailability, distribution, and metabolism determinants in humans. Almost one hundred years after the start of research on α-tocopherol, its biological properties are still under investigation. For several decades, researchers' interest in the biological importance of other forms of vitamin E has also been growing. Some of the functions, for instance the antioxidant functions of α- and γ-tocopherols, have been confirmed in humans, while others, such as the relationship with metabolic disorders, are still under investigation. Some studies, which analyzed the biological role and mechanisms of tocopherols and tocotrienols over the past few years described new and even unexpected cellular and molecular properties that will be the subject of future research.

Keywords: tocopherols; tocotrienols; vitamin E metabolites; α-tocopherol; bioaccessibility; bioavailability; bioactivity

Citation: Szewczyk, K.; Chojnacka, A.; Górnicka, M. Tocopherols and Tocotrienols—Bioactive Dietary Compounds; What Is Certain, What Is Doubt? *Int. J. Mol. Sci.* **2021**, *22*, 6222. https://doi.org/10.3390/ijms22126222

Academic Editors: Elena Azzini and David Arráez-Román

Received: 31 March 2021
Accepted: 5 June 2021
Published: 9 June 2021

1. Introduction

Vitamin E is a group of eight compounds: α-, β-, γ-, δ-tocopherols and α-, β-, γ-, δ-tocotrienols, which are lipid-soluble [1,2]. Only photosynthetic organisms—plants, algae, and cyanobacteria as well as fungi, corals, sponges, and tunicates—have the ability to synthesize these compounds [2,3]. The main natural source of tocopherols and tocotrienols is the oily fraction of nuts and oil seeds [4,5]. Data on their presence in fruits and vegetables are often contradictory. This is due to the variety of analytical methods that can be used for their determination in food products [6]. The main sources of tocopherols are almond oil and other nut oils, olive oil, sunflower oil, rapeseed oil, corn oil, linseed oil, and soybean oil. Tocotrienols, in turn, can be found in palm and rice bran oil, wheat germ, barley, oats, hazelnuts, maize, and in annatto oil [4,7]. Habitual diet supplies mainly tocopherols, especially α-tocopherol and γ-tocopherol. γ-tocopherol is the most common in the US diet due to the higher consumption of soybeans, sesame, and corn oil, and α-tocopherol is the most common in the European diet [8]. In spite of the similar structure and antioxidant activity, vitamin E isoforms differ in bioavailability and metabolism [9]. All isoforms are biologically active, but only α-tocopherol is retained at high levels in plasma and tissues. The selectivity is achieved by the action of the hepatic α-tocopherol transfer protein (α-TTP) [10]. Plasma and body tissues are 90% saturated with α-tocopherol while other forms of vitamin E are degraded and excreted [11,12].

Except for the above-mentioned antioxidative activity of all vitamin E isoforms, antiproliferative, pro-apoptotic, anti-angiogenic, and anti-inflammatory effects are indicated. The beneficial impact on human health may also result from the ability to modulate signal transduction and gene expression in inflammation and immune system disorders [13,14].

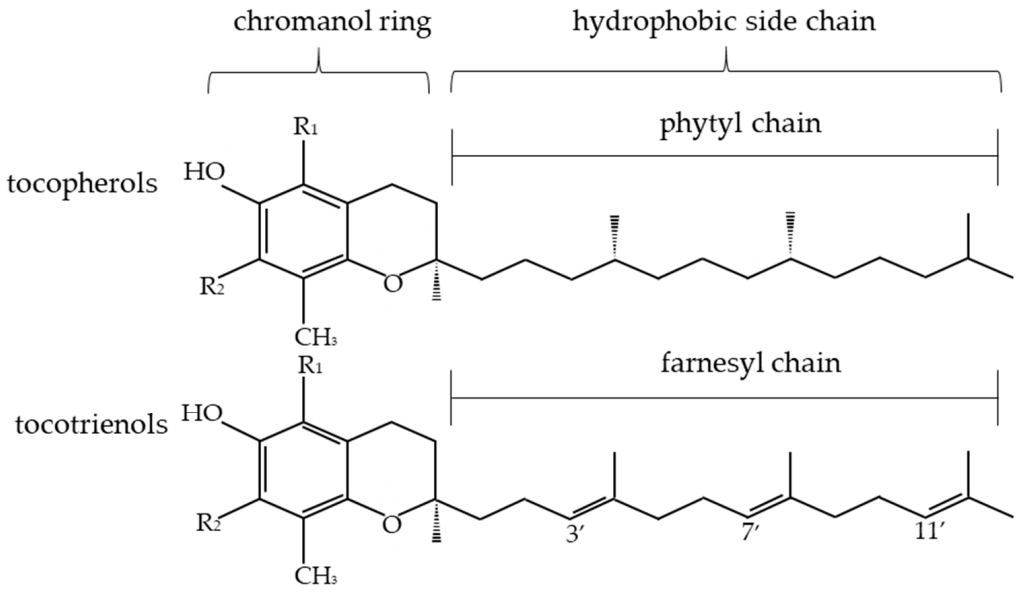
Therefore, the aim of the present work is to describe known activities of both tocopherols and tocotrienols. This work focused on a review of the factors that influence the activity of compounds belonging to the vitamin E family, and their roles for human health are described. Based on the current scientific research, this paper presents the known and sought mechanisms of action of tocopherols and tocotrienols in the prevention of diet-related diseases, their differentiated bioactivity as well as the determinants of distribution and metabolism in humans.

2. Literature Search

The presented review shows current information on the known and sought-after properties of tocopherols, tocotrienols, and their metabolites as well as their importance in human health. The literature search was conducted between September 2020 and February 2021 in PubMed and ScienceDirect for articles related to vitamin E, using specific keywords such as tocopherol, tocotrienol, vitamin E, vitamin E metabolites, and health. Regarding health, the strongest emphasis was based on the most common and discussed disease entities, such as cardiovascular diseases, cancer, and obesity. Original articles on tocopherols and tocotrienols, published in English, were used. The review included clinical trials, controlled trials, cohort studies, systematic reviews, and meta-analyses.

3. Vitamin E Isoforms and Their Bioactivity

Tocochromanols, known as vitamin E, are the most common and dominant chromanols in the nature. Tocochromanols belong to a group of lipid-soluble antioxidants present in the plastids of plants [15]. Tocochromanols are a group of compounds synthesized only by plants and photosynthetic microorganisms. Most often eight compounds are mentioned as vitamin E: four tocopherols and four tocotrienols. In the group of tocotrienols, two further homologues occurring in rice bran have been identified. These are desmethylotocotrienol (d-P21-T3) and didesmethylotocotrienol (d-P23-T3), which differ from other tocotrienols by the lack of methyl groups in the benzene ring [16]. Tocochromanols are synthesized by plants from homogentisic acid [15]. Tocopherols and tocotrienols contain a chromanol ring (they are bicyclic phenols) and a hydrocarbon side chain. They consist of homologues α-, β-, γ-, δ- and differ in the number and location of the methyl substituent in the hydrophilic head of 6-chromanol, which is responsible for the presence of various isomeric forms of tocopherols and tocotrienols. Tocopherols are characterized by a saturated side chain (an aliphatic phytyl side chain), and tocotrienols have three double bonds in the side chain (an unsaturated farnesyl side chain) (Figure 1) [3].



Tocopherol/ Tocotrienol	R1	R2
α-	CH3	CH3
β-	CH3	H
γ-	H	CH3
δ-	H	H

Figure 1. The structure of tocopherols, tocotrienols, and differences in the structure of their isoforms [17,18].

Due to their structure, they are incorporated into the amphipathic phospholipid bilayer of cell membranes. Thereby, they are able to protect membrane lipids, organs, and organs of photosynthesis in plants from oxidative stress [19,20]. Each of the tocopherols has three chiral centers, at C2′, C4′, and C8′ carbon atoms, which can have the R or S configuration, resulting in eight possible stereoisomers. The naturally occurring form of α-tocopherol is RRR-α-tocopherol [21]. The chemically synthesized α-tocopherol is a racemic mixture of all possible α-tocopherol isomers in equimolar concentrations (synthetic form: all-racemic-α-tocopherol) [22]. Results of many studies established that the animal organisms (including humans) preferentially absorb the natural stereoisomer RRR-α-tocopherol [23] due to the affinity of α-tocopherol transfer protein (α-TTP) to forms of R [19]. Studies have shown that only natural α-tocopherol (RRR-α-tocopherol) is the most biologically active, and the activity of non-α-tocopherol forms is expressed as the α-tocopherol equivalent (%), which accounts for 50% for β-tocopherol, 10% for γ-tocopherol, 3% for δ-tocopherol, 30% for α-tocotrienol, 8% for γ-tocotrienol, and 5% for β-tocotrienol. For δ-tocotrienol, the equivalent has not been established [24]. According to the Food and Nutrition Board of the Institute of Medicine, only α-tocopherol is recognized as a nutrient (vitamin) that is able to meet vitamin E requirements in humans. Additionally, the differences in antioxidant activities of vitamin E forms are relatively minor, while their biological activities are divergent and multidirectional [25].

4. Absorption, Bioavailability, and Biotransformation of Tocopherols and Tocotrienols

The amount of vitamin E absorbed depends on the differences in the food matrix that supplies this vitamin. It is known that retinoic acid, plant sterols, eicosapentaenoic acid, alcohol, and dietary fiber inhibit the absorption of vitamin E [26]. Studies have shown low bioavailability of vitamin E isoforms from the apples matrix and high bioavailability from the bananas, bread, and lettuce matrix. Additionally, they were more bioavailable from egg-

free durum wheat pasta than from pasta containing eggs. The fragmentation of the food matrix may increase the bioavailability of vitamin E isoforms and their transfer to micelles; no similar effect was found for technological treatment (either thermal or high-pressure treatments). The higher the amount of fat in a meal, the higher absorption of vitamin E compounds occurs [13]. In the gastrointestinal tract, tocopherols and tocotrienols are absorbed to a similar extent, but their absorption depends on adequate pancreatic function, bile secretion, and the formation of micelles [13]. It turned out that transmembrane proteins play a key role in the intestinal absorption of vitamin E [27]. Initially, it was thought that vitamin E absorption occurred by passive diffusion through the enterocyte membrane [13]. In later years, it was shown that absorption is also mediated, at least in part, by three groups of proteins: Niemann–Pick C1-like 1 protein (NPC1L1), scavenger receptor class B type 1 (SRB1), and a cluster of determinant 36 (CD36). These three proteins are mainly described as cholesterol transporters, but they can also bind to the other substrates [20,28]. The absorption of tocopherols and tocotrienols in the intestine varies from 20% to 80% of the total ingested amount and is lower than the other fat-soluble vitamins [29]. The concentration of tocopherols and tocotrienols in the plasma is influenced by their content in a diet, absorption, and their metabolism. Some of these factors may be modulated by emerging genetic changes in the genes, which encode proteins responsible for the above factors [30].

Vitamin E is transported in the blood by plasma lipoproteins and erythrocytes. After entering the circulation, chylomicrons undergo a reconstruction process consisting mainly of the hydrolysis of triglycerides by lipoprotein lipase, resulting in the formation of chylomicron residues. Part of vitamin E forms is taken up by extrahepatic tissue, and the remaining part of vitamin E incorporated in the chylomicron remnants is taken up by the liver [30]. Due to the presence of protein α-TTP in the liver, α-tocopherol is preferentially transported further, while the remaining tocochromanols are metabolized and excreted in the bile. α-TTP mediates the incorporation of α-tocopherol into very-low-density lipoproteins (VLDL) and the secretion of these complexes into circulation. In the blood, VLDLs are catabolized to low (LDL) and high-density (HDL) lipoproteins by lipoprotein lipase (LPL). VLDL catabolism causes α-tocopherol to occur simultaneously in all the above-mentioned types of lipoproteins. α-tocopherol that is delivered into LDL lipoproteins is transferred to the tissues, where it performs its functions (Figure 2) [31].

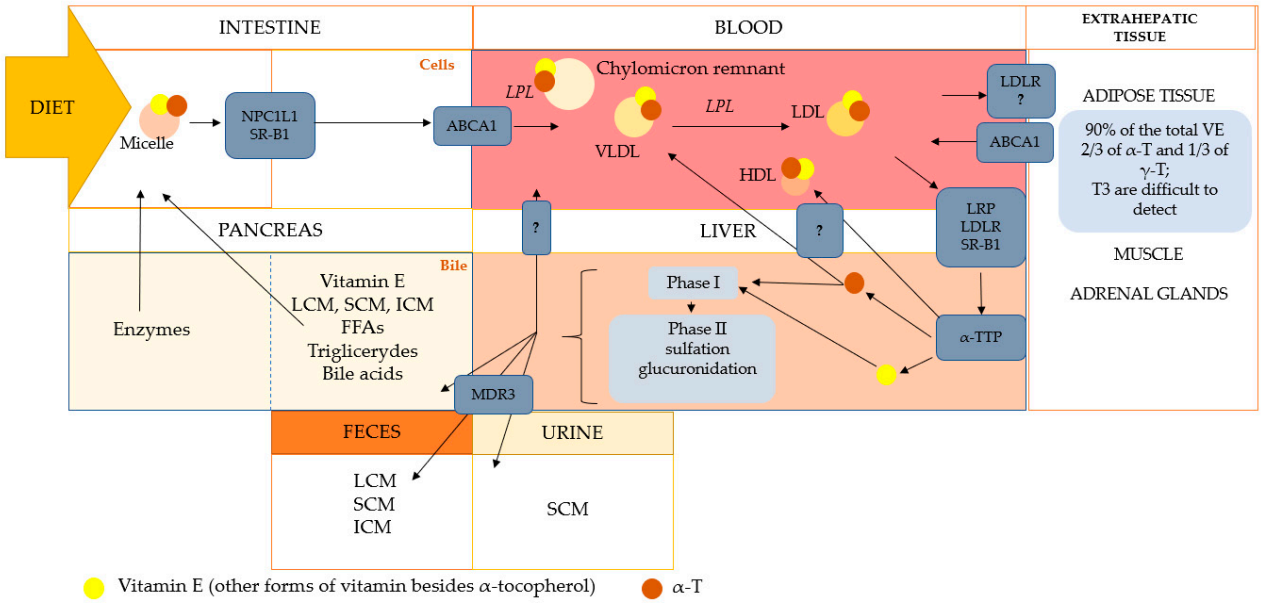


Figure 2. The simplified scheme of the transport and metabolism of vitamin E. The metabolism of vitamin E follows generally like other lipids species. In the intestine, vitamin E, along with other lipids, is packed into micelles, which are captured by receptors. In the intestinal epithelial cells, vitamin E is incorporated into chylomicrons or HDL via ABCA1. Vitamin E in the blood follows the lipoprotein transport route and is delivered to the liver or extrahepatic tissues. Vitamin E transport takes place with the participation of chylomicrons, which are subjected to hydrolysis by lipoprotein lipase. Further transport of vitamin E is through by chylomicron remnants, HDL, LDL, and VLDL. In the liver, vitamin E is sorted and directed to catabolism or to various lipoproteins (the mechanisms are not fully understood), returning to the bloodstream. The transport route is the same for all forms of vitamin E. Discrimination of the other forms in favor of α-tocopherol occurs in the liver by α-TTP, which protects against excessive degradation and excretion of α-tocopherol. The remaining forms of vitamin E are included in catabolism (phase I and II). NPC1L1, Niemann-Pick C1 Like 1 protein; SR-B1, scavenger receptor class B type 1; ABCA1, ATP-binding cassette transporter; VLDL, very-low-density lipoproteins; HDL, high-density lipoproteins; LDL, low-density lipoproteins; LDLR, LDL receptor; VE, vitamin E; α-T, alpha-tocopherol; γ-T, gamma-tocopherol; T3, tocotrienols; LRP, LDL receptor-related protein; LCM, long-chain metabolites (13′-COOH); ICM, intermediate-chain metabolites (11′-COOH, 9′-COOH); SCM, short-chain metabolites (7′-COOH, 5′-COOH, 3′-COOH); FFAs, free fatty acids; MDR3, multidrug resistance protein 3. The figure was modified from [1,32–36].

The uptake of different forms of vitamin E into the liver is probably nonspecific. The mechanisms that are involved are promiscuous in that these are general xenobiotic processes [37,38]. It is worth noticing that α-TTP has 100% affinity for α-tocopherol, 38% for β-tocopherol, 9% for γ-tocopherol, and 2% for δ-tocopherol [1]. Research results confirmed the existence of bio-discrimination against tocotrienols due to the affinity of tocopherols to the α-TTP protein [39]. Among the tocotrienols, the α- form is known for the highest oral bioavailability. A low level or lack of α-tocopherol, as well as the higher content of α-tocotrienol in the food matrix are the determinants of increased absorption of the ingested tocotrienols [29]. Due to their low affinities for hepatic α-TTP, non-α-tocopherol forms are less efficiently transferred to VLDL and are detected in the blood and tissues in low concentrations [37]. Both tocopherols and tocotrienols are accumulated in many tissues, among others, the liver, adrenal glands, and adipose tissue [40]. It is estimated that 90% of the total amount of vitamin E is accumulated in the adipose tissue, mainly in adipocyte lipid droplets [32]. Vitamin E that is accumulated in adipose tissue consists of about two-thirds of α-tocopherol and one-third of γ-tocopherol [33]. Accumulated tocotrienols are difficult to detect, but supplementation of tocotrienols in animals increased their pool in the adipose tissue [34]. With a vitamin E-free diet applied for four weeks in rats, a decrease of tocopherol and tocotrienol was observed but only in the liver, not in the adipose tissue [39].

Supplementation of α-tocopherol and tocotrienols significantly increased the level of α-tocopherol in the plasma, reaching a maximum concentration 8 h after supplementation and maintaining a high level even after 24 h. Tocotrienols reached their maximum concentration 4 h after supplementation. Then their level decreased significantly, and they completely disappeared from the plasma after 24 h [35]. Moreover, accumulation of α-tocotrienol in selected organs was observed in rats after supplementation with α-tocotrienol (5 mg/kg body weight) for over two years. When vitamin E-deficient diet was applicated, the accumulated α-tocotrienol was depleted after less than two months, while the loss of α-tocopherol was negligible [41].

Human studies also provide evidence for α-tocopherol retention and degradation of other non-α-tocopherol forms. Uchida et al. [42] even noted that γ-tocopherol was metabolized and excreted faster than α-tocopherol. Additionally, faster turnover of tocotrienols was seen in humans [43]. The action of non-α-tocopherol homologues in the human body is limited because of the fact that they are immediately metabolized in the liver and excreted in bile or urine. Recent studies have highlighted that tocotrienols exhibit higher antioxidant activity in in vivo systems. However, their oral bioavailability is significantly limited as they are not recognized by α-TTP. Additionally, their occurrence in food is low or even rare [44]. Due to their fast metabolism their lifetime is short. They quickly penetrate the skin, combating the effects of oxidative stress induced by UV radiation and ozone [16]. Non-α-tocopherol forms are recognized as xenobiotics and are metabolized and excreted. Consequently, their plasma concentrations are decreased, and formed metabolites concentrations are increased [43]. Tocotrienols metabolites formed in the liver are the promising forms of vitamin E in the prevention of diseases caused by acute inflammation and oxidative processes [44].

Recent studies have found that supplementation with a mixture of vitamin E isoforms resulted in a significant increase in the concentration of tocotrienols in the tissues. This may indicate a different mechanism of intracellular transport of α-tocotrienol, independent of α-TTP. Each cell type likely has a different selectivity for the tocotrienols uptake, e.g., it was found that sirtuin 1 (SIRT 1) proteins in human fibroblasts may regulate the uptake and bioavailability of tocotrienol isomers [40]. Additionally, other proteins—tocopherol-associated proteins 1, 2, 3 (TAP 1, 2, 3), human plasma protein afamin, albumin, and phospholipid transfer protein (PLTP)—show binding capacity to other forms of vitamin E [45]. An alternative explanation for the metabolism of other forms of vitamin E was proposed after identifying the metabolites of vitamin E—carboxyethyl-hydroxychroman (CEHC)—and discovering that this pathway preferentially metabolizes non-α-tocopherol forms in hepatic cells [1]. Vitamin E isoforms that are not transferred from the liver by α-TTP are metabolized in the phase I (catabolism and side-chain shortening) and phase II (sulfation and glucuronidation) [46]. The catabolism of α-tocopherol and other isoforms occurs when the amount of hepatic α-tocopherol exceeds α-TTP's transfer capacity. α-Tocopherol is endogenously converted to α-CEHC (2,5,7,8-tetramethyl-2-(2′-carboxyethyl)-6-hydroxychroman). Urinary excretion of α-CEHC positively correlates with α-tocopherol increase in both diet and plasma concentration in healthy subjects [47]. It is not clear whether the increase in urinary α-CEHC excretion is related to the increased consumption of α-tocopherol in a single meal or whether these changes reflect long-term higher consumption of this nutrient [38,47].

Initially, it was believed that the metabolism of α-tocopherol occurs through the opening of the chromanol ring and the subsequent degradation of the side chain, when only two metabolites are produced—namely, α-tocopheronic acid and its lactone, α-tocopheronolactone (α-TL), so-called Simon's metabolites [48,49]. In later years, additional metabolic pathways were defined following the discovery of other α-tocopherol metabolites with an intact chromanol ring. ω-Hydroxylation of the aliphatic side chain leads to the formation of 13′-hydroxychromanol (13′-OH), and 13′-carboxychromanols (13′-COOH) are formed as a result of oxidation. The subsequent stages of oxidation lead to shortening of the side chain, creating further metabolites—carboxydimethyldecylhydroxychromanol

(CDMDHC, 11′-COOH), carboxymethyloctylhydroxychromanol (CDMOHC, 9′-COOH), carboxymethylhexylhydroxychromanol (CDMHHC, 7′-COOH), and carboxymethylbutyl-hydroxyxhromanol (CMBHC, 5′-COOH). The end products of vitamin E metabolism are carboxyethyl-hydroxychromanols (CEHC), referred to as 3′-COOH or short-chain metabolites (SCM). Research confirms that tocotrienols follow the same metabolic pathway [26].

Non-α-tocopherol forms are preferentially catabolized in the oxidation of side chains with the formation of hydroxylated and carboxylated chromanols [50]. Vitamin E isoforms metabolism includes the phase I ω-hydroxylation to the alcohol derivative of 13′-OH, catalyzed by cytochrome P450 monooxygenase (CYP4F2), which takes place in the endoplasmic reticulum of liver cells. These products are considered as a phase I metabolic intermediate and limit the accumulation of the lipophylic vitamin [34,43]. The oxidized hydroxyl group leads to the formation of 13′-COOH long-chain metabolites (LCMs) under the action of aldehyde dehydrogenase. It is followed by a series of β-oxidations with the formation of medium and short-chain carboxychromanols—11′-COOH, 9′-COOH (in peroxisomes) and 7′-COOH, 5′-COOH (in the mitochondrial matrix)—and the final metabolite, 3′-COOH (CEHC). The metabolites are excreted from the body with urine and feces [46]. Metabolites occur in human urine in free form or as sulfates or glucuronides [51,52]. Because of the degradation of tocotrienols, CEHCs are formed, which suggests a similar metabolic mechanism as in tocopherols. The side-chain double bonds undergo a saturation step catalyzed by 2,4-dienoyl-CoA reductase and 3,2-enoyl-CoA isomerase (coenzyme A). They are also involved in the metabolism of unsaturated fatty acids [2].

5. The Bioactivity of Vitamin E Metabolites

Recent studies suggest that the properties of LCMs of vitamin E correspond to the functions of vitamins A and D. The structural similarity of vitamin E metabolites with vitamin A and D metabolites (9-cis-retinoic acid and 1,25(OH)2D3) allows us to reach the conclusion that there are also vitamin E-specific receptors, hitherto undiscovered. This hypothesis is likely confirmed by the findings on the regulatory activities of vitamin E metabolites. Although plasma concentrations of the metabolites are low, the results of animal and in vitro studies have indicated their strong biological potential [51]. Studies have reported that the functions of vitamin E metabolites can be divided in terms of anti-inflammatory activity, antitumor activity, participation in the regulation of cellular lipid homeostasis, drug interactions, and regulation of the metabolites' own metabolism [51,53].

Research on anti-inflammation activity often focuses on the possibility of regulating the pro-inflammatory enzymes by vitamin E metabolites [54,55]. Cyclooxygenase (COX) that catalyzes pro-inflammatory eicosanoid production plays an important role in regulation of the inflammatory response and contributes to the development of many chronic diseases, such as cancers. The inhibitory effect of α-tocopherol metabolites—α-9′-COOH and α-13′-COOH—on the activity of COX-1 and COX-2, catalyzing the production of pro-inflammatory eicosanoids, was demonstrated [56]. Studies have shown that α-13′-COOH is a competitive COX inhibitor and therefore competes to join to the substrate-binding site. It shows a greater affinity for cyclooxygenases than other metabolites and forms of tocopherols [54].

The α-tocopherol metabolite α-13′-COOH inhibits inflammation by targeting 5-lipoxygenase (5-LO), which catalyzes the initial stages of biosynthesis of strong immunomodulatory lipid mediators [57,58]. Leukotrienes, generated by the enzyme 5-LO through metabolism, play a major role in the development of asthma and allergic rhinitis. They also contribute to oxidative DNA damage and consequently to cardiovascular diseases, inflammatory liver diseases, neurodegenerative disorders, and cancers [59–61]. It has been shown that the α-13′-COOH is accumulated at sites of inflammation and suppresses acute inflammation in murine peritonitis [57].

Studies focused on antitumor properties have shown that α- and δ-tocopherol metabolites stopped proliferation in the human hepatocyte HepG2 cancer cell line [62]. Both

metabolites α-13′-COOH and δ-13′-COOH effectively inhibited cell growth while no such effect was noticed among the forms of hydroxy metabolites [53,62].

Additionally, Jang et al. [63] found a similar effect for 13′-COOH, derived from δ-tocopherol and δ-tocotrienol. In a mice model of colon cancer, they inhibited COX-2 and 5-LO and thus induced apoptosis and autophagy. They are seemingly promising factors that may act against cancer.

The metabolite of γ-CEHC, unlike α-CEHC, is attributed to natriuretic properties, including the regulation of the water–sodium balance and maintenance of cardiovascular homeostasis. Both γ-tocopherol and γ-CEHC also inhibit the activity of COX-2 [16].

Many of the studies have focused on vitamin E metabolites and their influence on the regulation of key pathways in the development of macrophage foam cells [64,65]. Macrophages bind oxidized LDL cholesterol (oxLDL) through many types of receptors, among them scavenger receptor CD36, which is also involved in the transport of tocopherol (as well as a number of lipid compounds) [8]. The expression of CD36 is reduced by α-tocopherol [65], while long-chain α-tocopherol metabolites (α-13′-OH and α-13′-COOH) influence oxLDL uptake independent of CD36 [64]. They can inhibit the formation of macrophage foam cells, thus having a positive effect on the prevention of atherosclerosis [64].

Torquato et al. [66], found that the α-13′-OH metabolite increased the expression of the CYP4F2 protein gene in human hepatic HepG2 cells. This protein is involved in the metabolism of vitamin E. This proves that derivatives of vitamin E metabolism, mainly α-13′-OH, may be responsible for the existence of a positive regulatory feedback loop that occurs in the metabolism of vitamin E.

The results of the research also indicate that α-13′-COOH and γ-tocotrienols activated the pregnane X receptor (PXR), which is a transcription factor of P-glycoprotein (P-gp), which is responsible for the intracellular concentration and transport of pharmaceuticals. What is more, it induced protein expression and P-gp transporter activity [67]. This means that in patients receiving γ-tocotrienol supplementation or high doses of α-tocopherol, drug levels that are P-gp substrates should be monitored.

6. Proven Antioxidant and Anti-Inflammatory Effects of α- and γ-Tocopherols, and What Is the Role of Other Isoforms?

α-Tocopherol is a specific non-enzymatic, chain-breaking antioxidant in aerobic organisms. It is present in cell membranes and plays a significant preventive role in the oxidative damage of molecules such as DNA or lipids [20,68]. α-Tocopherol neutralizes free radicals and breaks the chain reaction in the oxidation of the polyunsaturated fatty acids [69]. This activity is associated with the non-esterified hydroxyl groups of the chromanol ring. Moreover, the lipophilic tail of tocopherol can interact with cellular lipids and other molecules, protecting them from oxidation or peroxidation [8]. The α-tocopheroxyl radical is relatively long-lived, and it can be reduced to the α-tocopherol by water-soluble antioxidants such as ascorbic acid [43].

It is reported that the antioxidant activity of vitamin E isomers depends on the number of hydroxyl groups and is in the order of α > β > γ > δ [21]. Some research results indicate a greater antioxidant potential of tocotrienols than of tocopherols [70]. This may be due to their greater distribution in the phospholipid bilayer of cell membranes and more effective interaction with lipid peroxyl radicals [71]. However, compared to tocopherols they are less orally bioavailable [72]. Moreover, when they become radicals, they are more reactive and can readily form adducts that are potentially cytotoxic [43].

In addition, other forms of vitamin E, for instance, γ-tocopherol, δ-tocopherol, and γ-tocotrienol, have unique antioxidant properties. It has been observed that γ-tocopherol has the ability to scavenge reactive forms of nitrogen. This activity is not observed for α-tocopherol [50].

Moreover, at the molecular level, non-antioxidative functions of tocopherols and tocotrienols have been shown [45]. This activity is possible because of specific interactions with enzymes, structural proteins, structural lipids, and transcription factors [73]. The main

effect of α-tocopherol is the inhibition of the activity of protein kinase C (PKC). It affects the proliferation of monocytes, macrophages, and neutrophils of smooth muscle cells and reduces the production of superoxide free radicals in neutrophils and macrophages. α-Tocopherols can modulate of phospholipase A2 activity and inhibition of prostaglandin activity E2 and cyclooxygenase 2. In the regulation of gene expression, several genes have been described as modulated by tocopherol. Several possible regulatory pathways have been described for α-tocopherol, which can alter the activity of transcription factors and induction pathways through enzyme modulation and may ultimately affect gene expression. Vitamin E isoforms can directly modulate the activity of transcription factors through pregnane X receptor (PXR), peroxisome proliferator-activated receptors (PPARs), orphan nuclear receptors, or one of the three human α-tocopherol associated proteins (hTAPs). Additionally, they can affect gene expression by binding to human α-tocopherol-related hTAP proteins, which regulate tocopherol access to specific enzymes and transcription factors and control the level of "free" tocopherol. Ultimately, tocopherols and tocotrienols can be metabolized to bioactive compounds (metabolites) and affect the activity of transcription factors [71,74].

Both the anti-inflammatory and antioxidant functions of vitamin E may enhance the immune system [75]. In addition, vitamin E regulates the maturation and functioning of dendritic cells, whose role is to connect the innate and adaptive immune system to coordinate the immune response. Other main roles of vitamin E isoforms include the increase in NK cells (natural killer cells) activity, humoral response, antibody function, and the improvement of T lymphocyte synapses formation as well as the initiation of the T cell activation signal [76–78]. Later studies revealed that various forms of vitamin E act as signaling and gene-regulating molecules and indicated a nonantioxidant molecular function of α-tocopherol [79]. However, Traber and Atkinson [74] underlined that the mechanism of action of α-tocopherol results from its antioxidative role. It seems that this fundamental role of compounds with vitamin E activity is unquestionable, while its other properties require confirmation in further in vivo research.

Interest in tocopherols and tocotrienols has grown in recent years due to the emerging evidence that they can prevent common diseases. Both tocopherols and tocotrienols have been shown as compounds with the following properties: anti-atherosclerotic [80], anti-cancer [42,50,81,82], anti-allergic [83], improvement of immune functions [75], anti-cardiovascular disease [84], anti-lipidemic [85], anti-diabetic [86], antihypertensive [87], anti-inflammatory [88], anti-obesity [5], and anti-non-alcoholic steatohepatitis (NASH) [89]. On the other hand, the results of many clinical trials do not confirm the protective role of α-tocopherol in preventing disease in people with adequate nutritional status. Based on the current state of research, γ-tocopherol and tocotrienols, as well as metabolites of α- and γ-tocotrienol, are promising compounds in the prevention of diseases driven by acute inflammatory and oxidative damage. To verify their biological role, large-scale clinical trials are needed [44].

7. α-Tocopherol Status and Requirements

The assessment of the human requirement for α-tocopherol is hampered by the rare occurrence of clinical symptoms of deficiency [3]. Symptoms usually develop in premature babies, infants, and adults with fat malabsorption, liver disease, or genetic diseases [78]. Extremely low values of α-tocopherol in the body may lead to disease named ataxia with vitamin E deficiency (AVED). This is a rare disorder caused by a mutation in the gene encoding α-TTP. Patients with AVED have the ability to absorb vitamin E isoforms in the intestine, but they have extremely poor ability to retain it. Symptoms of the disease include as follows: progressive ataxia, clumsiness of the hands, loss of proprioception, areflexia, retinal atrophy, degeneration of the spine, accumulation of lipofuscin in neurons, and loss of Purkinje cells. The disease causes low blood levels of α-tocopherol, but it can be prevented by α-tocopherol supplementation. People who suffer from α-tocopherol deficiency because of genetic mutations also have impaired selectivity between α-tocopherol and γ-tocopherol,

and increased excretion of α-CEHC [45,90]. Other forms of vitamin E than α-tocopherol are not effective against the human deficiency disease [91]. Severe α-tocopherol deficiency causes significant neuronal disorders, such as ataxia and oxidative disorders, cardiovascular diseases, cancer, and cataracts. As pointed out by Azzi [91], only α-tocopherol should be called vitamin E, so consistently, both in the diet and in recommendations, only this form of the vitamin E family should be considered.

Now, 8–15 mg of α-tocopherol or an α-tocopherol equivalent for women and men, according to the different scientific institutions, is recommended [25,92–94]. There are significant differences in α-tocopherol intake in different countries, varying from 8 to 10 mg/person/day in Finland, Iceland, Japan, and New Zealand, to 20 to 25 mg/person/day in France, Greece and Spain [3]. However, in a study that focused on a comparison of vitamin E intake in different subpopulations, over 80% of the mean and the median data points were below the RDA of 15 mg/day [95].

It was also noted that the requirements for α-tocopherol depend on the content of polyunsaturated fatty acids (PUFA) in a diet [96]. Peroxyl radicals react 1000 times faster with α-tocopherol than with polyunsaturated fatty acids (PUFA), which prevent their further oxidation [97]. Therefore, to quantify the need for α-tocopherol, in addition to the basal need, additional amounts depending on PUFA intake should be considered. It has been estimated that the need for α-tocopherol should be in the range of 12–20 mg/day for the "typical" range of PUFA consumption [96].

The concentration of α-tocopherol in serum or plasma is the main method used for α-tocopherol status assessment. According to Traber et al. [98], levels of α-tocopherol in serum below 9 µmol/L in men and below 12 µmol/L in women are considered as deficiency. A large prospective cohort analysis with more than 30 years of follow-up has provided strong evidence that men with higher serum α-tocopherol levels (≥14.2 mg/L vs. <9.3 mg/L) had lower overall mortality and lower mortality from cardiovascular disease (CVD), heart disease, stroke, cancer, and respiratory disease [99]. In cancer prevention studies, higher blood levels of α-tocopherol were associated with lower mortality. The lowest total mortality was observed at the concentration of α-tocopherol at the level of 30 µmol/L in the blood serum [100,101]. Additionally, other results of observational studies showed that at the point of 30 µmol/L and above, the concentration of α-tocopherol in the serum has a positive effect on human health [102]. However, as it was summarized by Eggersdorfer [95], only 21% of the reported populations and subpopulations reached this threshold, which may indicate a generally low vitamin E (α-tocopherol) nutritional status worldwide. No reference values have been established for the other forms of vitamin E. According to Traber [43], plasma α-tocopherol concentration is not a reliable marker for the assessment of vitamin E status, especially in subjects with an abnormal lipids profile. Therefore, adjusting α-tocopherol to plasma lipids and lipoproteins is recommended. However, it is not widely used, and it may be a cause of overinterpretation and ambiguity of the described results about α-tocopherol status. Plasma α-tocopherol concentration may be modified by several factors such as age, gender, lifestyle, low circulating lipid levels, genetic variation and variation in the absorption, metabolism, and excretion of vitamin E, as well as by obesity, metabolic syndrome, or high levels of oxidative stress [5,12]. Urine or plasma α-CEHC has been suggested as a better biomarker of adequate vitamin E status, but the methodology was not sensitive enough to detect their low levels and thus they are not widely used [43]. Additionally, the assessment of vitamin E status is also difficult because it is a fat-soluble vitamin, which is stored in adipose tissue [12].

It is generally accepted that the concentration of α-tocopherol in adipose tissue reflects the long-term concentration of vitamin E [12]. Studies suggest that the α-tocopherol that is stored in the tissues would not be released on demand, but its concentration depends on the lipid content of the tissue. Adipose tissues were used to assess the long-term status of α-tocopherol, and it was determined that the typical concentration of α-tocopherol in adipose tissue in adults is about 100–300 µg/g (200–700 nmol/g). Additionally, the concentration of α-tocopherol in adipose tissue was used to determine the adequacy of

vitamin E supplementation in patients with deficiency and was related to the concentration in peripheral nerves [12]. Measuring this biomarker of vitamin E status requires a biopsy of adipose tissues that is not widely accepted and used.

In conclusion, validated questionnaires and appropriate biomarkers are needed to assess vitamin E intake and status. To assess the dietary intake, mainly dietary records are used; however, they may lead to losing the key sources of vitamin E such as nuts and fats. In turn, for a nutritional status assessment, an adjustment for plasma lipids should be applied. If possible, the concentration of metabolites in the plasma and urine should be measured.

8. Adiposity and Vitamin E status

The existing results indicated a significant relationship between the content of adipose tissue in the body and the demand and metabolism of tocopherols and tocotrienols, mainly α-tocopherol. An excessive level of adipose tissue generates chronic inflammation visible in the increased secretion of cytokines, proteins, and immune response mediators, leading to the activation of inflammatory signaling pathways. Chronic, low-grade inflammation in obesity leads to increased oxidative stress and a disturbed balance of oxidants and antioxidants in the body [103]. Bioactive dietary antioxidants such as tocopherols and tocotrienols can prevent damage caused by inflammation and reactive oxygen species, thereby reducing the negative effects of obesity. On the other hand, an excess of adipose tissue may generate in the body an increased demand for antioxidants, which may cause their greater utilization, leading to their decreased concentration in the blood [104].

Research into the relationship between obesity and plasma α-tocopherol concentration is inconclusive. In some studies, the concentration of tocopherols (α-, γ-) in blood plasma was significantly and positively associated with anthropometric indices, the strongest associations of which were with waist-to-hip ratio (WHR), body mass index (BMI), waist circumference (WC), waist-to-height ratio (WHtR), indicative of central obesity [104,105]. Some studies have found opposite results and a negative relationship between plasma α-tocopherol concentration and the incidence of central obesity [106–108]. The results of Barzegar-Amini and colleagues' studies [68] showed that low serum α-tocopherol levels were significantly associated with increased waist and hip circumference, body weight, and cholesterol and triglyceride levels. It can be assumed that all these associations show certain tendencies, but the clinical significance of the associations may be questionable.

More valuable for further research seem to be the results concerning the relationships between adiposity and plasma α-tocopherol, other vitamin E isomers, or their metabolites concentration. Chai et al. [109] found significantly higher levels of γ-tocopherol in the blood in people with obesity compared with subjects with normal body weight (classification based on BMI) and a lack of differences between the groups for α-tocopherol [109]. It has also been shown that in people with metabolic syndrome, which was associated, i.e., with excessive adipose tissue, the level of excreted α-tocopherol metabolites as well as the plasma level of α-tocopherol were lower, and the level of oxidative stress increased [110].

In patients with excess body weight who remained on a reduction diet for six weeks, significant reduction in blood plasma α-tocopherol was observed. Almost 80% of them had this level below 20 μmol/L. This may indicate an increased risk for cardiovascular diseases and low antioxidant protection [103]. Similar results were obtained after the eight-week weight reduction program [111]. Eighty-five subjects with initially normal levels of α-tocopherol (mean 28 μmol/L) reduced their body weight by an average of 13%, but a significant decrease in the level of α-tocopherol (to about 21 μmol/L) was observed [111]. It is unclear whether the above-mentioned differences in distribution of the α-tocopherol and other isoforms are due to insufficient intake, changes in metabolism in obese individuals, or due to these two factors simultaneously [112]. This is explained by decreased α-tocopherol catabolism (by slower turnover) in people with excess body weight, although it is unclear whether this is due to greater oxidative damage, decreased dietary vitamin intake, or impaired absorption. Any reduction in the absorption of α-tocopherol

limits its availability. In addition, inflammation and increased oxidation associated with metabolic disorders reduce the bioavailability of tocopherol [37].

Recently, the research results also indicated that tocotrienols may have a positive effect in reducing obesity. It has been shown that tocotrioenols inhibit adipogenesis by reducing the accumulation of triglycerides (TGs) and lipid droplets in murine Hepa 1-6 liver cancer cells, HepG2 human liver cancer cells, 3T3-L1 preadipocytes, and human adipose-derived stem cells (hASCs). γ-T3 was characterized by the greatest potency of the anti-adipogenic effect, followed by δ-, β-, and α- forms. Additionally, studies have reported that tocotrienols reduce body weight, especially fat mass in obese animals [112]. Uto-Kondo et al. [113] assessed the effect of a tocotrienol-rich fraction from palm oil on adipocyte differentiation in 3T3-L1 cells. The authors suggested that the α- and γ-tocotrienol fractions inhibited the differentiation of pre-adipocytes into adipocytes, potentially preventing obesity. A study in rats [114] showed that γ-tocotrienol (60 mg/kg body weight/day) decreased adipose tissue mass induced by various doses of glucocorticoid.

Based on those results, some authors indicate that strong epidemiological evidence is still needed regarding the relationship between vitamin E intake, status, and weight loss in humans [115]. However, on the other hand, given tocopherols and tocotrienols metabolism and the existence of the specific proteins for α-tocopherol, it may be supposed that vitamin E, despite its undoubted bioactivity, will be not a remedy for metabolic syndrome or obesity.

These results rather indicate the need for further research aimed at establishing the vitamin E requirements and desirable plasma concentrations for non-α-tocopherol forms for individuals with disorders associated with increased oxidative stress or inflammation [37,110]. Nonetheless, it is worth noting that people with excess body weight do not meet the requirements for many vitamins and minerals in their diet (the phenomenon of malnutrition in obesity), including vitamin E. It is often related to the reduction of fat in the diet, which is the main source of many lipid-soluble compounds, such as vitamin E [116].

9. Summary

This review presents the available evidence confirming the role of γ- and δ-tocopherol as well as α- and γ-tocotrienol as bioactive compounds that are defined to have documented health benefits beyond the normal nutritional effects and a positive effect on specific functions of the human body. The positive role of α-, γ-, and δ- tocopherol as well as α- and γ-tocotrienol and also their metabolites in cell homeostasis; modulation of signaling pathways, enzymes, and genes; and the mechanism of antioxidant action contributing to the reduction of inflammation was clearly documented. This activity is possible by specific interactions with enzymes, structural proteins, and structural lipids as well as by transcription. The metabolism of vitamin E is under constant control of the organism, and RRR-α-tocopherol as the most biologically active form is preferred. Although many studies confirm the multidirectional activity of vitamin E isoforms, the exact mechanisms require further research. In addition to its influence on fertility, early animal studies documented that this vitamin is a necessary factor for other vital functions and for the development of tissues and organs such as brain and nerves, muscles and bones, skin, bone marrow, and blood. Some of these functions have been confirmed in humans, while others are still under investigation. Over past few years, several laboratories have described new and even unexpected cellular and molecular properties of tocopherols, tocotrienols, and their metabolites (Figure 3).

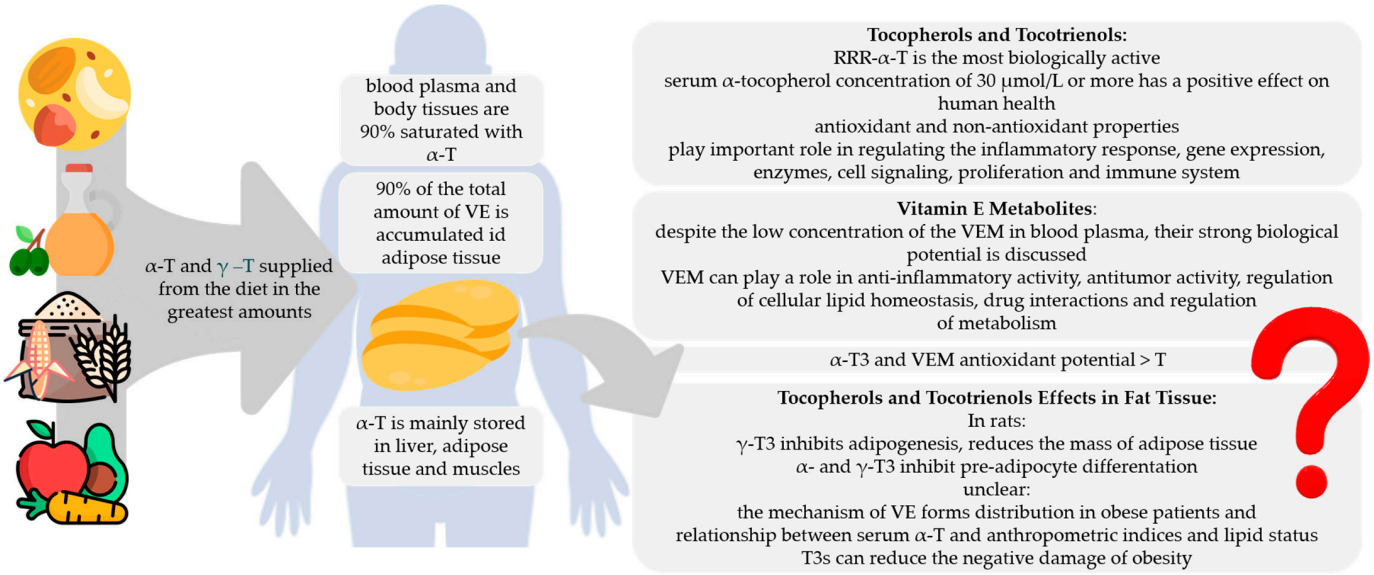


Figure 3. Summary of potential health benefits of tocopherols and tocotrienols. T, tocopherol; T3, tocotrienol; VE, vitamin E; VEM, vitamin E metabolites. The author's own study based on [4,8,10–12,35–37,74,99,104–107,112–114]; icons source: www.flaticon.com (accessed on 31 March 2021).

In many scientific studies, the terms α-tocopherol and vitamin E are used interchangeably, which can result in ambiguity and confusion. The need to specify nomenclature, to stop combining isoforms into one vitamin E compound, is highlighted. This is mainly due to the differences between the various forms of vitamin E as well as the differences in the mechanisms of action and biological activity between tocopherols and tocotrienols as well as the fact that α-tocopherol is the form of vitamin E that preferentially accumulates in blood and tissues This aspect, similar to the consideration of lipids/lipoproteins in the determination of plasma tocopherols levels, needs to be systematized. We believe that this updated knowledge is worth considering as a way of improving the nutritional recommendations and for creating the criteria for a new wave of research into tocopherols and tocotrienols and their relationship with health.

Author Contributions: Conceptualization, K.S. and M.G.; resources, K.S. and A.C.; writing—original draft preparation, K.S.; writing—review and editing, M.G.; visualization, K.S. and A.C.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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.

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Warszawa, 19.03.2025 r.

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OŚWIADCZENIE

Jako współautor pracy pt. „**Tocopherols and Tocotrienols—Bioactive Dietary Compounds; What Is Certain, What Is Doubt?**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

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Aleksandra Chojnacka

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Article

Nutritional Status of Vitamin E and Its Association with Metabolic Health in Adults

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Abstract: Background: Vitamin E is one of the key dietary antioxidants. However, current evidence remains insufficient to establish a definitive relationship between circulating vitamin E levels, body fat content, and their influence on metabolic health. This study aimed to assess and compare the vitamin E nutritional status in adults with normal and excess body fat and its determinants. Methods: Concentrations of vitamin E isoforms (α- and γ-tocopherols, α- and γ-tocotrienols) were assessed in 127 individuals. Body fat content and other anthropometric indices, as well as biochemical markers such as lipid profile, plasma fatty acid concentration and C-reactive protein, were identified as markers of metabolic health. Participants were divided into two groups: with normal and excess body fat (defined as more than 30% in women and more than 25% in men). Results: The determinants of higher α-tocopherol concentrations were lower body fat content and higher levels of circulating lipids as HDL and LDL (R^2 = 0.221, $p < 0.001$ in a model of multivariate linear regression). The level of circulating vitamin E isoforms correlated with the concentration of CRP (r = −0.464 for α-T, r = −0.453 for αT3, r = −0.270 for γ-T, r = −0.355 for γ-T3). Similarly, elevated concentrations of vitamin E isoforms are linked to lower adipose tissue content, which may contribute to lower inflammation and improved metabolic health (r = −0.359 for α-T, r = −0.333 for αT3, r = −0.276 for γ-T3, no significant correlation for γ-T). Conclusions: These results reveal that the vitamin E status of adults with excess body fat may be inadequate and linked to poorer metabolic health. We found that the determinants of lower plasma vitamin E were higher BF and lower TC and its fraction, with the strongest correlations being found for HDL.

Keywords: plasma α-tocopherols; plasma γ-tocopherols; plasma α-tocotrienols; plasma γ-tocotrienols; body fat; plasma fatty acids; lipid status; inflammation; adults

Academic Editor: Sita Aggarwal

Received: 19 December 2024
Revised: 18 January 2025
Accepted: 22 January 2025
Published: 23 January 2025

Citation: Szewczyk, K.; Bryś, J.; Brzezińska, R.; Górnicka, M. Nutritional Status of Vitamin E and Its Association with Metabolic Health in Adults. *Nutrients* **2025**, *17*, 408. https://doi.org/10.3390/nu17030408

1. Introduction

Vitamin E is a group of eight structurally similar compounds comprising four tocopherols (α-; β-; γ-; δ-forms) with single bonds between carbon atoms and four tocotrienols (α-; β-; γ-; δ-forms), each featuring a single double bond in the side chain [1]. This family of compounds, classified as vitamin E, is synthesized by plants and is primarily found in dietary sources such as vegetable oils, seeds, kernels, and nuts [2]. Current findings suggest that various forms of vitamin E may protect cell membranes from oxidative damage, contribute to the inhibition of inflammatory pathways leading to atherosclerosis and non-alcoholic fatty liver disease, and reduce inflammatory biomarkers in individuals with insulin resistance [3,4]. Vitamin E plays a multifaceted role in adipose tissue, influencing its

metabolic and structural characteristics. Evidence suggests that vitamin E may modulate adipose tissue function by reducing oxidative stress, inflammation, and fibrosis, thereby improving metabolic profiles in the context of obesity [5]. Circulating levels of vitamin E, particularly α- and γ-tocopherol, have been positively associated with increased visceral and subcutaneous adipose tissue volumes, as well as a higher likelihood of metabolic syndrome. This relationship indicates a complex interplay in which vitamin E levels correlate with specific features of excess body fat [6].

Results of our previous studies have shown that only 40–57% of the studied group met the level of adequate intake (AI) for vitamin E [7]. The predominant dietary forms were α- and γ-tocopherols and tocotrienols [4]. Among these, α-tocopherol is the most biologically active and extensively studied due to the presence of a specific transport protein, α-tocopherol transfer protein (α-TTP). However, research suggests that other forms of vitamin E may be preferentially absorbed or utilized when circulating α-tocopherol levels are low [8].

In recent years, other forms of vitamin E have gained attention for their superior antioxidant and anti-inflammatory properties compared to α-tocopherol, leading to a shift in research focus [4,7,9]. Tocotrienols are also believed to promote angiogenesis and regulate enzyme activity and transcriptional pathways, which may contribute to cancer prevention [10]. Vitamin E has shown also promising results in reducing inflammation measured by the C-reactive protein (CRP) level and/or tumor necrosis factor-α (TNF-α) concentration, suggesting its potential as a therapeutic agent in the treatment of inflammation-related diseases [4].

Vitamin E is absorbed in the small intestine, often enhanced by the presence of dietary fats, and is incorporated into chylomicrons for transport through the lymphatic system [11]. In the liver, α-tocopherol is preferentially incorporated into very low-density lipoproteins (VLDL) and secreted into the bloodstream [12]. Vitamin E is distributed in the plasma primarily associated with lipoproteins, including low-density lipoproteins (LDL) and high-density lipoproteins (HDL) [13]. The absorption of vitamin E is increased when consumed with dietary fat. Low-fat diets may result in reduced vitamin E levels within the body [14].

Thus, vitamin E metabolism is associated with dietary lipids (found in vegetable oils), body lipids (transported by HDL and magazine in fat tissue) and cell lipids (cell membranes) [15]. The bioavailability and utilization of vitamin E are influenced by many age-related factors, including dietary intake, absorption, transport, and metabolism [16].

Plasma α-tocopherol is commonly used as a marker of vitamin E status, despite the lack of a definitive correlation between its plasma levels and tissue concentrations [17]. Since α-tocopherol is primarily associated with lipoproteins in the lipid fraction, minor fluctuations in circulating lipids can significantly affect its plasma concentration. Therefore, lipid-adjusted α-tocopherol—normalized to cholesterol or triglycerides—is considered a more reliable indicator [18]. Although plasma concentrations of vitamin E in older adults are comparable to those in younger adults, older adults have increased oxidative stress and subsequent cellular damage. This is thought to be one of the mechanisms underlying the age-related dysregulation of immune and inflammatory responses, resulting in increased incidence, morbidity, and mortality from infections and chronic noncommunicable diseases [16].

The relationship between fatty acid profiles and vitamin E availability primarily arises from their common dietary sources, such as oils and nuts, which are rich in both nutrients [19,20]. These sources provide essential nutrients, including polyunsaturated fatty acids (PUFAs) and vitamin E, with the latter's concentration increasing in parallel with the levels of unsaturated fatty acids [19]. The coexistence of these nutrients in dietary sources highlights a potential relationship between their intake and associated health benefits [20].

Fatty acids in plasma serve as biomarkers for short-term dietary fat intake, exhibiting rapid responses to changes in dietary composition [21]. High-fat diets significantly influence lipid management parameters by altering the concentrations of total cholesterol, LDL cholesterol, and triglycerides, thereby modifying the overall lipid profile in the bloodstream [22].

Moreover, emerging evidence suggests that circulating fatty acids, a cause of oxidative stress, are related to metabolic disorders and could play a role in obesity [23,24]. The inflammatory potential of fatty acids in obesity varies significantly depending on their type, with distinct effects on inflammation and metabolic health. Saturated fatty acids (SFA) are generally pro-inflammatory, whereas omega-3 polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) exhibit anti-inflammatory properties [25,26]. Dietary fats are critical determinants of plasma lipid concentrations and play a central role in lipid metabolism [27]. Among these, non-esterified fatty acids (NEFAs), often referred to as fatty acids, are of particular interest due to their involvement in obesity-related metabolic dysfunction [28].

The metabolism of fatty acids is closely linked to the activity of key enzymes, including delta-5 desaturase (D5D), delta-6 desaturase (D6D), and stearoyl-CoA desaturases (SCD-16 and SCD-18), which play crucial roles in metabolic health. D5D activity is generally associated with favorable metabolic effects, such as a reduced risk of metabolic syndrome (MetS), lower triglyceride levels, decreased diastolic blood pressure, and reduced waist circumference [29]. The activities of D6D, SCD-16 and SCD-18 are associated with adverse metabolic outcomes, such as elevated triglyceride levels and reduced HDL cholesterol, contributing to the development of MetS [29–31].

The relationship between plasma vitamin E isoforms and metabolic health is not yet fully understood. In our study, we considered 'good' metabolic health as the absence of the metabolic syndrome components [32] and lower risk of cardiometabolic diseases, evaluated through higher D5D and lower D6D, SCD-16, SCD-18 activity, as well as decreased levels of inflammation. Therefore, the aim of this study was to assess and compare the nutritional status of vitamin E in adults with normal and excess body fat content and its determinants. Furthermore, it examined the hypothesis that lower concentrations of α-tocopherol, γ-tocopherol, α-tocotrienol, and γ-tocotrienol are linked with poorer metabolic health (including excess body fat and central adiposity, abnormal profile of lipid compounds, and elevated inflammation).

2. Materials and Methods

2.1. Ethical Approval

The research adhered to the principles outlined in the Declaration of Helsinki, and the Ethics Committee of the Institute of Human Nutrition Sciences of the Warsaw University of Life Sciences (Resolution No. 05/2019), approved all procedures involving human subjects. Before participating in the study, all subjects provided their written informed consent, before the survey.

2.2. Individuals Recruitment

The recruitment for this observational, cross-sectional study took place between October 2021 and October 2022. Participants were recruited from social media users through advertisements in social media groups. Patients came from both the Warsaw agglomeration and other towns from the entire territory of Poland. A total of 148 people expressed their willingness to participate in the study. The study involved one meeting where anthropometric data, blood samples, and sociodemographic data were collected. The study group selection process and the inclusion criteria are outlined in Figure 1. After applying the criteria, the data from 127 subjects were included in the study.

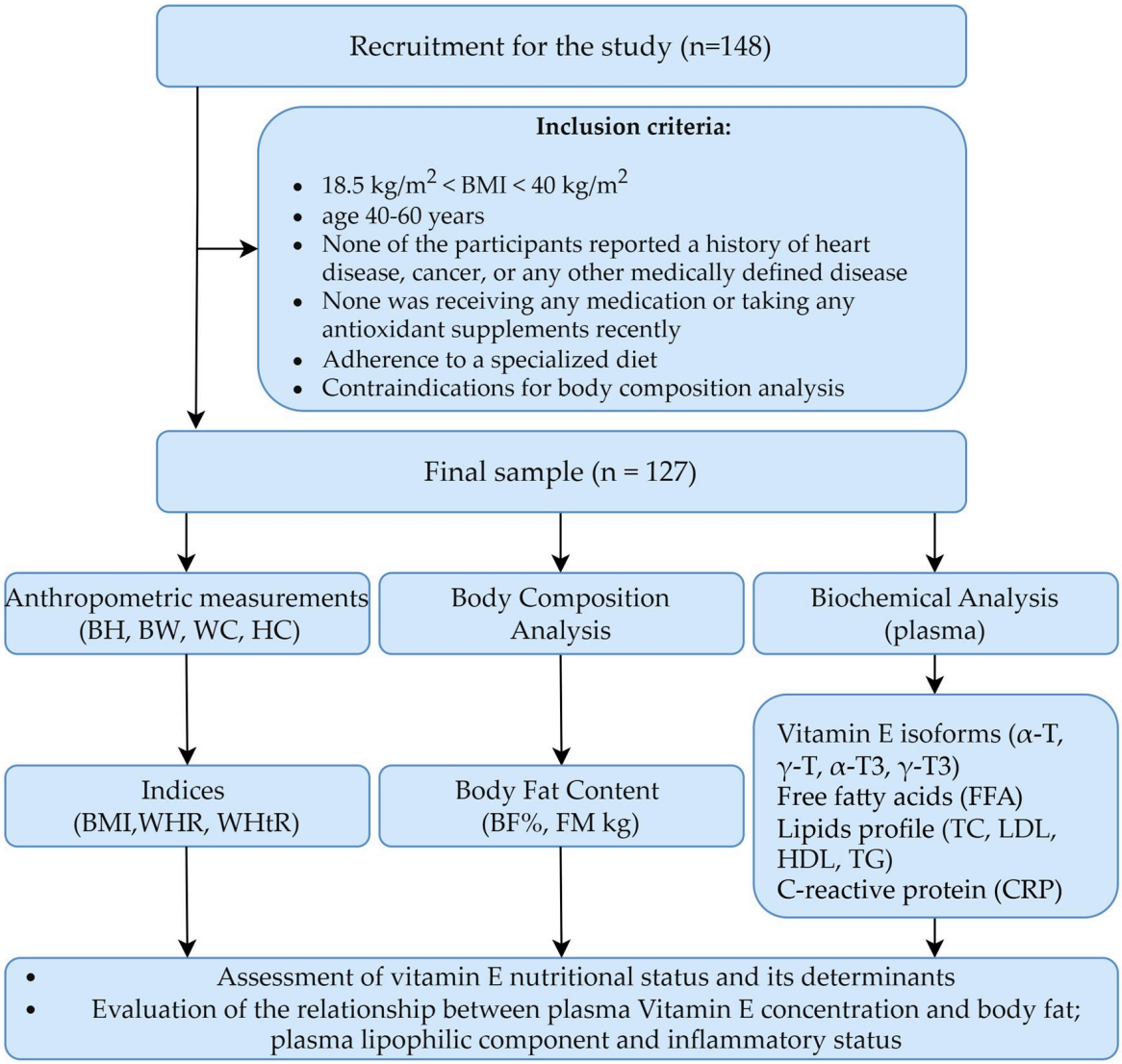

Figure 1. Study design. BH, body height; BW, body weight; WC, waist circumference; HC, hip circumference; BF, body fat content; BMI, body mass index; WHR, waist–hip ratio; WHtR, waist to height ratio; FM, fat mass; T, tocopherol; T3, tocotrienol; FA, fatty acids; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerols; CRP, C-reactive protein.

2.3. Anthropometric Measurements

Anthropometric measurements, including body height (BH), body weight (BW), waist circumference (WC), and hip circumference (HC), were conducted following the standardized protocols outlined in the International Standards for Anthropometric Assessment (ISAK) guidelines [33]. The measurements employed professional-grade equipment and a calibrated measuring tape. Body mass was assessed using a digital electronic scale with a precision of 0.1 kg (SECA 799, Hamburg, Germany). BH was determined with a stadiometer, ensuring the head was positioned in the Frankfurt horizontal plane, and recorded with an accuracy of 0.1 cm (SECA 220, Hamburg, Germany). WC was measured using a stretch-resistant tape that applies a constant tension of 100 g (SECA 201, Hamburg, Germany), positioned midway between the iliac crest and the lower rib at the anterior axillary line during a relaxed exhalation. HC was recorded at the widest part of the buttocks, with the measuring tape held parallel to the floor.

Based on the anthropometric measurements, several key anthropometric indices commonly used in screening studies were calculated: the Body Mass Index (BMI) calculated

as body mass in kilograms divided by the square of body height in meters (kg/m^2). The range from 18.5 to 24.99 was considered a normal body weight, whereas a BMI above 25 was classified as overweight [34]. Additionally, the waist-to-hip ratio (WHR) and the waist-to-height ratio (WHtR) were computed, serving as indices of central obesity and metabolic risk assessment. WHR above 0.8 for women and men was interpreted as an accumulation of adipose tissue in the android area, while the opposite results as the accumulation of adipose tissue in the gynoid area [34]. For WHtR, a cut-off point of 0.5 was adopted, above which results indicate excess adipose tissue accumulation in the abdominal area [34].

2.4. Body Composition Analysis

Body composition, including Body Fat Content (BF) and Fat Mas (FM), was assessed using the bioelectrical impedance technique using a Tanita eight-point multifrequency analyzer (Tanita BC-418 MA, Tanita Co., Tokyo, Japan). Measurements were performed under standardized conditions according to the manufacturer's protocol: fasting for at least four hours, avoiding vigorous physical activity for at least 12 h before the study, abstaining from alcohol for 24 h and caffeine for four hours before the study, and voiding urine before BIA analysis [35]. For further analyses, the cut-off points of 30% of body fat in women and 25% in men were assumed to indicate excess body fat [36].

All measurements were performed under strictly standardized conditions (room temperature 22 °C, air humidity 45%) by one well-trained researcher (dietitian) using the same device. Measurements were performed twice in light clothing and without shoes, and averages were calculated.

2.5. Biochemical Analysis

In assessing the level of the lipophilic compounds, the research material was venous blood collected by qualified personnel according to the standard procedure in laboratory conditions. Blood was collected from patients after a 12-h fast, after overnight rest, from a peripheral vein in the lower elbow joint. The total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols, and CRP as a marker of inflammation were assessed in cooperation with an external laboratory by the procedure in force at a given facility.

Based on the reference values used in the laboratory, the following cut-off points for the lipid profile were adopted and interpreted as correct levels:

- Total Cholesterol (TC) < 190 mg/dL;
- High-density lipoprotein (HDL) > 50 mg/dL for women and 40 mg/dL for men;
- Low-density Lipoprotein (LDL) < 115 mg/dL;
- Triacylglycerols (TG) < 150 mg/dL.

Additionally, blood was taken to test for vitamin E and FA. After blood was collected from the study participants, the samples were centrifuged in a rotary centrifuge for 10 min at +4 °C, 8000 rpm. The plasma from the sediment was transferred to plastic tubes. All collected plasma samples were protected from light and frozen at −80 °C until analysis.

2.5.1. Plasma Vitamin E Analysis

Drawing from our study, which evaluated the intake of all eight vitamin E isoforms, four with the highest consumption levels in the Polish population were prioritized for further analysis [7]. Research indicates that typical diets predominantly provide tocopherols, particularly α-tocopherol and γ-tocopherol [37]. Consequently, the selected isoforms for detailed examination included α-tocopherol (α-T), γ-tocopherol (γ-T), α-tocotrienol (α-T3), and γ-tocotrienol (γ-T3).

High-performance liquid chromatography and a diode array detector (HPLC-DAD) were used to determine selected tocopherols and tocotrienols. In brief, 200 µL of plasma

was transferred to a 2 mL Eppendorf tube and deproteinized with 400 µL of a methanol solution containing 0.04% BHT as an antioxidant. Subsequently, 800 µL of n-hexane solution with 0.04% BHT was added to the deproteinized sample and extracted for 3 min in a shaker. Following this step, the samples underwent centrifugation for 10 min in a rotary centrifuge (MPW Med. Instruments, Warsaw, Poland) at a shaking speed of 8000 rpm and a temperature of 4 °C. Post-centrifugation, 700 µL of supernatant was transferred to a new tube, followed by evaporation in a vacuum evaporator (Labconco Corporation, Kansas City, MO, USA), for 10 min at 30 °C. Then the obtained sample was reconstituted in 180 µL of methanol. The sample injection volume was 50 µL. The HPLC analysis was conducted using a specialized HPLC system (Knauer Azura, Berlin, Germany) consisting of a P6.1L pump, DAD 2.1L detector, HT310L autosampler, and CT 2.1 thermostat. An analytical column C18 Grace Vydac 201TP54 (5 µm particle size, 250 mm length × 4.60 mm) was utilized to perform the chromatographic separation. The HPLC methodology was adopted from established techniques reported by Abidi [38]. The elution was performed in isocratic mode using a 9/1, (*v*/*v*) mixture of methanol and water at the constant flow rate of 1.0 mL/min. ClarityChrom chromatographic 9.1. software was used for instrument control, data acquisition, and data processing. Detection of α-tocopherol and α-tocotrienol, γ-tocotrienols, and γ-tocopherol was performed at 292 nm, 295 nm, and 298 nm, respectively. Vitamin E compounds were identified based on the retention time values compared to standards of tocopherols and tocotrienols (LGC Standards Sp. z o.o., Kiełpin, Poland), and their concentrations were recalculated and expressed as µmol/L. Each sample underwent separate duplicate analyses, and the average of the two measurements was utilized for subsequent analysis.

Based on the plasma concentration of α-tocopherol, the nutritional status was assessed as (1) low for values below 12 µmol/L, (2) adequate/optimal for the range between 12 and 30 µmol/L interpreted, and (3) pro-healthy for values above 30 µmol/L [39].

The ratio α-tocopherol/total lipid concentration (cholesterol + triacylglycerols) was also taken into account for further analyses as a marker of vitamin E status [18].

2.5.2. Plasma Fatty Acids Analysis as a Marker of Dietary Fat Intake

The plasma fatty acid (FA) composition can be used as an objective biomarker of dietary FA intake [40,41], and lipid metabolism [42].

Total plasma lipids were extracted via direct transesterification to fatty acid methyl esters (FAME), as described by Nikolic Turnic et al. [43]. Briefly, 1.5 mL of 3 M HCl in methanol was added to 100 µL of plasma, and the mixture was heated to 85 °C for 45 min. The samples were then cooled to room temperature, after which 1 mL of hexane was added. The mixture was subsequently centrifuged at 4000 rpm for 10 min at 4 °C, and the upper fraction was collected and evaporated using a CentriVap concentrator. The fatty acid profile was assessed using gas chromatography (GC) with a YL6100 GC gas chromatograph, equipped with a flame ionization detector (FID) and a BPX 70 capillary column (60 m length, 0.25 µm film thickness, and 0.25 mm internal diameter). The oven temperature was programmed as follows: 70 °C for 0.5 min, increased by 15 °C/min to 160 °C, then by 1.1 °C/min to 200 °C, held at 200 °C for 12 min, followed by a final increase of 30 °C/min to 225 °C, where it was maintained for 0.5 min. The injector and detector temperatures were set to 225 °C and 250 °C, respectively. Nitrogen was used as the carrier gas with a flow rate of 1.2 mL/min. The relative abundance of each fatty acid was quantified, and each sample was analyzed in duplicate. Results were expressed as relative percentages, determined by external normalization of the chromatographic peak areas. Fatty acids were identified by comparing the relative retention times with those of an external standard fatty acid methyl ester mixture (Supelco 37 Component FAME Mix, Sigma-Aldrich, Hamburg, Germany).

The enzymatic activity associated with fatty acid (FA) synthesis was assessed by calculating the ratios of the relative abundance of products to their respective precursors for each enzyme. Specifically, the activity of stearoyl-CoA desaturase (SCD) was evaluated using the palmitoleic-to-palmitic acid ratio (16:1*n*-7/16:0) for SCD-16, and the oleic-to-stearic acid ratio (18:1*n*-9/18:0) for SCD-18. In a similar manner, the activity of Δ6-desaturase (D6D) was determined from the dihomo-γ-linolenic-to-linoleic acid ratio (20:3*n*-6/18:2*n*-6), while Δ5-desaturase (D5D) activity was derived from the arachidonic to the dihomo-γ-linolenic acid ratio (20:4*n*-6/20:3*n*-6). Additionally, the ratio of stearic to palmitic acids (18:0/16:0) was used to estimate elongase activity [44].

2.6. Data Analysis

The data were reported as percentages for categorical variables and as means with standard deviations (SD) for continuous variables. Prior to conducting statistical tests, the normality of the data distribution was evaluated using the Shapiro–Wilk test. Group differences were assessed using the Pearson Chi-square test for categorical variables and either the Kruskal–Wallis test with post-hoc analysis for comparisons involving more than two groups or the Mann–Whitney U test for two-group comparisons of continuous variables. We used Spearman rank correlation analyses to investigate the link between the concentrations of vitamin E isoforms and potential determinants, including anthropometric variables, enzyme activities, fatty acid profiles, and lipid parameters. For variables showing significant correlations, we comprehensively evaluated all effects using univariate and multivariate regression analysis to identify the potential and the most robust predictors of α-tocopherol and other vitamin E isoforms. Additionally, we conducted stepwise forward multiple regression analysis to identify the potential determinants of vitamin E. The models were adjusted for age, gender and smoking status. The analyses were conducted using untransformed variables and were stratified by the total group, as well as separately for women and men. A significance threshold of $p \leq 0.05$ was adopted for all statistical tests. The analyses were performed using STATISTICA software version 13.0 (StatSoft Inc., Tulsa, OK, USA; StatSoft, Krakow, Poland). Additionally, the sample size for each group was initially estimated to be 64 participants (128 total) using G*Power 3.1.9.7. software. The calculations were based on the assumption of medium effects (Cohen's d = 0.5), the difference between two independent means (two groups), statistical power 0.8, and alpha significance level 0.05. Finally, for the obtained results of the main examined factor which was the percentage of body fat, the effect size was obtained in the form of Cohen's d = 2.8 (it was correctly calculated on the basis of the difference of means and common standard deviation), and the statistical power of the test was 0.9.

3. Results

3.1. Participant Characteristics

The study included 127 adult participants, of whom 61% were women (Table 1). The mean age was 49 ± 6 years. Over 80% had at least a secondary education, 77% lived in big cities, and 67% were employed full-time. Additionally, 79% indicated non-use of tobacco products, and almost half (49%) rated their health status as good or very good. Based on body fat content (Table 1), 47% of the studied group was classified as having normal body fat levels, while 53% as excess body fat. Individuals with excess body fat content were statistically significantly older than those with normal body fat levels (52 years vs. 46 years) and more frequently reported their health status as "bad" or "not bad not good" compared to those with normal body fat content (24% and 61% vs. 2% and 12%, respectively). A higher proportion of women was classified as having excess body fat (56%) than men (44%).

Table 1. Baseline characteristics of the study subjects and according to body fat content.

Variables	Total (n = 127)	Body Fat Content (%)		*p*-Value
		Normal (n = 60)	Excess (n = 67)	
Sociodemographic (n, %)				
Women	77 (61)	34 (57)	43 (64)	NS
Men	50 (39)	26 (43)	24 (36)	
Age in years (mean ± SD)	49 ± 6	46 ± 5	52 ± 6	<0.001
Education (n, %)				
Primary and basic vocational	15 (12)	3 (5)	12 (18)	<0.001
Secondary	42 (33)	7 (12)	35 (52)	
University	70 (55)	50 (83)	20 (30)	
Place of living (n, %)				
Village	10 (8)	3 (5)	7 (10)	NS
City < 100,000 inhab.	19 (15)	5 (8)	14 (21)	
City > 100,000 inhab.	98 (77)	52 (87)	46 (69)	
Professional status (n, %)				
Not working	24 (19)	6 (10)	18 (27)	NS
Work part-time	18 (14)	10 (17)	8 (12)	
Work full time	85 (67)	44 (73)	41 (61)	
Smoking (n, %)				
Yes	27 (21)	13 (22)	14 (21)	NS
No	100 (79)	47 (78)	53 (79)	
Health status self-assessment (n, %)				
Bad	17 (13)	1 (2)	16 (24)	<0.001
Not bad not good	48 (38)	7 (12)	41 (61)	
Good or very good	62 (49)	52 (87)	10 (15)	

NS, not significant; results of the *t*-Student test or the U-Mann–Whitney test; *p*-value < 0.05.

3.2. Lipid Profile, CRP and Fatty Acids Composition

Statistically significant lowest HDL and LDL cholesterol levels were identified in the group with excess body fat (Table 2). The vast majority (92%) of individuals with normal BF met the reference value for HDL cholesterol. In contrast, in 66% of individuals with excess BF HDL cholesterol was under recommendation. Similarly, TG concentration was higher in individuals with excess BF compared with those with normal BF (64% vs. 17%).

A significantly lower inflammation, as measured by CRP concentration, was found in individuals with normal BF compared to those with excess BF (0.118 mg/dL for women and 0.805 mg/dL for men vs. 1.8 mg/dL and 3.4 mg/dL, respectively) (Table 2).

Individuals with excess BF showed different plasma FA profiles. Significantly higher of MUFA (30.5% for women and 33.8% for men vs. 22.7% and 25.8%, respectively), lower PUFA (32.2% for women and 31.1% for men vs. 37.6% and 37.0%, respectively), lower of *n*-3 (5.18% for women and 4.46% for men vs. 4.01% and 3.71%, respectively), and *n*-6 (27.1% for women and 26.7% for men vs. 33.6% and 33.3%, respectively), compared to individuals with excess BF.

Table 2. Lipid profile and fatty acid composition and body fat content.

Variables	Total (n = 127)	Body Fat Content (%)						
		Normal (n = 60)		*p*-Value	Excess (n = 67)		*p*-Value	*p*-Value
		Women (n = 34)	Men (n = 26)		Women (n = 43)	Men (n = 24)		Normal vs. Excess
Lipid profile								
TC (mg/dL)	199.7 ± 33.4	197.3 ± 28.2	204.5 ± 37.1	NS	201.3 ± 34.7	194.8 ± 34.7	NS	NS
<190 mg/dL, n (%)		13 (38)	11 (42)	NS	12 (28)	12 (50)	NS	NS
≥190 mg/dL, n (%)		21(62)	15 (58)		31 (43)	12 (50)		
HDL (mg/dL)	53.2 ± 16.8	71.2 ± 10.1	54.8 ± 14.5	<0.001	46.2 ± 11.0	38.6 ± 12.6	0.002	<0.001
>50 mg/dL for women and 40 mg/dL for men, n (%)	78 (61)	33 (97)	22 (85)	NS	15 (35)	8 (33)	NS	<0.001
≤50 mg/dL for women and 40 mg/dL for men, n (%)	49 (39)	1 (3)	4 (15)		28 (65)	16 (67)		
LDL (mg/dL)	118.2 ± 32.0	121.5 ± 28.5	132.2 ± 35.2	NS	113.1 ± 30.8	107.4 ± 31.0	NS	<0.001
<115 mg/dL, n (%)	61 (48)	14 (41)	10 (38)	NS	22 (51)	15 (63)	NS	NS
≥115 mg/dL, n (%)	66 (52)	20 (59)	16 (62)		21 (49)	9 (37)		
TG (mg/dL)	139.3 ± 78.6	77.7 ± 35.0	118.0 ± 81.1	NS	168.5 ± 55.0	197.4 ± 91.2	NS	<0.001
<150 mg/dL, n (%)	74 (58)	31 (91)	19 (73)	NS	16 (37)	8 (33)	NS	<0.001
≥150 mg/dL, n (%)	53 (42)	3 (9)	7 (27)		27 (63)	16 (67)		
Inflammation								
CRP (mg/dL)	1.5 ± 3.4	0.118 ± 0.055	0.805 ± 2.5	0.034	1.8 ± 2.6	3.401 ± 6.03	NS	<0.001
Plasma fatty acids (results for selected FA)								
Σ SFA (%)	37.5 ± 7.5	39.6 ± 10.3	37.2 ± 5.5	NS	37.3 ± 7.1	35.1 ± 4.5	NS	NS
C 15:0	0.82 ± 3.7	1.04 ± 4.9	0.23 ± 0.1	NS	1.32 ± 4.7	0.26 ± 0.1	NS	0.024
C 16:0	21.8 ± 5.5	20.8 ± 5.3	22.8 ± 3.2	NS	21.5 ± 7.0	22.6 ± 4.8	NS	NS

Table 2. *Cont.*

Variables	Total (n = 127)	Body Fat Content (%)						
		Normal (n = 60)		*p*-Value	Excess (n = 67)		*p*-Value	*p*-Value
		Women (n = 34)	Men (n = 26)		Women (n = 43)	Men (n = 24)		Normal vs. Excess
C 17:0	0.26 ± 0.2	0.16 ± 0.2	0.21 ± 0.1	NS	0.33 ± 0.3	0.30 ± 0.2	NS	<0.001
C 18:0	9.5 ± 2.8	9.75 ± 1.7	8.65 ± 1.5	0.011	10.3 ± 4.1	8.67 ± 1.8	0.028	NS
Σ MUFA (%)	28.1 ± 6.6	22.7 ± 4.6	25.8 ± 5.7	0.025	30.5 ± 5.1	33.8 ± 5.4	0.015	<0.001
C 16:1	1.89 ± 1.0	1.22 ± 0.75	1.65 ± 0.8	0.037	2.28 ± 1.0	2.39 ± 1.0	NS	<0.001
C 18:1 *n*-9	25.8 ± 5.9	21.4 ± 4.25	24.1 ± 5.1	0.033	27.4 ± 5.2	31.0 ± 4.9	0.007	<0.001
Σ PUFA (%)	34.4 ± 7.3	37.6 ± 8.4	37.0 ± 6.4	NS	32.2 ± 6.5	31.1 ± 5.2	NS	<0.001
Σ *n*-3 (%)	4.43 ± 2.4	4.01 ± 2.4	3.71 ± 1.8	NS	5.18 ± 2.6	4.46 ± 2.0	NS	0.005
C 18:3 *n*-3	0.61 ± 0.4	0.482 ± 0.4	0.52 ± 0.3	NS	0.66 ± 0.3	0.82 ± 0.5	NS	0.0004
C 20:5 *n*-3	1.01 ± 1.0	0.958 ± 0.9	0.75 ± 0.7	NS	1.16 ± 1.0	1.14 ± 1.3	NS	0.029
C 22:6 *n*-3	2.80 ± 1.6	2.573 ± 1.7	2.44 ± 1.3	NS	3.37 ± 1.8	2.51 ± 1.1	0.039	0.039
Σ *n*-6 (%)	30.0 ± 6.8	33.6 ± 7.7	33.3 ± 6.1	NS	27.1 ± 5.4	26.7 ± 4.3	NS	<0.001
C 18:2 *n*-6	21.4 ± 7.1	25.7 ± 7.9	24.8 ± 6.6	NS	17.7 ± 4.8	18.4 ± 5.0	NS	<0.001
C 18:3 *n*-6	0.49 ± 1.8	0.210 ± 0.2	0.29 ± 0.2	NS	0.49 ± 0.6	1.14 ± 4.0	0.016	0.006
C 20:3 *n*-6	2.32 ± 1.5	1.66 ± 1.1	2.31 ± 1.5	NS	2.99 ± 1.6	2.06 ± 1.4	0.021	0.009
C 20:4 *n*-6	5.76 ± 1.7	6.02 ± 1.9	5.84 ± 2.3	NS	5.89 ± 1.5	5.05 ± 1.0	0.005	NS
n-3/*n*-6	0.154 ± 0.09	0.121 ± 0.08	0.115 ± 0.06	NS	0.197 ± 0.1	0.168 ± 0.07	NS	<0.001

NS, not significant; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerol; CRP, C-reactive protein, FA, fatty acids; Σ, sum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; *n*-3, omega 3; *n*-6, omega6; results of the Chi^2 or *t*-Student test or the U-Mann–Whitney test; *p*-value < 0.05.

3.3. Vitamin E Status and Isoforms in Dependence of Body Fat Content

Table 3 presents the results regarding plasma levels of vitamin E isoforms (α-tocopherol, γ-tocopherol, α-tocotrienol, γ-tocotrienol) and their total concentration by BF content (normal vs. excess). A low nutritional status of vitamin E was observed in 21% of the study participants, while a health-promoting status was identified in 30%. Individuals with a normal BF exhibited significantly higher concentrations of all examined vitamin E isoforms than individuals with excess BF (32.9 vs. 19,6 µmol/L for α-tocopherol, 12.6 vs. 8.69 µmol/L for γ-tocopherol, 7.79 vs. 4.41 µmol/L for α-tocotrienol, 10.7 vs. 7.47 µmol/L for γ-tocotrienol and 64.0 vs. 40.2 µmol/L for sum of Ts and T3s, $p < 0.001$). A pro-healthy status of α-T (>30 µmol/L) was observed in 50% of individuals with normal BF content and in 12% with excess BF. Individuals with excess BF were significantly more likely to exhibit a low nutritional status of vitamin E (30% vs. 12%) and less likely to display a health-promoting status (12% vs. 50%) compared to individuals with normal BF.

Table 3. Plasma Vitamin E isoforms and assessment of vitamin status and body fat content.

		Body Fat Content (%)		
Variables	**Total (n = 127)**	**Normal (n = 60)**	**Excess (n = 67)**	***p*-Value**
α—T (µmol/L)	25.9 ± 17.6	32.9 ± 19.5	19.6 ± 12.9	<0.001
Low, n (%) Adequate, n (%) Pro-Healthy, n (%)	27 (21) 62 (49) 38 (30)	7 (12) 23 (38) 30 (50)	20 (30) 39 (58) 8 (12)	<0.001
γ—T (µmol/L)	10.5 ± 9.1	12.6 ± 9.5	8.69 ± 8.3	<0.001
α—T3 (µmol/L)	6.0 ± 4.5	7.79 ± 4.1	4.41 ± 4.2	<0.001
γ—T3 (µmol/L)	9.0 ± 6.8	10.7 ± 5.7	7.47 ± 7.4	<0.001
Sum of Ts and T3s (µmol/L)	51.5 ± 31.4	64.0 ± 31.4	40.2 ± 26.9	<0.001

T, tocopherol; T3, tocotrienol; results of the Chi^2 or *t*-Student test or the U-Mann–Whitney test; *p*-value < 0.05.

3.4. Association Between Plasma Ts and T3s Concentration and Anthropometrics, Plasma Fatty Acids, Lipid Profile, Enzymes Activity and Inflammation

Negative correlations (Table 4) were observed between plasma α-T concentration and WHtR (r = −0.394), FM (r = −0.387), WC (r = −0.382), BF (r = −0.359), BMI (r = −0.346), BW (r = −0.340), HC (r = −0.334), and WHR (r = −0.277). Similar patterns were identified for other isoforms of vitamin E (α- and γ-) and their combined total.

Table 4. Correlation coefficients of anthropometric variables, fatty acids, enzyme activity and lipid profile in different vitamin E isoforms.

Variables	**α-T**	**α-T3**	**γ-T**	**γ-T3**	**Sum of Ts and T3s**	**α-T/TL**
BW (kg)	−0.340	−0.237	−0.219	-	−0.296	-
WC (cm)	−0.382	−0.287	−0.231	−0.227	−0.343	−0.241
HC (cm)	−0.334	−0.226	-	-	−0.279	−0.327
BF (%)	−0.359	−0.333	-	−0.276	−0.348	−0.584
FM (kg)	−0.387	−0.333	−0.230	−0.261	−0.365	−0.416
BMI (kg/m^2)	−0.346	−0.288	-	−0.228	−0.321	−0.289
WHR	−0.277	−0.233	−0.203	−0.183	−0.259	-
WHtR	−0.394	−0.339	−0.211	−0.291	−0.381	−0.408
Σ SFA (%)	0.192	-	-	-	-	-

Table 4. *Cont.*

Variables	α-T	α-T3	γ-T	γ-T3	Sum of Ts and T3s	α-T/TL
Σ MUFA (%)	−0.376	−0.294	−0.227	−0.238	−0.339	−0.384
Σ PUFA (%)	0.184	0.247	0.294	0.276	0.242	0.254
TC (mg/dL)	-	-	-	-	-	−0.396
HDL (mg/dL)	0.487	0.393	0.331	0.349	0.459	0.232
LDL (mg/dL)	0.255	-	-	-	-	-
TG (mg/dL)	−0.295	−0.337	−0.192	−0.268	−0.307	−0.625
CRP (mg/dL)	−0.464	−0.453	−0.270	−0.355	−0.454	−0.373
D5D	-	-	-	-	-	-
D6D	-	-	-	-	-	−0.234
SCD16	−0.265	−0.247	−0.194	-	−0.235	−0.377
SCD18	−0.237	−0.197	−0.182	-	−0.236	−0.232
Elongase	-	-	-	-	-	-

T, tocopherol; T3, tocotrienol; TL, total lipids; BW, body weight (kg), WC, waist circumference (cm), HC, hip circumference (kg), BF, body fat content (%); FM, fat mass (kg); BMI, body mass index (kg/m^2), WHR, waist–hip ratio; WHtR, waist to height ratio; Σ, sum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerol; CRP, C-reactive protein; D5D, delta-5-desaturase; D6D, delta-6-desaturase; SCD16, stearoyl-CoA desaturase 16; SCD18, stearoyl-CoA desaturase; -, the result is statistically insignificant; results of the Spearman rank correlations, $p < 0.05$.

The level of sum Ts and T3s showed a negative correlation with the sum of MUFA (r = −0.339), but a positive correlation with PUFA (r = 0.242).

A positive correlation was observed between α-T and HDL (r = 0.487) as well as LDL (r = 0.255), while a negative correlation was noted with TG (r = −0.295). Similar trends were seen for other isoforms of vitamin E (α- and γ-) and their combined total. However, TC and LDL were not significant for the other vitamin E forms. All vitamin E isoforms had the strongest correlation with HDL. Moreover, the strongest correlation was found between α-T/TL and TG (r = −0.625).

The concentration of all vitamin E isoforms strongly correlated negatively with CRP levels. Although the correlations for γ- isoforms were weaker, but still negative.

Significant negative correlations were also identified for SCD-16 with various vitamin E isoforms (except γ-tocotrienol), their combined total, and lipid-adjusted α-tocopherol concentration (e.g., r = −0.265 for α-T, r = −0.247 for α-T3, r = −0.194 for γ-T, r = −0.235 for the sum of tocopherols and tocotrienols, and r = −0.377 for α-T/TL). SCD-18 exhibited similar trends, albeit with slightly weaker correlations, showing negative relationships with α-T (r = −0.237), α-T3 (r = −0.197), γ-T (r = −0.182), the sum of tocopherols and tocotrienols (r = −0.236), and α-T/TL (r = −0.232). Furthermore, D6D negatively correlated with lipid-adjusted α-tocopherol concentration (r = −0.234). In contrast, no significant associations were observed between vitamin E levels and the activities of D5D or Elongase.

Univariate linear regression (Table 5) showed that significant predictors for higher vitamin E isoform concentration were higher HDL, lower: MUFA, CRP, WC, WHtR, FM and BMI. Stronger associations were found for α- forms.

Results of multivariate linear regression analysis (Table 6) revealed that the most significant predictors of α-T were the sum of MUFA (β = 0.476, 95% CI: −0.933; −0.019, p = 0.041) and HDL (β = 0.371, 95% CI: 0.071; 0.671, p = 0.016). This model explained 29% of the variance in α-T concentration. The most significant predictors for α-T3 were HDL (β = 0.347, 95% CI: 0.011; 0.684, p = 0.043). This model explained 22% of the variance in α-T3 concentration. For γ-Tand for γ-T3, HDL was a significant predictor (β = 0.330,

95% CI: 0.014; 0.646, $p = 0.041$ and β = 0.364, 95% CI: 0.023; 0.705, $p = 0.036$); however, the overall models were not statistically significant ($R^2 = 0.017$, $p = 0.118$ and $R^2 = 0.172$, $p = 0.105$).

Table 5. Results of univariate linear regression between vitamin E isoforms and significant variables.

Variables	α-T	α-T3	γ-T	γ-T3
	β, *p*-Value	β, *p*-Value	β, *p*-Value	β, *p*-Value
BW (kg)	−0.258, 0.003	−0.249, 0.005	−0.242, 0.006	−0.130, 0.144
WC (cm)	−0.318, <0.001	−0.312, <0.001	−0.243, 0.006	−0.205, 0.021
HC (cm)	−0.292, 0.001	−0.191, 0.031	-	−0.106, 0.236
FM (kg)	−0.347, <0.001	−0.303, <0.001	−0.223, 0.012	−0.192, 0.030
BF (%)	−0.336, <0.001	−0.292, <0.001	-	−0.202, 0.023
BMI (kg/m^2)	−0.315, <0.001	−0.290, <0.001	−0.204, 0.022	−0.176, 0.048
WHR	−0.223, 0.012	−0.302, <0.001	−0.203, 0.022	−0.213, 0.016
WHtR	−0.339, 0.001	−0.324, <0.001	−0.211, 0.017	−0.225, 0.011
Σ SFA (%)	0.069, 0.442	-	0.0006, 0.99	0.011, 0.905
Σ MUFA (%)	−0.239, 0.007	−0.322, <0.001	−0.250, 0.005	−0.234, 0.008
Σ PUFA (%)	0.143, 0.108	0.179, 0.044	0.223, 0.012	0.199, 0.024
TC (mg/dL)	0.283, <0.001	−0.033, 0.712	0.071, 0.431	−0.104, 0.245
HDL (mg/dL)	0.381, <0.001	0.383, <0.001	0.331, <0.001	0.294, 0.001
LDL (mg/dL)	0.320, <0.001	-	0.135, 0.129	−0.086, 0.335
TG (mg/dL)	−0.114, 0.201	−0.271, 0.002	−0.153, 0.085	−0.212, 0.017
CRP	−0.254, 0.004	−0.230, 0.009	−0.148, 0.097	−0.113, 0.206
D5D	-	-	−0.084, 0.345	−0.059, 0.513
D6D	-	-	0.047, 0.602	0.036, 0.689
SCD16	−0.127, 0.153	−0.126, 0.157	−0.143, 0.108	−0.004, 0.964
SCD18	−0.066, 0.463	−0.186, 0.036	−0.137, 0.124	−0.154, 0.084
Elongase	-	-	−0.030, 0.734	0.077, 0.388

T, tocopherol; T3, tocotrienol; BW, body weight (kg), WC, waist circumference (cm), HC, hip circumference (kg), BF, body fat content (%); FM, fat mass (kg); BMI, body mass index (kg/m^2), WHR, waist–hip ratio; WHtR, waist to height ratio; Σ, sum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerol; CRP, C-reactive protein; D5D, delta-5-desaturase; D6D, delta-6-desaturase; SCD16, stearoyl-CoA desaturase 16; SCD18, stearoyl-CoA desaturase; -, variables not included in the analyses; Adjusted for gender, age and smoking.

Since previous results revealed significant predictors for α-tocopherol, and considering that this form of vitamin E is recognized as the primary biologically active form, further analyses were conducted using stepwise multiple linear regression. This approach aimed to determine whether the automatic elimination of variables would uncover new associations. Model 1, without adjustments, indicates (Table 7) that plasma α-tocopherol concentration is associated with decreased BF (β = −0.201, 95% CI: −0.389; −0.012, $p = 0.037$). Additionally, α-tocopherol concentration is positively associated with TC (β = 0.234, 95% CI: 0.073; 0.394, $p = 0.005$) and HDL cholesterol (β = 0.230, 95% CI: 0.038; 0.421, $p = 0.019$). After adjustment for age, sex, and smoking status (model 2), an increase in plasma α-tocopherol concentration is associated with higher HDL cholesterol (β = 0.389, 95% CI: 0.201; 0.578, $p < 0.001$) and LDL cholesterol (β = 0.231, 95% CI: 0.068; 0.395, $p = 0.006$). However, in women, only HDL cholesterol was significantly associated with the α-tocopherol concentration (β = 0.443, 95% CI: 0.203; 0.684, $p < 0.001$), whereas in men, a significant association was observed with LDL cholesterol (β = 0.429, 95% CI: 0.148; 0.800, $p = 0.004$).

Table 6. Multivariate linear regression between plasma vitamin E isoform concentration and selected predictors (all effects).

Variables	α-T	α-T3	γ-T	γ-T3
	β (95% CI), *p*-Value	β (95% CI), *p*-Value	β (95% CI), *p*-Value	β (95% CI), *p*-Value
Σ SFA (%)	−0.068 (−0.298; 0.162), 0.560	-	-	-
Σ MUFA (%)	−0.476 (−0.933; −0.019), 0.041	−0.333 (−0.744; 0.079), 0.112	−0.383 (−0.800; 0.035), 0.072	−0.097 (−0.367; 0.173), 0.476
Σ PUFA (%)	-	0.053 (−0.178; 0.285), 0.648	0.138 (−0.088; 0.364), 0.230	0.109 (−0.109; 0.328), 0.324
SCD16	0.164 (−0.062; 0.389), 0.154	0.084 (−0.152; 0.320), 0.481	0.071 (−0.163; 0.305), 0.549	-
SCD18	0.318 (−0.035; 0.672), 0.077	0.226 (−0.142; 0.593), 0.226	0.227 (−0.142; 0.597), 0.225	-
BW (kg)	1.182 (−1.996; 4.360), 0.462	1.316 (−1.999; 4.632), 0.433	0.460 (−0.418; 1.339), 0.301	-
WC (cm)	−0.887 (−7.656; 5.882), 0.796	−0.422 (−7.451; 6.606), 0.905	−1.367 (−3.037; 0.304), 0.108	0.629 (−0.997; 2.255), 0.445
HC (cm)	−0.742 (−2.595; 1.111), 0.429	−0.696 (−2.629; 1.237), 0.477	-	-
FM (kg)	−0.390 (−1.872; 1.092), 0.603	−0.523 (−2.069; 1.022), 0.504	−0.148 (−0.883; 0.587), 0.691	−0.509 (−2.057; 1.039), 0.516
BF (%)	0.213 (−0.695; 1.121), 0.642	0.174 (−0.773; 1.122), 0.716	-	0.278 (−0.633; 1.189), 0.546
BMI (kg/m^2)	−0.471 (−3.665; 2.724), 0.771	−0.799 (−4.133; 2.534), 0.635	-	0.484 (−0.651; 1.619), 0.340
WHR	−0.618 (−2.757; 1.521), 0.568	−0.959 (−3.188; 1.269), 0.395	0.185 (−0.259; 0.629), 0.410	−0.122 (−0.565; 0.320), 0.583
WHtR	1.511 (−3.802; 6.824), 0.574	1.717 (−3.808; 7.244), 0.539	1.058 (−0.106; 2.221), 0.074	−0.613 (−2.249; 1.023), 0.459
CRP (mg/dL)	−0.110 (−0.305; 0.084), 0.263	−0.131 (−0.333; 0.071), 0.201	−0.059 (−0.264; 0.145), 0.565	−0.071 (−0.272; 0.131), 0.487
TC (mg/dL)	0.020 (−0.377; 0.417), 0.921	−0.015 (−0.364; 0.070), 0.182	−0.085 (−0.307; 0.136), 0.445	−0.065 (−0.284; 0.154), 0.559
HDL (mg/dL)	0.371 (0.071; 0.671), 0.016	0.347 (0.011; 0.684), 0.043	0.330 (0.014; 0.646), 0.041	0.364 (0.023; 0.705), 0.036
LDL (mg/dL)	0.166 (−0.016; 0.347), 0.413	-	-	-
TG (mg/dL)	0.181 (−0.063; 0.425), 0.144	−0.071 (−0.319; 0.177), 0.571	0.141 (−0.107; 0.390), 0.263	−0.055 (−0.307; 0.197), 0.666
R^2, *p*-Value	0.293, 0.003	0.221, 0.048	0.017, 0.118	0.172, 0.105

T, tocopherol; T3, tocotrienol; BW, body weight (kg), WC, waist circumference (cm), HC, hip circumference (kg), BF, body fat content (%); FM, fat mass (kg); BMI, body mass index (kg/m2), WHR, waist–hip ratio; WHtR, waist to height ratio; Σ, sum; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triacylglycerol; CRP, C-reactive protein; D5D, delta-5-desaturase; D6D, delta-6-desaturase; SCD16, stearoyl-CoA desaturase 16; SCD18, stearoyl-CoA desaturase; -, variables not included in the analyses; Adjusted for gender, age and smoking.

Table 7. Multivariate models between plasma Vitamin E isoform concentration and selected predictors.

Model	Variable	Total (n = 127)		Women (n = 77)		Men (n = 50)	
		β (95% CI), *p*-Value	R^2, *p*-Value	β (95% CI), *p*-Value	R^2, *p*-Value	β (95% CI), *p*-Value	R^2, *p*-Value
α-tocopherol							
1	BF (%)	−0.201 (−0.389; −0.012), 0.037	0.221, <0.001	0.170 (−0.397; 0.283), 0.739	0.257, <0.001	−0.158 (−0.494; 0.179), 0.350	0.310, <0.001
	TC (mg/dL)	0.234 (0.073; 0.394), 0.005		0.002 (−0.201; 0.204), 0.988		0.462 (0.196; 0.727), 0.001	
	HDL (mg/dL)	0.230 (0.038; 0.421), 0.019		0.461 (0.120; 0.801), 0.009		0.081 (−0.275; 0.435), 0.652	
2	HDL (mg/dL)	0.389 (0.201; 0.578), <0.001	0.230, <0.001	0.443 (0.203; 0.684), <0.001	0.270, <0.001	0.209 (−0.096; 0.514), 0.174	0.288, 0.004
	LDL (mg/dL)	0.231 (0.068; 0.395), 0.006		0.085 (−0.118; 0.290), 0.407		0.429 (0.148; 0.800), 0.004	

β, regression coefficient; CI, confidence interval; BF, body fat content (%); TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Model 1 without adjustment. Model 2 adjustment for age and smoking as well as by gender for the total group.

4. Discussion

The low nutrition status of vitamin E measured by α-tocopherol plasma concentrations was found in 27% of our group. We found that the determinants of lower plasma vitamin E were higher BF and lower TC and its fraction, with the strongest correlations being found for HDL. This partially confirmed our hypothesis that a lower concentration of vitamin E isoforms is linked with poorer metabolic health. Vitamin E isoform concentration was negatively correlated with CRP, but in-depth analyses did not confirm this association. Our findings indicated the need for further studies to explain these associations.

In our study, 12% of participants with normal body fat and 30% of participants with excess body fat had low plasma α-tocopherol concentrations. Only 30% of participants had pro-healthy plasma status vitamin E, and only 12% of those with excess body fat. The average plasma vitamin E concentrations in our research were comparable to those of other studies [45], or slightly higher [46,47]. This is consistent with the results of Peter et al. [39], who found that α-tocopherol status was inadequate in a significant part of the studied populations, with only 21% globally reaching a plasma concentration of ≥30 μmol/L, which is considered beneficial for health.

Among subjects with excess body fat, we observed significantly lower plasma concentrations of all vitamin E isoforms compared to healthy controls, both in terms of mean concentrations and the frequency of optimal levels. Participants with lower vitamin E concentrations also exhibited higher values of anthropometric indices, such as body weight, waist circumference, BMI, WHR, and WHtR. Other studies have reported similarly low levels of vitamin E in overweight or obese individuals [48,49].

Additionally, research involving postmenopausal women showed a strong positive correlation between α-tocopherol and waist-to-hip ratio, with no significant associations observed with waist circumference or BMI. This may indicate that α-tocopherol is more closely associated with fat distribution than total body fat [50]. However, Meulmeester et al. [51] found no significant relationship between adiposity measures and plasma α-tocopherol levels in middle-aged individuals, though they report that higher BMI and total

body fat were associated with lower levels of oxidized α-tocopherol metabolites, suggesting reduced antioxidant activity in individuals with increased abdominal fat. Although no overall differences in vitamin E isoform concentrations were observed between men and women, gender-specific patterns emerged. Women with excess body fat exhibited significantly lower concentrations of all four tested vitamin E isoforms compared to women with normal body fat. In contrast, significant differences in vitamin E levels were observed only in α-tocopherol and tocotrienol concentrations among men. These findings suggest that body fat may influence vitamin E metabolism differently in men and women, potentially due to variations in fat distribution and metabolic pathways.

Gender-specific differences in vitamin E metabolism are influenced by hormonal factors, particularly estrogen and testosterone, which regulate fat distribution and lipid metabolism. These hormones play a pivotal role in storing, mobilizing, and utilizing vitamin E in men and women. Estrogen promotes subcutaneous fat accumulation, which is more prevalent in women, potentially affecting the storage and release of vitamin E. Testosterone, on the other hand, is associated with visceral fat accumulation in men, influencing lipid metabolism and vitamin E utilization [52]. Sex hormones also differentially regulate lipid metabolism genes in male and female hepatocytes, impacting vitamin E metabolism. For instance, 17β-estradiol modulates the expression of genes involved in lipid metabolism in female hepatocytes, whereas testosterone influences distinct gene pathways in males. Recognizing these sex-based metabolic differences is crucial for developing effective vitamin E supplementation strategies. Tailoring dosages or delivery methods based on individual fat distribution patterns and hormonal profiles may enhance the outcomes of nutritional interventions [53].

Nutritional guidelines for vitamin E intake differ by gender, which could potentially affect plasma vitamin E levels [54]. Additionally, differences in body fat composition between men and women may also play a significant role in vitamin E distribution and metabolism [55]. Despite these differences, optimal plasma vitamin E concentrations are generally consistent for both genders, making it essential to explore whether gender impacts these levels [54]. Furthermore, age groups need to be taken into account to verify current recommendations. Studies have shown that one of the main functions of vitamin E is to correct impaired immune and inflammatory cell functions in older adults, which has not been considered in recommendations to date [16]. However, it is well documented that older adults (>65 years) have impaired immune and inflammatory responses, as well as enzymatic antioxidant defense mechanisms, which increase the risk of chronic infectious and noncommunicable diseases compared to younger adults [56]. The aging process is associated with a dysregulation of immune function, a phenomenon often referred to as "immunosenescence," characterized by hyporesponsive cellular immune responses and pathogen defenses, coupled with a prolonged inflammatory state known as "inflammaging" [16]. Among the issues to consider is the efficacy of other forms of vitamin E. In this context, there is emerging evidence that other vitamin E homologues are unique and may potentially have higher efficacy. Based on the current state of research, γ-T and T3, as well as the hepatic metabolites α- and γ-T, are promising forms of vitamin E in the prevention of diseases caused by acute inflammatory and oxidative processes [16,56].

Our findings confirmed that plasma concentrations of total cholesterol, HDL, and LDL were the main predictors of vitamin E concentration, and HDL cholesterol played a pivotal role across all vitamin E isoforms [57,58]. Interestingly, individuals with normal body fat exhibit significantly higher HDL and LDL levels compared to those with excess body fat, which may further explain elevated vitamin E levels. Despite this, the literature presents divergent findings regarding the direction and strength of lipid vitamin E correlations [57,58]. Nevertheless, HDL is consistently identified as positively associated with vitamin E concen-

trations. Given that dyslipidemia, characterized by high TG and LDL levels, and low HDL levels, is a primary contributor to cardiovascular disease (CVD) [58] this relationship is crucial. Dyslipidemia is strongly linked to obesity, non-alcoholic fatty liver disease (NAFLD), and other metabolic disorders [59]. Vitamin E, a crucial antioxidant, has been shown to mitigate CVD risk through mechanisms such as inhibiting LDL cholesterol oxidation and reducing oxidative stress in NAFLD [60]. Given the strong association between dyslipidemia, obesity, and oxidative stress, vitamin E supplementation is particularly recommended for individuals with elevated LDL levels and obesity. Studies suggest that vitamin E may prevent LDL particle oxidation, a critical factor in atherosclerosis progression [61]. Consequently, its role in CVD prevention, particularly in populations with metabolic disorders, confirmed the potential of vitamin E as a complementary therapeutic agent.

Our study found that participants with higher adiposity exhibited significantly elevated levels of monounsaturated fatty acids (MUFA) and omega-3 fatty acids, whereas individuals with normal body fat demonstrated higher concentrations of polyunsaturated fatty acids (PUFA), particularly omega-6 fatty acids. This divergence in fatty acid composition suggests an underlying disruption in lipid metabolism associated with obesity, which may influence both metabolic health and oxidative stress levels [62]. Additionally, fatty acids serve as potential dietary intake markers, reflecting the consumption of specific fats in the diet. The observed variations in fatty acid profiles across different body fat levels may indicate distinct dietary patterns or metabolic responses to fat intake. Elevated MUFA and omega-3 levels in individuals with excess body fat could be a consequence of altered lipid processing or increased dietary fat intake. In contrast, the higher PUFA and omega-6 levels in individuals with normal body fat may be indicative of a diet richer in these essential fatty acids, which are known to play a critical role in cellular integrity and metabolic regulation [63,64].

We also observed that individuals with adiposity had exhibited elevated activities of some enzyme activity involved in fatty acid metabolism. The activity of Δ6-desaturase, stearoyl-CoA desaturase-16, and stearoyl-CoA desaturase-18 was higher, whereas Δ5-desaturase (D5D) was more active in those with normal body weight. Increased D6D activity may enhance pro-inflammatory eicosanoid synthesis, commonly linked to chronic inflammation in obesity [65]. Elevated stearoyl-CoA desaturase (SCD) activity significantly influences vitamin E concentrations and its antioxidant function through its role in lipid metabolism [66]. SCD catalyzes the conversion of SFAs into MUFAs, such as oleic acid, which are key components of triglycerides, phospholipids, and cholesterol esters that transport and store vitamin E [67]. Changes in SCD activity can alter the lipid microenvironment, potentially impacting vitamin E bioavailability and efficacy. MUFAs are less susceptible to peroxidation than polyunsaturated fatty acids, reducing the oxidative demand on vitamin E and potentially modifying its antioxidant requirements [68]. However, elevated SCD activity is often linked to lipid accumulation in tissues, which may alter local vitamin E concentrations and impair its protective role. This lipid accumulation is associated with insulin resistance and metabolic disturbances, as MUFAs contribute to triacylglycerol synthesis, potentially leading to fat deposition in the liver and peripheral tissues [68]. Conversely, D5D converts dihomo-γ-linolenic acid (DGLA) into anti-inflammatory PUFAs, suggesting that higher D5D activity in normal-weight individuals supports a healthier lipid profile and metabolic health. However, in our study, vitamin E isoforms negatively correlated with SCD-16 and SCD-18 activity, but their association was not confirmed by multivariate linear regression, which requires further study.

Our study demonstrates inflammation was linked with excess body fat. Vitamin E isoforms showed significant negative correlations with CRP, and confirmed a potential anti-inflammatory role for vitamin E. Similarly, Mazidi et al. [69] found that higher antioxidant

levels, including vitamin E, were associated with lower CRP concentrations, with obesity moderately influencing this relationship. Moreover, results of meta-analyses indicated that α-tocopherol supplementation can reduce CRP by 0.52 to 0.62 mg/L, underscoring its anti-inflammatory properties [4]. Furthermore, observational meta-analyses report that over half (17/27) of randomized controlled trials (RCTs) identified statistically significant protective associations between vitamin E and various health parameters, including CRP, further supporting its role in inflammation reduction [70].

Several limitations of our study should be acknowledged. Firstly, we did not collect dietary data to assess the intake of different vitamin E isoforms and fatty acids. According to other studies, we assumed that their consumption is related to the consumption of plant fats, for which the plasma fatty acid profile is a better biomarker. Secondly, our findings concern people aged 40–60 years and do not apply to other age groups. Additionally, more precise biomarkers of vitamin E status, such as α-tocopherol concentrations in adipose tissue obtained through biopsies or vitamin E metabolites, should be considered for future research, as plasma α-tocopherol levels alone may not fully reflect long-term vitamin E status. Nutritional status of vitamin E is affected by numerous factors, e.g., dietary intake of vitamin E, other antioxidant and pro-oxidant compounds, absorption efficiency, and vitamin E catabolism, but bioavailability has been shown to be its key determinant. Significant variability in plasma vitamin E isoform levels among participants was observed, potentially stemming from differences in individual absorption, metabolism, or dietary habits that were not accounted for. We recognize that genetic variation, including single nucleotide polymorphisms associated with fasting blood vitamin E concentration and α-tocopherol bioavailability, could have influenced plasma vitamin E levels [71]. The interindividual variability in α-tocopherol bioavailability is associated with a combination of 28 SNPs in or near 11 candidate genes. Four of these genes SLC10A2 (solute carrier family 10 (sodium/bile acid cotransporter), member 2), PNLIP (pancreatic lipase), SREBF2 (sterol regulatory element binding transcription factor 2), and ABCG1 (ATP-binding cassette, subfamily G (WHITE), member 1) play a specific role in α-tocopherol bioavailability [71]. This variability could affect the generalizability of the findings, as outcomes may differ across diverse populations or settings. Furthermore, the observational nature of the study limits causal inferences between α-tocopherol levels and metabolic health. While correlations were identified, they do not confirm causality, as various confounding factors—such as diet, genetic predispositions, and health behaviors—may have influenced the results. Although C-reactive protein (CRP) is a well-established marker of inflammation, the study could benefit from incorporating more sensitive and specific biomarkers for detecting inflammatory or metabolic conditions. Additionally, the potential influence of other nutrients on the function and status of vitamin E was not assessed in this study. The synergistic antioxidant effects and mutual regeneration capacity of vitamins C and E are particularly significant, as they are crucial in maintaining oxidative balance within the body [72]. Additionally, the combined antioxidant activity of vitamin E with carotenoids, and also selenium, and zinc as cofactors of antioxidant enzymes further underscores the importance of nutrient interactions in modulating oxidative stress [73]. Moreover, the interplay between vitamins E and K warrants attention, as vitamin E may interfere with vitamin K's essential role in blood coagulation [74]. Including such parameters in future research could provide a more comprehensive understanding of the interplay between antioxidants and metabolic health.

Indices of central obesity, such as waist circumference (WC) and waist-to-height ratio (WHtR), did not reveal significant associations with vitamin E status in our study. While these measures are commonly used to estimate the central fat distribution and associated metabolic risks, their inability to differentiate between visceral and subcutaneous fat might limit their predictive value for specific outcomes like vitamin E metabolism. Advanced

imaging techniques, such as DEXA, MRI, or CT scans, could provide a more precise assessment of visceral and subcutaneous fat depots. The ability to distinguish between these types of fat could enhance our understanding of their specific roles in vitamin E metabolism and health outcomes.

The undoubted strength of this study is the comprehensive assessment of multiple lipophilic components related to vitamin E metabolism. Moreover, the calculated effect size allows us to assume that the sample size was sufficient to statistically confirm the obtained relationships. In our research, more isoforms of vitamin E were analyzed, extending beyond alpha-tocopherol, to provide a more comprehensive assessment of the nutritional status of vitamin E.

5. Conclusions

In our study, 12% of participants with normal body fat and 30% of participants with excess body fat had low plasma α-tocopherol nutritional status. Only 30% of participants had a pro-healthy plasma status of vitamin E, and only 12% of those with excess body fat. These results found that the vitamin E status of adults with excess body fat may be inadequate and linked to poorer metabolic health. We found that the determinants of lower plasma vitamin E were higher BF and lower TC and its fraction, with the strongest correlations being found for HDL. This partially confirmed our hypothesis that a lower concentration of vitamin E isoforms is linked with poorer metabolic health. Considering that the risk of metabolic diseases increases with age and with adiposity, but is also associated with a biological change in body composition towards increasing fat mass, the pro-healthy nutritional status of vitamin E through would be beneficial for health.

Author Contributions: Conceptualization, M.G. and K.S.; methodology K.S., M.G., J.B. and R.B.; formal analysis, K.S., J.B. and M.G.; investigation, K.S.; data curation, K.S.; writing—original draft preparation, K.S.; writing—review and editing, M.G., K.S., J.B. and R.B.; visualization, K.S. and R.B.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: The publication was (co)financed by the science development fund of the Warsaw University of Life Sciences—SGGW.

Institutional Review Board Statement: This study was approved by the Ethics Committee of the Institute of Human Nutrition Sciences of the Warsaw University of Life Sciences, Poland (Resolution No. 05/2019, approved on 19 February 2019).

Informed Consent Statement: The participants gave informed consent to participate in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Acknowledgments: Thanks are expressed to the participants for their contributions to the study.

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

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Warszawa, 19.02.2025 r.

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Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults

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Abstract: Due to the documented health benefits of tocopherols and tocotrienols as bioactive compounds, it seems important to assess their intake. The aim of this study was to develop a new tool and its application for assessment of tocopherol and tocotrienol intake in adults. Dietary data were collected by semiquantitative FFQ (VitE-FFQ) and by a 1-day dietary record in a group of 447 subjects. The database of the US Department of Agriculture (USDA) was used to calculate the individual isoforms of vitamin E and develop the tool—VIT_E.CAL. The assessment of measuring agreement between the two methods was conducted by analysis of the correlations and Bland–Altman plots. The average α-tocopherol intake was 11.3 mg/day for the data obtained using the FFQ method and 12.8 mg/day for the results obtained using the 1-day dietary record. Depending on the adopted recommendation, only 40–57% of the subjects had adequate vitamin E intake. The intake of α-tocopherol did not exceed the UL value in any of the respondents. The dominant forms of vitamin E in the diet of the studied group were α- and γ- forms (55% and 38% of the total sum) among tocopherols and β- and γ- forms (49% and 24% of the total sum) among tocotrienols. VIT_E.CAL allows us to calculate not only the total amount of vitamin E but also its eight isoforms. It can be a useful tool to assess individual and group intake of various forms of vitamin E in the diet. The use of VIT_E.CAL enables the proper assessment of vitamin E (as α-tocopherol and not α-tocopherol equivalent) in the diet of Poles, and most likely also in the European diet. The obtained results indicate the need to take into account the content of individual forms of vitamin E in food/diet, which will allow for a reliable assessment of its consumption. It also seems necessary to standardize the nomenclature regarding the name of vitamin E and its use for correct nutritional assessment.

Keywords: vitamin E; dietary intake; dietary assessment; adequate intake

Citation: Szewczyk, K.; Górnicka, M. Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults. *Nutrients* **2023**, *15*, 3759. https://doi.org/10.3390/nu15173759

Academic Editor: Hongbing Sun

Received: 27 July 2023
Revised: 19 August 2023
Accepted: 23 August 2023
Published: 28 August 2023

1. Introduction

Tocopherols and tocotrienols belonging to the vitamin E family are antioxidants and play specific roles in the human body [1,2]. There are eight different natural forms in the vitamin E family: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols [3]. The most biologically active form of vitamin E is α-tocopherol because of the presence of a specific α-tocopherol transfer protein (α-TTP). So far, α-TTP is the only known protein that precisely recognizes α-tocopherol in the liver and transfers it to lipoproteins for further circulation in the blood [4]. A mutation in the gene encoding the α-TTP protein can lead to the development of ataxia with vitamin E deficiency (AVED) [5]. The assessment of the human requirement for α-tocopherol is challenged by the rare occurrence of clinical symptoms of deficiency. Symptoms usually appear in premature babies, infants, and adults with fat malabsorption, liver disease, or genetic conditions [6]. The recommended dietary allowance (RDA) of vitamin E varies between countries, depends on age and gender, and these values often differ from each other. Current research shows that there is a constant need to review, define, and assess the dietary requirements of different populations for vitamin E or only α-tocopherol [7,8]. The US National Institute of Health (NIH) and US

Institute of Medicine (IoM), based on evidence from clinical trials, recommend the intake of α-tocopherol for adults at the level of 15 mg per day (RDA) regardless of gender [9,10]. Many health policy makers, like the Nordic Council of Ministers [11], European Food Safety Authority (EFSA) [6], French Agency for Food, Environment, and Occupational Health and Safety (ANSES) [12], recommend intake at the level of 7.5–10 mg α-tocopherol/day. Polish recommendations have been developed by the National Institute of Public Health—National Institute of Hygiene—National Research Institute (NIZP–PZH–PIB) [13] as the adequate intake (AI) expressed in α-tocopherol equivalents, which takes into account the biological activity of all eight isoforms, at the level of 8 mg per day for women and 10 mg for men. Our national AI values [13] are slightly lower than those of the European Food Safety Authority (EFSA) [6], which are, respectively, 13 mg of α-tocopherol equivalent per day and 11 mg per day for men and women. The upper level intake (UL) for vitamin E has been set at 300 mg α-tocopherol per day for adults [6,13].

Tocopherols and tocotrienols neutralize free radical scavengers in membranes and lipoproteins [8]; of these isoforms, α-tocopherol is the most studied [14]. It is used in research on diseases with increased inflammation, mainly cancer, and diseases of the central nervous system, the immune system, and the cardiovascular system [15–18]. In recent years, other forms of vitamin E have been shown to have better antioxidant and anti-inflammatory properties than α-tocopherol, and research started focusing on these forms [19]. Much attention is also paid to γ-tocopherol, which is the dominant form of vitamin E in the US diet. γ-tocopherol shows higher activity in capturing reactive oxygen and nitrogen species than α-tocopherol, and its concentration in the blood may be related to a lower risk of cancer and cardiovascular diseases [19]. Research on tocotrienols is expanding worldwide due to their interesting biological properties that tocopherols do not have, including neuroprotective, radioprotective, anticancer, anti-inflammatory, and lipid-lowering properties [20]. Tocotrienols are also credited with angiogenic effects and participation in the regulation of the activity of enzymes and transcription pathways, which may be reflected in the prevention of cancer [21].

There are studies indicating the possible health benefits of consuming different forms of vitamin E [22–25]. The main food sources of tocopherols and tocotrienols are the lipid components of oilseeds and nuts. Sunflower, rapeseed, corn, linseed, soybean, almond, peanut, and olive oils are the most abundant sources of tocopherols [26]. The main rich, natural sources of tocotrienols are palm oil, rice bran, wheat germ oil, coconut oil, and annatto seeds. An extract from the annatto seeds of the achiote tree (*Bixa orellana* L.) consists of 90% δ-tocotrienol and 10% γ-tocotrienol; palm oil, extracted from the reddish pulp of the fruits of the palm tree (*Elaeis guineensis*), consists mainly of 46% γ-tocotrienol and 22% α-tocotrienol [20,27,28].

To the best of our knowledge, no previous study in Europe has assess the intake of individual forms of vitamin E with different biological activities. Considering that vitamin E is contained in products that are often skipped in methods using current records, i.e., added fats, sauces, nuts, or oilseeds, it is important to include them when developing tools for nutrition assessment. Moreover, taking into account that only α-tocopherol has the vitamin property and can be called vitamin E [8], tools are needed to evaluate the different forms currently included as vitamin E.

The above conditions were an argument for the development of a tool that would allow us to assess the consumption of individual forms of vitamin E. The aim of this study was to develop a new tool for the assessment of tocopherol and tocotrienol intake in adults by (i) developing a semiquantitative food frequency questionnaire (VitE-FFQ), (ii) creating a database of tocopherol and tocotrienol content, (iii) providing a tool (VIT_E.CAL) for the calculation of vitamin E content, and (iv) applying it to the assessment of tocopherol and tocotrienol intake.

2. Materials and Methods

2.1. Dietary and Sociodemographic Data

This study was conducted for a period of 12 months, from September 2021 to September 2022, using random sampling in a group of adult Poles. During this period, participants were asked to complete the VitE-FFQ questionnaire and a 1-day dietary record immediately after completing the FFQ. Both the VitE-FFQ and the 1-day dietary record were self-reported. In addition, data on basic sociodemographics such as age, education, and place of living as well as the anthropometrics of body height and weight were collected. The inclusion criteria for this study were ages 18–65 and the use of a diet that did not eliminate any of the food groups. The exclusion criteria were gestation, vitamin E supplementation, and lack of or incomplete data about food consumption. The computer-assisted web-based interviewing (CAWI) method was used to collect all data. In this study, 504 participants took part. Finally, completed questionnaires from 447 respondents were included; 57 questionnaires were rejected due to nonfulfilment of the inclusion criteria and lack of data on consumption (Figure 1).

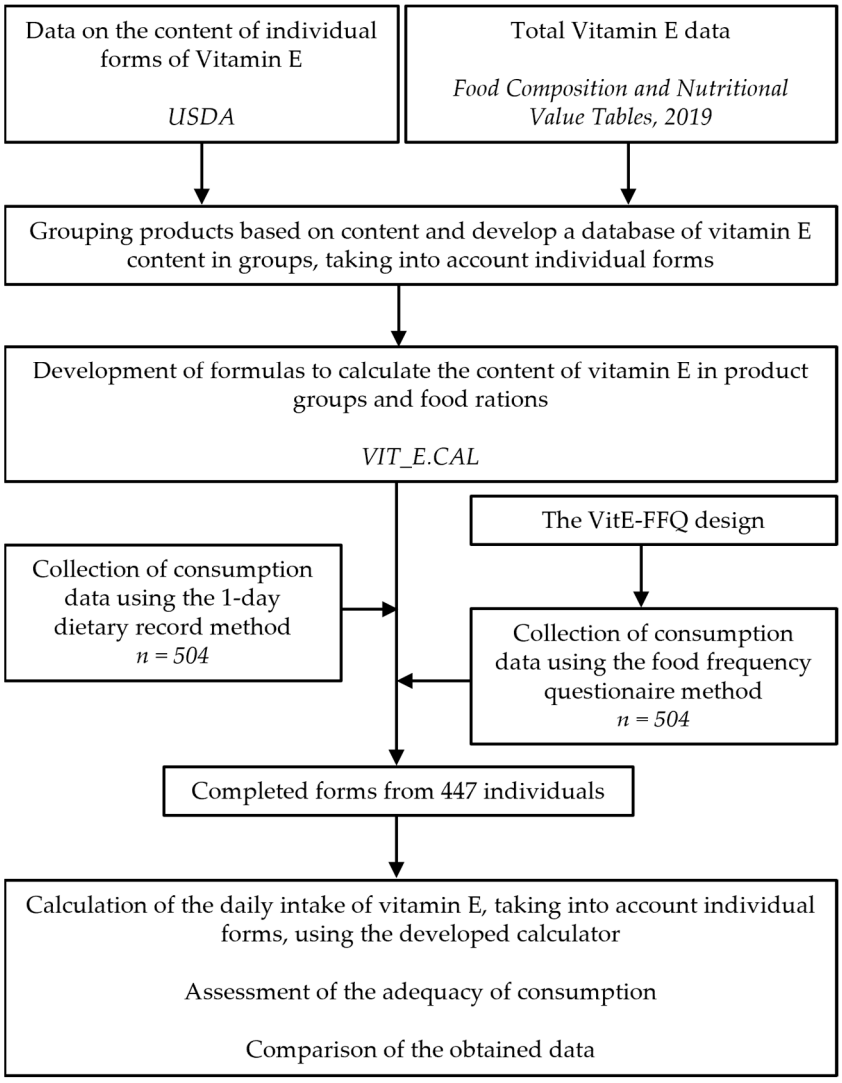

Figure 1. The design of the study.

2.2. The VitE-FFQ Design

The developed semiquantitative FFQ to evaluate the consumption of tocopherols and tocotrienols in the diet with VitE-FFQ covers 8 groups of food products, such as vegetables, fruits and fruit products, legumes and legume products, nuts and oilseeds, fats, cereals, fish

and fish products, and snacks and "others". The VitE-FFQ questionnaire consists of a list of 67 food products that are sources of these compounds and their usual portion size according to the Polish Atlas of Portion Sizes of Food Products and Dishes [29]. The questionnaire was developed for assessing tocotrienol consumption in an initial study but has been improved for this investigation [30]. All food products were selected based on the content of vitamin E marked as the equivalent of α-tocopherol (Polish Food Composition and Nutritional Value Tables) [31]. In the developed VitE-FFQ, we asked respondents to indicate the usual number of servings of the listed products consumed in a week (Table 1). The questions concerned the 6-month period preceding the completion of the questionnaire. Moreover, in the information for the respondent, it was indicated that the respondent should recall all the ingredients of the dishes, such as the fats used or added nuts or oilseeds. The VitE-FFQ specifies that the usual number of servings should include portions of products eaten separately and added to prepared dishes, and it also indicates that not only whole numbers but fractions can be provided.

Table 1. Design of the VitE-FFQ—food items, portion sizes, and household measures.

Food Product	Serving Size	Household Measures
Vegetables		
Broccoli/brussels sprouts/kale/asparagus/leeks	100 g	2/3 cup; 2 handfuls of leaves
Carrots/parsley root	100 g	2/3 cup
Green peas/tomatoes	100 g	2/3 cup
Beetroot/beet greens/pumpkin/red peppers/turnip	100 g	2/3 cup; 2 handfuls of leaves
Lettuce/romaine lettuce/spinach/chicory/rucola	100 g	2/3 cup; 2 handfuls
Corn	20 g	1 tablespoon
Carrot juice/multivegetable juices	200 mL	1 cup
Other vegetables	100 g	2/3 cup; 2 handfuls of leaves
Fruit and Fruit Products		
Kiwifruit	100 g	2/3 cup
Raspberries/blackberries/blueberries	100 g	2/3 cup
Avocado	70 g	1/2 medium piece
Other fruit	100 g	2/3 cup
Legumes and Legume Products		
Peas/lentils—dry seeds	15 g	1 tablespoon
Hummus	10 g	1 teaspoon
Nuts and Oilseeds		
Pumpkin seeds	30 g	2 tablespoons
Linseeds	30 g	2 tablespoons
Pistachios/pecans	30 g	2 tablespoons
Almonds/sunflower seeds	30 g	2 tablespoons
Almond drink	200 mL	1 cup
Hazelnuts	30 g	2 tablespoons
Peanuts	30 g	2 tablespoons
Pine nuts	30 g	2 tablespoons
Other nuts, e.g., walnuts, cashews	30 g	2 tablespoons
Fats		
Canola oil	10 g	1 tablespoon
Sunflower oil	10 g	1 tablespoon
Olive oil	10 g	1 tablespoon
Grape-seed oil	10 g	1 tablespoon
Peanut oil	10 g	1 tablespoon
Wheat germ oil	10 g	1 tablespoon
Soybean oil	10 g	1 tablespoon
Coconut oil	10 g	1 tablespoon
Margarine	10 g	1 tablespoon

Table 1. *Cont.*

Food Product	Serving Size	Household Measures
Mayonnaise	10 g	1 tablespoon
Pesto	10 g	1 teaspoon
Eggs	50 g	1 piece
Cereals		
Wholemeal bread, whole wheat pasta/brown rice/cornflakes	bread 35 g or 75 g other	1 slice or cup of dry product
Fish and Fish Products		
Fresh fish, e.g., salmon, trout, mackerel, herring	100 g	1 piece
Canned fish in oil, e.g., mackerel, tuna, herring, sardines	150 g	1 medium can
Other fish, e.g., canned fish in water/in tomatoes, flounder, carp, fish fingers	150 g	1 medium can
Snacks and Others		
Dark chocolate	5 g	1 cube
Chips/crackers/nachos	10 g	1 handful

2.3. Development of the Tool Vit_E.CAL

The developed questionnaire VitE-FFQ, by taking into account the same products, is compatible with the calculator Vit_E.CAL. The tool was developed in MS Excel in 2 sheets (Supplementary Materials, VIT_E.CAL). Due to the fact that the Polish Food Composition and Nutritional Value Tables [31] only provide the total content of vitamin E expressed as the equivalent of α-tocopherol, the database of the United States Department of Agriculture (USDA) was used to calculate individual tocopherols and tocotrienols [32]. In the first sheet, "Product group database", data on the content of tocopherols and tocotrienols were entered: in the rows, the products in each group were entered, while in the columns, the mean content of α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols was entered. The added column "vitamin E added" refers to the amount of vitamin E added to the product by the manufacturer (data from the USDA database). The initial selection of products was made on the basis of the Food Composition and Nutritional Value Tables [31], and then this database was extended to include other products from the USDA database which are not included in the Food Composition and Nutritional Value Tables. Food products were organized into groups based on similar content of individual forms of vitamin E, where possible. Food products were grouped into the following categories: vegetables, fruit and fruit products, legumes and legume products, nuts and oilseeds, fats, cereals, fish and fish products, and snacks and others.

The second sheet, "VIT_E.CAL", allows us to calculate the daily intake of individual forms of vitamin E. After entering information on the average number of servings of each group consumed during the week in the column "Number of portions", the total amount of this product per week (column "Product consumption per 7 days [g]") is calculated. The average daily consumption of the product in these groups is computed next (by dividing by the number of days—column "Average product consumption [g]"). Based on that, in the appropriate columns, the daily content of tocopherols and tocotrienols in the consumed product (mg) is calculated from the "Product group database" sheet. In the rows with the names of food product categories, formulas have been entered that allow the calculation of the sum of individual tocopherols consumed in a given product category. To simplify, the calculator uses the following procedure to calculate the daily intake of tocopherols and tocotrienols from particular groups of food products: consumption of the selected form of tocopherols or tocotrienols (mg) = daily number of portions x average content of the selected form of tocopherols or tocotrienols in one portion of a group of food products.

It is also possible to calculate the sum of all forms of vitamin E as mg of α-tocopherol equivalents as follows:

Vit. E [mg α-tocopherol equivalents/100 g] = mg α-tocopherol + 0.4 × mg β-tocopherol + 0.1 × mg γ-tocopherol + 0.01 × δ-tocopherol + 0.3 × α-tocotrienol + 0.05 × β-tocotrienol + 0.01 × γ-tocotrienol (the formula in "VIT_E.CAL" sheet).

This makes it possible to compare the obtained data with the values from the Food Composition and Nutritional Value Tables [31] or refer to the AI in α-tocopherol equivalents. The "Total of vitamin E isoforms" row indicates the total intake of all isoforms of vitamin E.

2.4. Assessment of Adequecy of Vitamin E and α-Tocopherol Intake

Due to the fact that dietary reference values refer to α-tocopherol equivalents in mg/person/day or α-tocopherol in mg/person/day, the obtained results from the VitE-FFQ and the 1-day dietary record were compared for each respondent with reference values from the RDA levels (NIH) [9], and with AI values according to NIPH–NIH–NRI [13] and EFSA for the European [6] population (Table 2).

Table 2. Adult dietary reference intakes (DRIs) for α-tocopherol (α-T) and α-tocopherol equivalents (α-T Eq) by different institution.

	NIPH–NIH–NRI		**NIH**		**EFSA**	
Vitamin E	**α-T Eq [mg/d]**		**α-T [mg/d]**		**α-T [mg/d]**	
DRIs	AI	UL	RDA	UL	AI	UL
Men	10	300	15	1000	13	300
Women	8				11	

NIPH–NIH–NRI—National Institute of Public Health–National Institute of Hygiene–National Research Institute; NIH—US National Institute of Health; EFSA—European Food Safety Authority; Eq—equivalents; AI—adequate intake; UL—upper level intake; RDA—recommended dietary allowances (based on: [6,9,13]).

2.5. Statistical Analysis

Assessment of the usefulness of this tool was carried out by comparing the results of the VitE-FFQ with the results of the 1-day dietary record. Statistical analysis included the following:

- Calculation of differences between the intake levels of tocopherols and tocotrienols obtained from the two methods: the normality of the distribution of results was analyzed using the Shapiro–Wilk test, and then the Mann–Whitney U test was applied.
- Analysis of the correlations between results: the normality of distribution of the results was analyzed using the Shapiro–Wilk test, and, afterwards, Spearman's rank correlation was applied for nonparametric distribution.
- Analysis of the Bland–Altman plots in the assessment of agreement (VitE-FFQ vs. 1-day dietary record); the results were interpreted using the Bland–Altman index, whereas the limits of agreement values (LOA) were calculated as the sum of the mean absolute difference in the consumption of individual forms of tocopherols and tocotrienols, measured by the two methods, and the ± standard deviation of the absolute difference of the assessment compounds intake recorded by the two methods and magnified by 1.96; the Bland–Altman index (%) was calculated as a percentage of persons beyond the limits of agreement (LOA). Good reproducibility of the measurement was proved by a minimum of 95% difference within the ±2 SD limits, which corresponds to the Bland–Altman index amounting to no more than 5%.

A level of significance at $p \leq 0.05$ was accepted. Statistical analysis was carried out using Statistica software version 13.0.0. (StatSoft, Tulsa, OK, USA) and the Bland–Altman Statistica software macro by Matt Coates version 2009 (StatSoft, Inc., Tulsa, OK, USA).

3. Results

3.1. Characteristics of the Participants

A total of 447 adults were included in this study; 73% were women and 76% were people with secondary and university education. More than half of the study group (58%) lived in a city of >100,000 inhabitants. The largest share of the study sample (60%) comprised individuals aged 18–25, 62% of which were women and 52% of which were men, respectively. The smallest were the age groups of 41–60 years (10% of the total) and >60 years (1% of the total) (Table 3).

Table 3. Characteristics of the participants.

Variables	Total *n* = 447	Women *n* = 327 (73%)	Men *n* = 120 (27%)
Age group, %			
18–25 years	60	62	52
26–40 years	29	27	32
41–60 years	10	9	15
>60 years	1	2	1
Education, %			
Primary and vocational	3	18	5
Secondary	24	7	25
University	52	53	48
While studying	21	22	22
Place of living, %			
Village	21	20	25
City < 100,000 inhab.	21	21	22
City > 100,000 inhab.	48	50	41
City > 500,000 inhab.	10	9	12
Anthropometrics (mean ± SD)			
Height, cm	171 ± 9.0	166.2 ± 6.0	181.1 ± 6.4
Body weight, kg	70.3 ± 33.2	65.1 ± 36.0	84.6 ± 16.9
BMI	24.1 ± 11.3	23.5 ± 12.8	25.8 ± 5.2

3.2. Intake of Tocopherols, Tocotrienols, and α-tocopherol Equivalents

The average intake of α-tocopherol equivalents in the study group was 11.3 mg based on the data computed using the FFQ method and 12.8 mg based on the 1-day dietary record (Table 4). The α- and γ-tocopherol forms had the greatest share in the total content of vitamin E (55% and 38% of the total sum of tocopherols, respectively).

Table 4. Comparison of tocopherols, tocotrienols, their sums, and α-tocopherol equivalents intake (mg/day) estimated by VitE-FFQ and 1-day dietary record methods.

Vitamin E Isoforms	Methods						*p* *
	VitE-FFQ (mg/day)			1-Day Dietary Record (mg/day)			
	Mean ± SD	Median	Min.–Max.	Mean ± SD	Median	Min.–Max.	
α-T	12.0 ± 8.5	9.7	0.4–50.6	13.3 ± 11.7	10.1	0.2–73.5	NS
β-T	0.3 ± 0.2	0.2	0.1–1.6	0.3 ± 0.4	0.2	0.1–3.3	NS
γ-T	8.3 ± 6.6	6.5	0.2–42.3	9.8 ± 9.8	6.3	0.1–45.7	NS
δ-T	1.1 ± 0.9	0.7	0.1–6.9	1.1 ± 1.4	0.6	0.1–7.7	NS
Sum of Ts	21.7 ± 15.1	17.7	0.7–87.9	24.6 ± 20.5	18.3	0.2–118.7	NS
α-T3	0.3 ± 0.2	0.2	0.1–1.4	0.4 ± 0.3	0.3	0.1–1.9	NS
β-T3	0.6 ± 0.6	0.4	0.1–3.6	0.8 ± 0.7	0.7	0.0–3.6	NS
γ-T3	0.3 ± 0.3	0.2	0.1–1.9	0.5 ± 0.4	0.4	0.0–1.9	NS
δ-T3	0.1 ± 0.1	0.1	0.0–0.6	0.1 ± 0.1	0.1	0.0–0.2	NS
Sum of T3s	1.3 ± 1.2	1.0	0.1–6.8	1.6 ± 1.4	1.4	0.1–6.7	NS
α-T Eq	11.3 ± 7.6	9.2	0.4–40.9	12.8 ± 11.6	9.5	0.1–83.0	NS

* U Mann–Whitney test; FFQ—food frequency questionnaire; SD—standard deviation T/s—tocopherol/s; T3/s—tocotrienol/s; Eq—α-tocopherol equivalents; NS—not significant.

The mean intake values of tocopherols, tocotrienols, their sums, and α-tocopherol equivalents calculated using these two methods did not differ significantly ($p > 0.05$) (Table 4). The dominant forms of tocopherols were α- and γ-, regardless of the data collection method. The major sources of α-tocopherol in the diet were almonds and sunflower seeds, while chips, crackers, nachos, and rapeseed oil were the major sources of γ-tocopherol. Among the tocotrienols, the β- form (49% of the total sum of tocotrienols) and α- and γ- forms (24% of the total sum of tocotrienols) dominated. Their main sources in the diet were wholemeal bread, wholegrain pasta, brown rice, and cornflakes. Tocopherols accounted for 94.3% of the total pool of consumed vitamin E, whereas tocotrienols accounted for only 5.7%.

Only 40–57% of respondents had an adequate vitamin E intake (Table 5). In more than half (55–57%), the requirement for vitamin E according to DRIs in Poland was met [13], regardless of the estimation method. The intake of α-tocopherol did not exceed the UL value for any of the respondents.

Table 5. Assessment of adequacy of intake of α-T and α-T equivalents.

DRIs		Percentage (%) of Individuals According to Method	
		FFQ	1-D
		***n* = 447**	***n* = 447**
α-T; RDA (NIH)	Adequate intake	42	45
	Inadequate intake	58	55
α-T equivalents; AI (NIPH–NIH–NRI)	Adequate intake	57	55
	Inadequate intake	43	45
α-T equivalents; AI (EFSA)	Adequate intake	40	47
	Inadequate intake	60	53
α-T equivalents; UL	Excessive intake	0	0

DRIs—daily recommended intakes; FFQ—food frequency questionnaire; 1-D—1-day dietary record.

3.3. Analyzing the Agreement between Dietary Data Obtained by FFQ and Dietary Record Methods

The analysis of the correlation between the results for the intake of all forms of tocopherols and tocotrienols, the sum of tocopherols and tocotrienols, and the α-tocopherol equivalents obtained using the VitE-FFQ and the 1-day dietary record is presented in Figures 2–4. Spearman's rank correlation coefficient revealed a statistically significant association for data obtained using the VitE-FFQ and the 1-day dietary record for δ-tocotrienol ($p < 0.01$, R = 0.545), α-tocopherol ($p < 0.01$, R = 0.409), α-tocotrienol ($p < 0.01$, R = 0.405), sum of tocotrienols ($p < 0.01$, R = 0.393), α-tocopherol equivalents ($p < 0.01$, R = 0.382), β-tocotrienol ($p < 0.01$, R = 0.375), β-tocopherol ($p < 0.01$, R = 0.374), sum of tocopherols ($p < 0.01$, R = 0.367), γ-tocotrienols ($p < 0.01$, R = 0.358), γ-tocopherols ($p < 0.01$, R = 0.340), and δ-tocopherols ($p < 0.01$, R = 0.320).

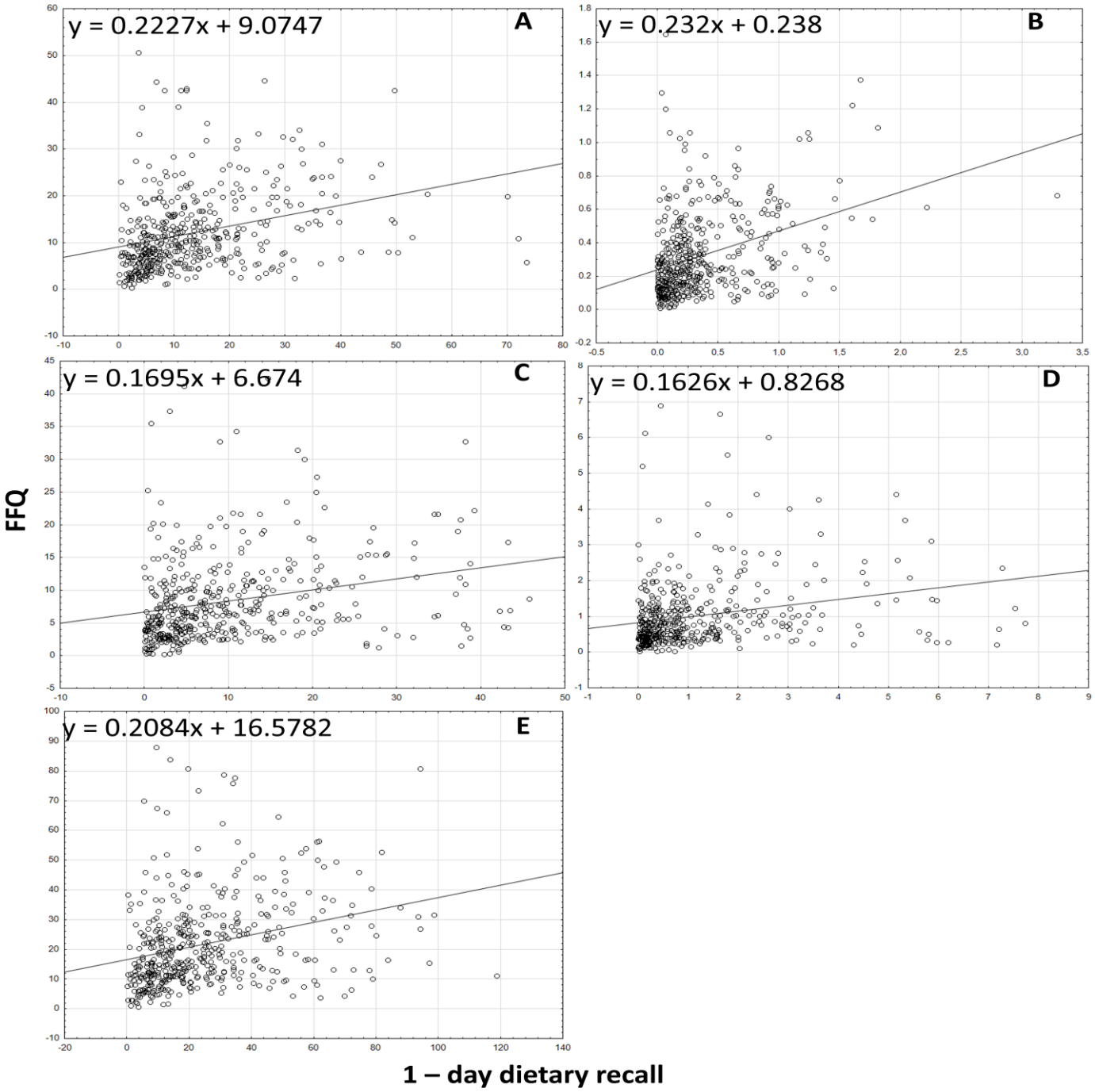

Figure 2. Correlation analysis between VitE-FFQ results and 1-day dietary record for daily intake of α-tocopherol (**A**), β-tocopherol (**B**), γ-tocopherol (**C**), δ-tocopherol (**D**), and sum of tocopherols (**E**).

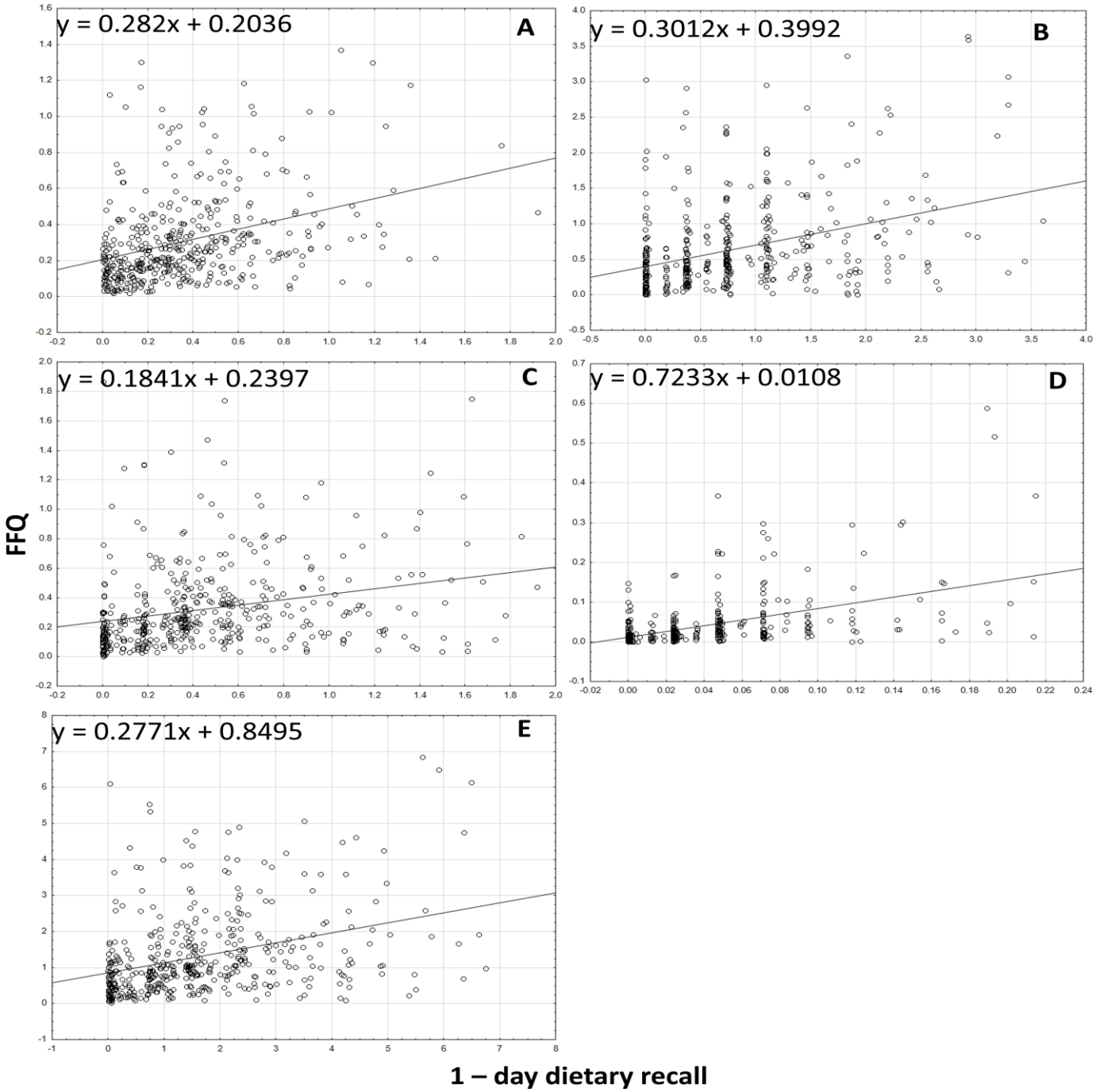

Figure 3. Correlation analysis between VitE-FFQ results and 1-day dietary record for daily intake of α-tocotrienol (**A**), β-tocotrienol (**B**), γ-tocotrienol (**C**), δ-tocotrienol (**D**), and sum of tocotrienols (**E**).

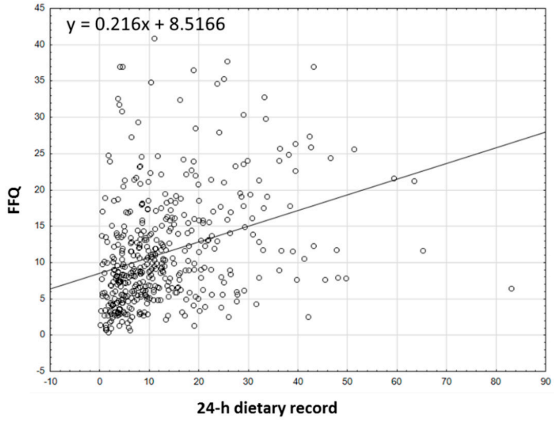

Figure 4. Correlation analysis between VitE-FFQ results and 1-day dietary record for daily intake of α-tocopherol equivalents.

Bland–Altman plots comparing VitE-FFQ results with a 1-day dietary record of daily tocopherol intake with diet are shown in Figure 5.

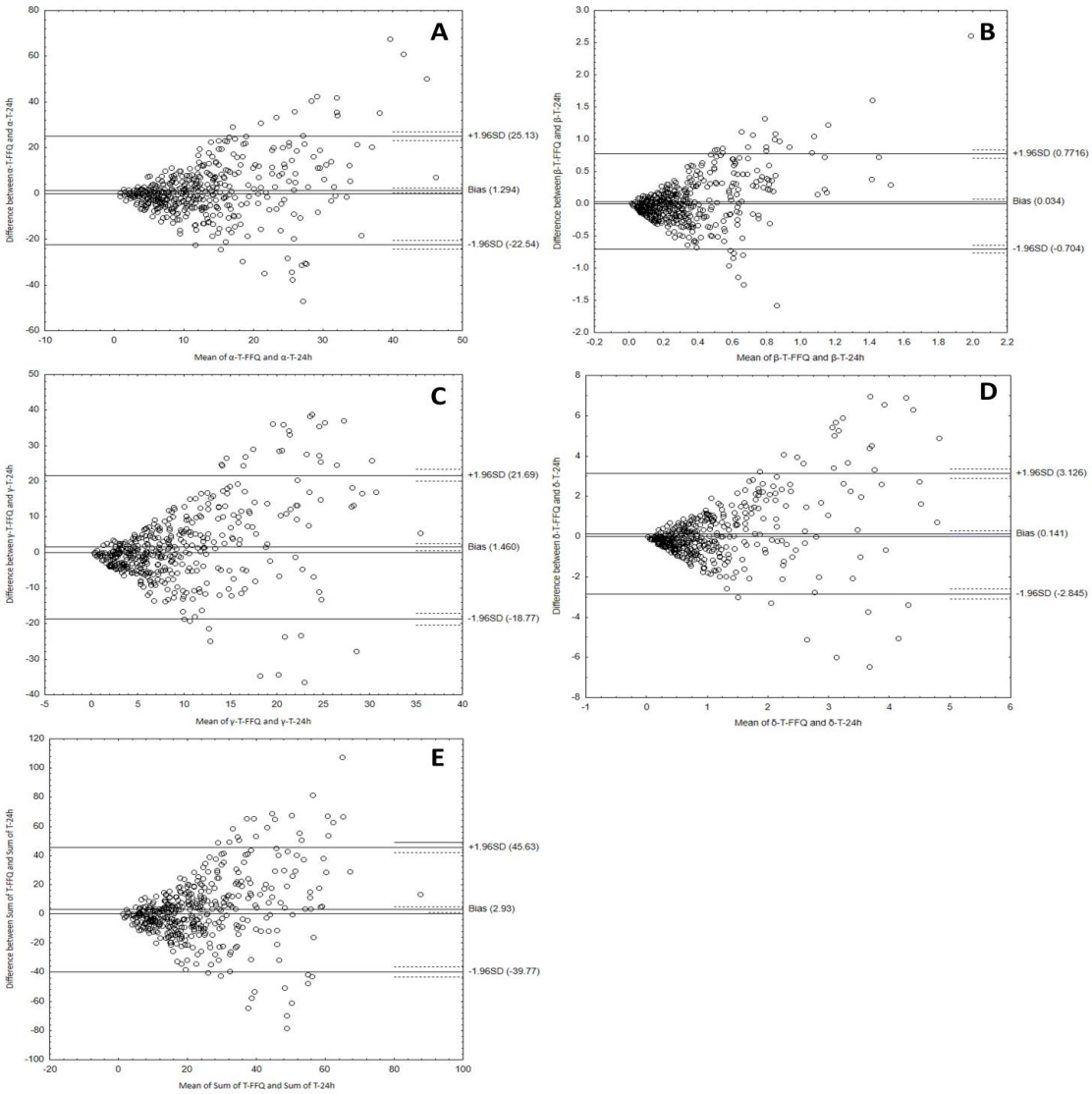

Figure 5. Bland–Altman plots comparing results of α-tocopherol (**A**), β-tocopherol (**B**), γ-tocopherol (**C**), δ-tocopherol (**D**), and sum of tocopherols (**E**) intake using the VitE-FFQ and 1-day dietary record.

The results for the comparison of the two methods for assessing the intake of tocopherols from the Bland–Altman analysis are presented in Table 6.

Table 6. Bland–Altman results for tocopherols.

Vitamin E Isoforms	Mean Absolute Difference (mg)	Lower LOA	Upper LOA	Number of Individuals beyond the LOA	Bland–Altman Index (%)
α-tocopherol	1.29	−22.54	25.13	422 out of 447	5.6
β-tocopherol	0.034	−0.704	0.772	413 out of 447	7.6
γ-tocopherol	1.46	−18.77	21.69	416 out of 447	6.9
δ-tocopherol	0.141	−2.845	3.126	420 out of 447	6.04
Sum of tocopherols	2.93	−39.77	45.63	415 out of 447	7.2

LOA—limits of agreement.

The Bland–Altman plots comparing data collected with VitE-FFQ and a 1-day dietary record of daily tocotrienols intake are shown in Figure 6.

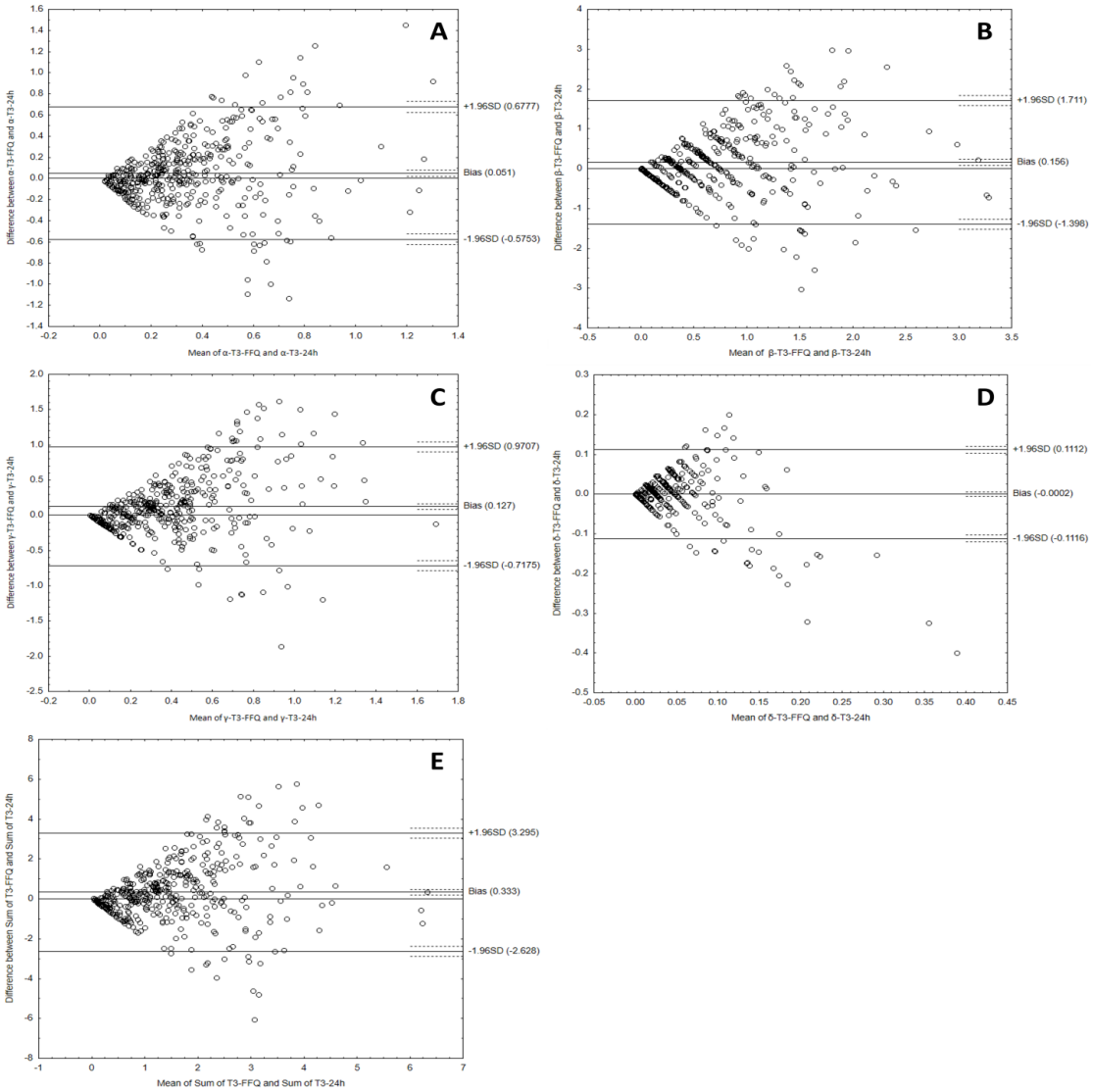

Figure 6. Bland–Altman plots comparing consumption data for α-tocotrienol (**A**), β-tocotrienol (**B**), γ-tocotrienol (**C**), δ-tocotrienol (**D**), and sum of tocotrienols (**E**) using the VitE-FFQ and 1-day dietary record.

The Bland–Altman analysis results for the comparison of both methods for determining tocotrienol consumption are shown in Table 7.

Table 7. Bland–Altman results for tocotrienols.

Vitamin E Isoforms	Mean Absolute Difference (mg)	Lower LOA	Upper LOA	Number of Individuals beyond the LOA	Bland–Altman Index (%)
α-tocotrienol	0.051	−0.5753	0.6777	415 out of 447	7.2%
β-tocotrienol	0.156	−1.398	1.711	412 out of 447	7.8%
γ-tocotrienol	0.127	0.7175	0.9707	415 out of 447	7.2%
δ-tocotrienol	0.0002	−0.1116	0.1112	417 out of 447	6.7%
Sum of tocotrienols	0.333	−2.628	3.295	415 out of 447	7.2%

LOA—limits of agreement.

The Bland–Altman plot comparing data obtained from the VitE-FFQ and a 1-day dietary record of α-tocopherol equivalent intake is shown in Figure 7.

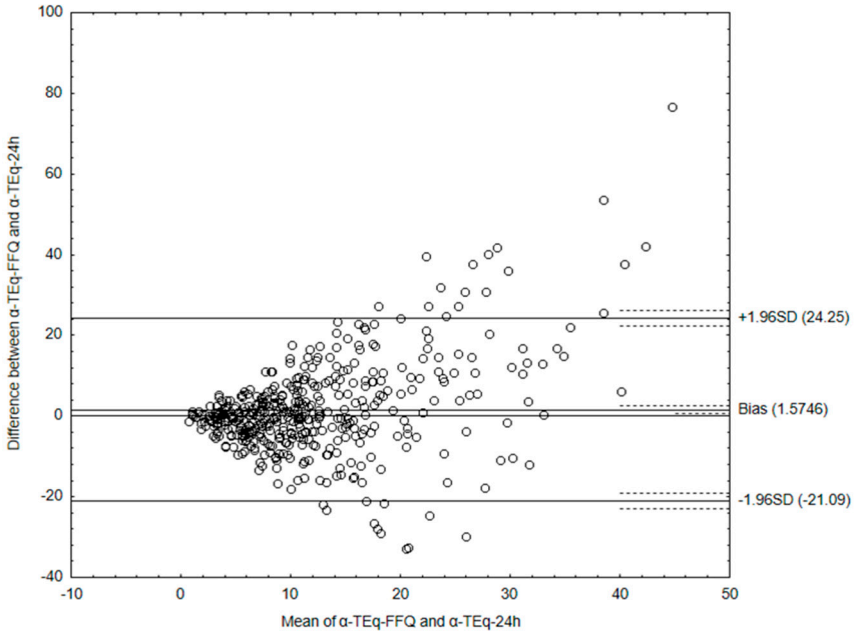

Figure 7. Bland–Altman plot comparing α-tocopherol equivalent intake data obtained from the VitE-FFQ and 1-day dietary record.

The mean absolute difference in α-tocopherol equivalent (Figure 7) intake was observed to amount to 1.57 mg. After adding ± 1.96 standard deviation for the LOA, an interval from −21.1 (lower agreement limit) to 24.25 (upper agreement limit) was obtained. The number of individuals observed to be within the LOA value was 420 out of 447, which confirmed the Bland–Altman index of 6.04%.

To sum up, the Bland–Altman indexes of less than 10% indicate a high agreement of the results regarding the consumption of tocopherols and tocotrienols, obtained using both methods and using the developed calculator, which confirms its usefulness.

4. Discussion

Tocopherols and tocotrienols are important biological active compounds. For this reason, the developed VIT_E.CAL may prove to be helpful in adequately assessing vitamin E and also α-tocopherol intake, in arranging a balanced diet and dietotherapy, and in the prevention of diseases. In our study group, the mean tocopherol intake was about 22–25 mg/d and the mean tocotrienol intake was about 1.3–1.6 mg/d. The dominant

forms of vitamin E in the diet were α- and γ- forms (55% and 38% of the total sum) among tocopherols and β- and γ- forms (49% and 24% of the total sum) among tocotrienols. Although the mean intake of vitamin E in α-tocopherol equivalents was about 12 mg per day, which is close to the DRI, only 55% of the study group had adequate intake. Considering α-tocopherol alone, only diets in 42–45% of the group met the recommendations.

Vitamin E is a nutrient derived from a limited number of products in the Polish diet, and the richest sources of vitamin E are vegetable oils (rapeseed, sunflower, and wheat germ). In addition, it is found in some cereal products, nuts, and vegetables, mainly dark-green ones [13]. In this study, the most abundant form of vitamin E in the diet of the studied group was α-tocopherol, and its primary sources were almonds and sunflower seeds. These data are supported by results from a multicenter, randomized, double-blind, prospective intervention study that assessed, among other things, the intake of vitamin E in the diet of older Europeans [33]. The second predominant form of vitamin E in the assessed diet in this study was γ-tocopherol, and its main sources were potato chips, crackers, nachos, and rapeseed oil. It seems that this is due to the fact that these products are highly processed foods, and during their processing, for example, frying, vegetable oils are used. A rich dietary source of γ-tocopherol are plant seeds and derived products such as vegetable oils, e.g., from corn, soybean, sesame, walnuts, pecans, and peanuts [26,34]. According to Dietrich et al. [35], γ-tocopherol is the most prevalent form of vitamin E in the American diet.

The main part of tocotrienol intake comprised β- and next α- and γ- forms, but these were small amounts compared to the sum of tocopherols and tocotrienols (21.7 mg vs. 1.3 mg). Their main sources in the diet were wholemeal bread, wholegrain pasta, brown rice, and cornflakes. Research indicates that the sources of tocotrienols could also be wheat germ, barley, oats, rye, corn, and others [27]. Tocotrienols are found in significant amounts in palm oil and rice bran oil, but they are less common than tocopherols in other vegetable oils popular in the Polish local diet [19]. Comparing the obtained values with the results of other authors allows us to assess the developed tool as correct. Sookwong et al. [36,37] estimated the daily intake of tocotrienols in the Japanese population at 1.9–2.1 mg T3/day/person. Data from the Korea National Health and Nutrition Examination Survey (KNHANES) 2016–2019 showed an average daily intake of tocotrienols of 1.61 mg/person. The main known sources of tocotrienols (annatto, palm oil, and rice bran oil), widely used in Asian countries also as a health supplement, are not included in this calculator as they are not commonly found in the local diet. It seems that their consumption level in the present study is related to the reported consumption of processed products (chips, crackers, and nachos), for which palm oil is used, for example.

The results of this study showed that only 42% of the subjects consumed α-tocopherol in the amount of at least 15 mg/day and 57% in the amount of at least 8 mg vitamin E/day for women and 10 mg for men, respectively. This is in line with the results published in the Global Systematic Review of Vitamin E Intake, which indicated that in most of the 176 studies included from 46 countries, the average intake levels of α-tocopherol and the average intake of all eight vitamin E isomers were below recommended intakes in all reported countries and regions. The lowest consumption of α-tocopherol in the world was reported in the USA, Spain, Brazil, and Poland and averaged below or close to 5 mg per day [7]. According to EFSA data [6], the average consumption of α-tocopherol in European adults ranged from 7.8 to 12.5 mg/day in women and from 8.2 to 16.0 mg/day in men. In adults, the average α-tocopherol equivalent intakes ranged between 8.9 and 13.5 mg/day in females and between 10.1 and 16.0 mg/day in males. In previous studies [38–41], the intake of vitamin E by Poles was assessed as sufficient and varied from 44% to even 199% of the DRI. Notably, food consumption in these studies was reported using various types of food records. These methods are often described as burdensome for respondents and interviewers, time-consuming, and difficult [42]. Moreover, misreporting in a food record is often observed as a result of a change in eating behavior during the reporting period. Consequently, this leads to underestimation or overestimation of energy in the

diet [43,44] and fat content, which is the primary source of vitamin E. Studies comparing food-recording methods noticed a problem with the underestimation of fat intake by the respondents. The OPEN study noted a trend toward lower fat intake for both men and women [45]. In the study by Goris et al. [46], obese men underestimated their fat intake. It seems that the semiquantitative FFQ method for a short period, relating to the consumption of a main source of micronutrients, might be more accurate. The FFQ is the method most commonly used to record food consumption in large-scale studies [47]. It can be used to assess the consumption of individual products, product groups, and the amount of nutrients present in food as well as to determine dietary patterns [48]. Questionnaires are defined as quantitative when they take into account the portion size of consumed products or their groups [49]. FFQs are often used in diet assessment because they are practical, easy to use, accessible, inexpensive to use, and engage the respondent, better describing the usual diet than other methods [50]. In the FFQ reproducibility study, cross-match was checked (test–retest), and the lowest cross-classification ability for fat-source products (vegetable oil, margarine, and butter blend) was noted at 63.8% [48]. These products are a source of vitamin E, which implies the need to refine the tool that will take into account such food products.

There are attempts to create and validate food frequency questionnaires taking into account tocopherols and tocotrienols or α-tocopherol equivalent intake [48,51–56].

In this study, the values of the obtained Bland–Altman indexes for all forms of vitamin E, the sum of tocopherols and tocotrienols, and the α-tocopherol equivalents were below 10%, which proves a good result of the questionnaire agreement with the 1-day dietary record. The VitE-FFQ includes the usual food products grouped to minimize the risk of respondents underestimating their consumption of fat, which is the primary source of various forms of vitamin E in the diet. Moreover, since the questionnaire was designed with typical European food products in mind, it would be relatively easy to modify. The VIT_E.CAL tool can be used as a practical, quick tool to assess the consumption of individual forms of tocopherols, tocotrienols, vitamin E, and α-tocopherol equivalents in the adult Polish population. The tool can be applied to assess vitamin E consumption among Europeans after introducing appropriate modifications. At a later stage, it is necessary to validate the developed questionnaire, preferably using the biochemical concentration of the consumed forms of vitamin E in the blood or metabolites of various forms of vitamin E, which will be the most reliable. A validated questionnaire combined with the calculator has the potential to provide a reliable intake assessment.

The results of this study should not be extrapolated to the Polish population in general because random sampling of the study group was used. The limitation of this study is the lack of assessment of the reproducibility of the FFQ questionnaire, which limits the possibilities of drawing conclusions and may increase the risk of overinterpretation of the results. A validation study is required to confirm the dependability of the obtained results. In addition, data on the content of various forms of vitamin E from the American database were used for Polish food products. The main advantage of this study is the relatively large number of respondents (n = 447), which leverages the importance and statistical power of the results. In addition, the Bland–Altman plot analysis was applied, which is considered the gold standard for validation of food intake questionnaires. However, further research on a representative Polish sample is necessary. This will allow for the extrapolation of the results on the structure of consumption of various forms of vitamin E in the country and at the same time will facilitate the assessment of the repeatability of the presented questionnaire.

5. Conclusions

Tocopherols and tocotrienols are important bioactive compounds and can play an important role in the prevention and treatment of many diseases, such as cardiovascular, neurodegenerative, and cancer diseases. The obtained results indicated insufficient intake of vitamin E in the study group and the need to optimize the structure of the diet in terms

of the selection of sources of its individual forms. For this reason, the developed tool, VIT_E.CAL, can be used not only in arranging a balanced diet but also in diet therapy and the prevention of these diseases. Our provided database and proposed algorithm can help professionals calculate the intake of individual forms of tocopherols and tocotrienols. The use of VIT_E.CAL enables the proper assessment of vitamin E (as α-tocopherol and not α-tocopherol equivalent) in the diet of Poles, and most likely also in the European diet. The tool may also be helpful in determining the relationship between individual forms of vitamin E intake and their plasma concentrations, which in the long run may allow for the development of individualized nutritional recommendations. The obtained results indicate the need to take into account the content of individual forms of vitamin E in food/diet, which will allow for a reliable assessment of its consumption. It also seems necessary to standardize the nomenclature regarding the name of vitamin E and its use in correct nutritional assessment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu15173759/s1, VIT_E.CAL.

Author Contributions: Conceptualization, K.S. and M.G.; methodology, K.S. and M.G.; software, K.S.; validation, K.S.; investigation, K.S. and M.G.; resources, K.S. and M.G.; data curation, K.S.; writing—original draft preparation, K.S.; writing—review and editing, M.G.; visualization, K.S.; supervision, M.G. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by the Polish Ministry of Education and Sciences within funds of the Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (WULS) for scientific research.

Institutional Review Board Statement: This study was approved by the Ethics Committee of the Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences, Poland (Resolution No. 05/2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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Warszawa, 19.03.2025 r.

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OŚWIADCZENIE

Jako współautor pracy pt. „**Dietary Vitamin E Isoforms Intake: Development of a New Tool to Assess Tocopherols and Tocotrienols Intake in Adults**" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

współtworzenie koncepcji pracy, współopracowanie metodyki, pomoc w przeprowadzeniu badań, analiza krytyczna roboczej wersji artykułu

Mój udział procentowy w przygotowaniu publikacji określam jako 30 %.

Wkład Kacpra A. Szewczyka w powstawanie publikacji określam jako 70 %,

obejmował on:

współtworzenie koncepcji pracy, współopracowanie metodyki, opracowanie narzędzi do przeprowadzenia badań, przeprowadzenie badań, analizę statystyczną uzyskanych wyników, interpretację uzyskanych wyników, gromadzenie danych, przygotowanie oryginalnego projektu, wizualizację uzyskanych danych.

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej mgr Kacpra A. Szewczyka.

Magdalena Górnicka

(podpis oświadczającego)