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„Wpływ 24-tygodniowej suplementacji L-karnityną i treningu oporowego na mięśnie szkieletowe oraz wybrane wskaźniki krwi u kobiet po 65 roku życia”

"Effects of 24-week L-carnitine supplementation and resistance training on skeletal muscle and selected blood markers in women aged over 65 years"

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1. Streszczenie w języku polskim

Masa mięśni szkieletowych zależy od tempa syntezy i degradacji białek. U ludzi w starszym wieku dysproporcja między tymi dwoma procesami jest bardzo wysoka. Co za tym idzie w starzeniu odnotowuje się zmniejszenie masy i siły mięśniowej, co przekłada się bezpośrednio na funkcjonalność i jakoś życia osób starszych.

Wpływ mają na to m.in. zachodzące z wiekiem zmiany w składzie ciała (procentowy wzrost tkanki tłuszczowej) oraz brak aktywności fizycznej. Z wiekiem wzrasta poziom krążących czynników prozapalnych we krwi, takich jak interleukina-6 (IL-6), czynnik martwicy nowotworów-α (TNF- α), czy białko C-reaktywne (CRP), a spada poziom czynników wzrostu.

W starszym wieku obserwuje się również istotny spadek zawartości L-karnityny w mięśniach. Badania pokazują, że L-karnityna może skutecznie obniżać poziom czynników prozapalnych, a także zwiększać poziom krążącego insulinopodobnego czynnika wzrostu (IGF-1), który jest kluczowy w procesie syntezy białek mięśniowych. Dodatkowo czynnikiem pozytywnie wpływającym na tkankę mięśniową jest odpowiednio dobrana aktywność fizyczna. W kontekście budowania tkanki mięśniowej szczególnie polecany jest trening oporowy. Oddziałuje on na tkankę mięśniową poprzez czynniki wzrostu, m.in. wspomniany już IGF-1, czy dekorynę i miostatynę.

Celem niniejszych badań było wskazanie skutecznej interwencji w zapobieganiu procesowi utraty masy i funkcji tkanki mięśniowej w starszym wieku.

Pierwszy etap badań, miał wskazać skuteczność suplementacji L-karnityną na poziom krążących czynników prozapalnych: IL-6, TNF- α, CRP, poziom IGF-1, poziom wolnej karnityny we krwi oraz siłę mięśni szkieletowych i skład ciała w grupie kobiet po 65 roku życia. W kolejnym etapie badań zastosowano interwencję łączoną, aby wskazać, czy suplementacja L-karnityną w połączeniu z leucyną zwiększy efektywność 24-tygodniowego treningu siłowego w kontekście zmian wielkości i funkcjonalności mięśni szkieletowych, a także związanych z tym procesem poziomów miostatyny, dekoryny, IGF-1, karnityny całkowitej (TC) i N-tlenku trimetyloaminy (TMAO) we krwi.

Do badań zostały zrekrutowane zdrowe osoby w wieku między 65 a 70 rokiem życia. Pierwszy etap badań ukończyło 20 kobiet, które losowo były przydzielone do grupy suplementowanej L-karnityną (1500 mg/doba; n=11) lub grupy przyjmującej placebo (n=9). Zastosowane interwencje wykazały, że 24-tygodniowa suplementacja L-karnityną zwiększa

poziom krążącej wolnej karnityny (o 22 ± 9%), jednak nie wpływa to na wyniki mierzonych markerów we krwi. Nie stwierdzono różnic między suplementowanymi grupami w stężeniu czynników prozapalnych, ani IGF-1. Podobnie nie zaobserwowano zmian w masie ciała, ani jej poszczególnych komponentach oraz sile mięśni.

Drugi etap badań ukończyło 37 kobiet, które przez 24 tygodnie uczestniczyły w treningu oporowym dwa razy w tygodniu. Część kobiet miała uzupełnioną interwencję o suplementację L-karnityną i leucyną (LC+L; 1000mg+3000mg/doba; n=12), lub leucyną (L; 4000mg/doba; n=13), natomiast grupa kontrolna (n=12) ćwiczyła bez przyjmowania suplementów. Wyniki uzyskane po 24 tygodniowej interwencji wskazały na efektywność treningu oporowego w kontekście hipertrofii mięśni, a także siły mięśni mierzonych w testach izokinetycznych i izometrycznych. Niemniej jednak zastosowane protokoły suplementacyjne nie wpłynęły na większą efektywność uzyskanych zmian (mimo zaobserwowanego wzrostu TC we krwi w grupie suplementowanej LC+L; $p=0.009$). Poziom dekoryny wzrósł po interwencji ($p=0.012$) w analizie obu grup suplementowanych łącznie (LC+L i L), jednak nie różnił się istotnie od wyniku w grupie CON ($p=0.231$). Trening, także połączony z suplementacją nie wpłynął na poziom IGF-1, ani poziom miostatyny. Nie zaobserwowano również korelacji między poszczególnymi parametrami wielkości i siły mięśni, a parametrami oznaczanymi we krwi (z wyjątkiem korelacji między poziomem TC i TMAO; $p<0.001$).

Na cykl publikacji składających się na niniejszą rozprawę doktorską składa się również praca przeglądowa, opisująca problematykę stosowania suplementacji L-karnityną u ludzi, ze szczególnym uwzględnieniem jej działania na metabolizm mięśni szkieletowych. Zestawione badania pozwalają wyciągnąć wnioski na temat najefektywniejszego sposobu suplementacji L-karnityną w procesie zmiany metabolizmu mięśniowego. Powinien on przebiegać w połączeniu z węglowodanami oraz trwać nie krócej niż 12 tygodni (z uwagi na niską przyswajalność L-karnityny w tkance mięśniowej).

Podsumowując zebrane dane, można wyciągnąć wnioski, że 24 tygodniowa suplementacja samą L-karnityną nie jest efektywną strategią dla zmiany komponentów składu ciała oraz wzrostu masy i siły mięśniowej u zdrowych kobiet po 65 roku życia. Połączenie suplementacji L-karnityny z leucyną nie wpływa na poziom krążącego IGF-1 i miostatyny. Mimo wzrostu poziomu TC i dekoryny we krwi, nie zwiększa efektywności treningu oporowego. Co istotne, w każdym z przypadków suplementacja L-karnityną powoduje wzrost poziom krążącego TMAO.

Słowa kluczowe: siła mięśniowa, pole przekroju poprzecznego mięśni, trening siłowy, starzenie się, insulinopodobny czynnik wzrostu-1, cytokiny, dekoryna, miostatyna, N-tlenek trimetyloamino

2. Streszczenie w języku angielskim

Skeletal muscle mass depends on the rate of protein synthesis and degradation. However, the imbalance between these two processes is very high in older people. Consequently, a reduction in muscle mass and strength is noted in ageing, directly impacting older people's functionality and quality of life.

Age-related changes in body composition (percentage increase in body fat) and lack of physical activity, among other things, impact this disproportion. With age, levels of circulating pro-inflammatory factors in the blood, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF- α), and C-reactive protein (CRP), increase, while levels of growth factors decrease.

There is also a significant decline in muscle L-carnitine content in older age. Studies show that L-carnitine can effectively reduce levels of pro-inflammatory factors and increase circulating insulin-like growth factor (IGF-1) levels, which is crucial for muscle protein synthesis. In addition, a factor that positively influences muscle tissue is appropriately selected physical activity. In the context of building muscle tissue, resistance training is particularly recommended. It affects muscle tissue through growth factors, such as the IGF-1 mentioned above or decorin and myostatin.

The aim of this study was to identify an effective intervention to prevent the loss of muscle mass and function in old age.

The first stage of the study was to indicate the efficacy of L-carnitine supplementation on levels of circulating pro-inflammatory factors: IL-6, TNF- α, CRP, IGF-1 levels, free carnitine levels and skeletal muscle strength and body composition in a group of women over 65 years of age. In the next stage of the study, a combined intervention was used to indicate whether L-carnitine supplementation combined with leucine would increase the effectiveness of 24-week strength training in the context of skeletal muscle mass, size and strength, as well as the associated blood levels of myostatin, decorin, IGF-1, total carnitine (TC) and trimethylamine N-oxide (TMAO).

Healthy subjects aged between 65 and 70 years were recruited for the study. Twenty women completed the study's first phase and were randomly allocated to either the 1500 mg/day L-carnitine supplementation group (n=11) or the placebo group (n=9). The interventions showed that 24-week L-carnitine supplementation increased circulating free carnitine levels (by 22 ± 9%) but did not affect the results of measured markers in the blood. No differences were found

between the supplemented groups in the levels of pro-inflammatory factors or IGF-1. Similarly, no changes were observed in components of body mass, or muscle strength.

The study's second phase was completed by 37 women who participated in resistance training twice a week for 24 weeks. Some women had the intervention supplemented with L-carnitine and leucine (LC+L;1000mg+3000mg/day; n=12) or leucine alone (L; 4000mg/day; n=13), while the control group exercised (n=12) without taking supplements. The results obtained after the 24-week intervention indicated the effectiveness of resistance training in muscle hypertrophy and muscle strength measured by isokinetic and isometric tests. Nevertheless, the supplementation protocols did not increase the effectiveness of the changes achieved (despite the observed increase in TC in the LC+L supplemented group; $p=0.009$). Decorin levels increased after the intervention in the analysis of both supplemented groups combined (LC+L and L; $p=0.012$), but were not significantly different from the CON group ($p=0.231$).Training, also combined with supplementation, did not affect IGF-1 or myostatin levels. There was also no correlation between individual muscle size and strength parameters and those determined in the blood (except for the correlation between TC and TMAO levels; $p<0.001$).

The series of publications comprising this doctoral thesis also includes a review paper that looks at using L-carnitine supplementation in humans, focusing on its effect on skeletal muscle metabolism. The research gathered in the review allows conclusions to be drawn on the most effective way of L-carnitine supplementation in changing muscle metabolism. L-carnitine should be supplemented with carbohydrate supplementation and last no less than 12 weeks (due to the low bioavailability of L-carnitine in muscle tissue).

Summarizing the data collected, 24 weeks of L-carnitine supplementation alone is ineffective for changing body composition components and increasing muscle mass and strength in healthy women over 65. Similarly, L-carnitine supplementation with leucine does not affect circulating levels of IGF-1 and myostatin Furthermore, despite increasing blood levels of TC and decorin, it does not increase the effectiveness of resistance training. Significantly, in each case, L-carnitine supplementation increases circulating TMAO levels.

Key words: muscle strength, muscle cross-section area, strength training, aging, Insulin-like growth factor-1; cytokines, decorin, myostatin, trimethylamino-N-oxide

3. Lista stosowanych skrótów

1RM – test jednego maksymalnego powtórzenia (ang. *one repetition maximum*)

ACSP – Amerykańskie Kolegium Medycyny Sportowej (ang. *American College of Sports Medicine*)

Akt – kinaza białkowa B (ang. *protein kinase B)*

AP – średnia moc (ang. *average power*)

BM – masa ciała (ang. *body mass*)

CHO – węglowodany (ang. *carbohydrates*)

CL – przedział ufności (ang. *confidence limit*)

CRP – białko C-reaktywne (ang. *C-reactive protein*)

CSA KE – przekrój poprzeczny prostowników kolana (ang. *cross section area of knee extensors*)

CSA TM - przekrój poprzeczny mięśni uda (ang. *cross section area of total muscle*)

FFM – beztłuszczowa masa ciała (ang. *fat free mass*)

IGF-1 – insulinopodobny czynnik wzrostu (ang. *insulin-like growth factor 1*)

IL-6 – interleukina 6 (ang. *interleukin-6*)

mTOR – kinaza białkowa treoninowo-serynowa (ang. *mammalian target of rapamycin kinase*)

MVC – maksymalny skurcz izometryczny (ang. *maximum voluntary contraction*)

NSCA – Amerykańskie Stowarzyszenie Kondycji Siłowej (ang. *National Strength and Conditioning Association*)

PI3K – kinaza 3-fosfoinozytydu (ang. *phosphoinositide-3-kinase*)

RET – trening oporowy (ang. *resistance exercise training*)

SMM – masa mięśni szkieletowych (ang. *skeletal muscle mass*)

TC – karnityna całkowita (ang. *total carnitine*)

TMAO – N-tlenek trimetyloaminy (ang.*trimethylamine-N-oxide*)

TNF- α – czynnik martwicy guza (ang. *tumor necrosis factor*)

TW – praca całkowita (ang. *total work)*

4. Wykaz prac wchodzących w skład rozprawy doktorskiej

Przedstawiona rozprawa doktorska składa się z cyklu trzech prac (dwóch prac oryginalnych i jednej pracy przeglądowej) opublikowanych w recenzowanych czasopismach zagranicznych o sumarycznej punktacji Impact Factor (IF) równej 14.615 oraz Ministerstwa Nauki i Szkolnictwa Wyższego (MNiSW) równej 235 pkt.

Publikacja nr 1: Sawicka, A. K., Hartmane, D., Lipinska, P., Wojtowicz, E., Lysiak-Szydlowska, W., Olek, R. A. (2018). L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function. Nutrients, 10(2), 255, 1-11.

IF: 4,171 / MNiSW: 35 / artykuł oryginalny

Publikacja nr 2: Sawicka, A. K., Jaworska, J., Brzeska, B., Sabisz, A., Samborowska, E., Radkiewicz, M., Szurowska, E., Winklewski, P. J., Szarmach, A., & Olek, R. A. (2022). L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women. The journal of nutrition, health & aging, 26(10), 945–953.

IF: 5.285 / MNISW: 100 / artykuł oryginalny

Publikacja nr 3: Sawicka, A. K., Renzi, G., & Olek, R. A. (2020). The bright and the dark sides of L-carnitine supplementation: a systematic review. Journal of the International Society of Sports Nutrition, 17(1), 49.

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„Suplementacja karnityną a funkcja mięśnia szkieletowego w starzeniu”

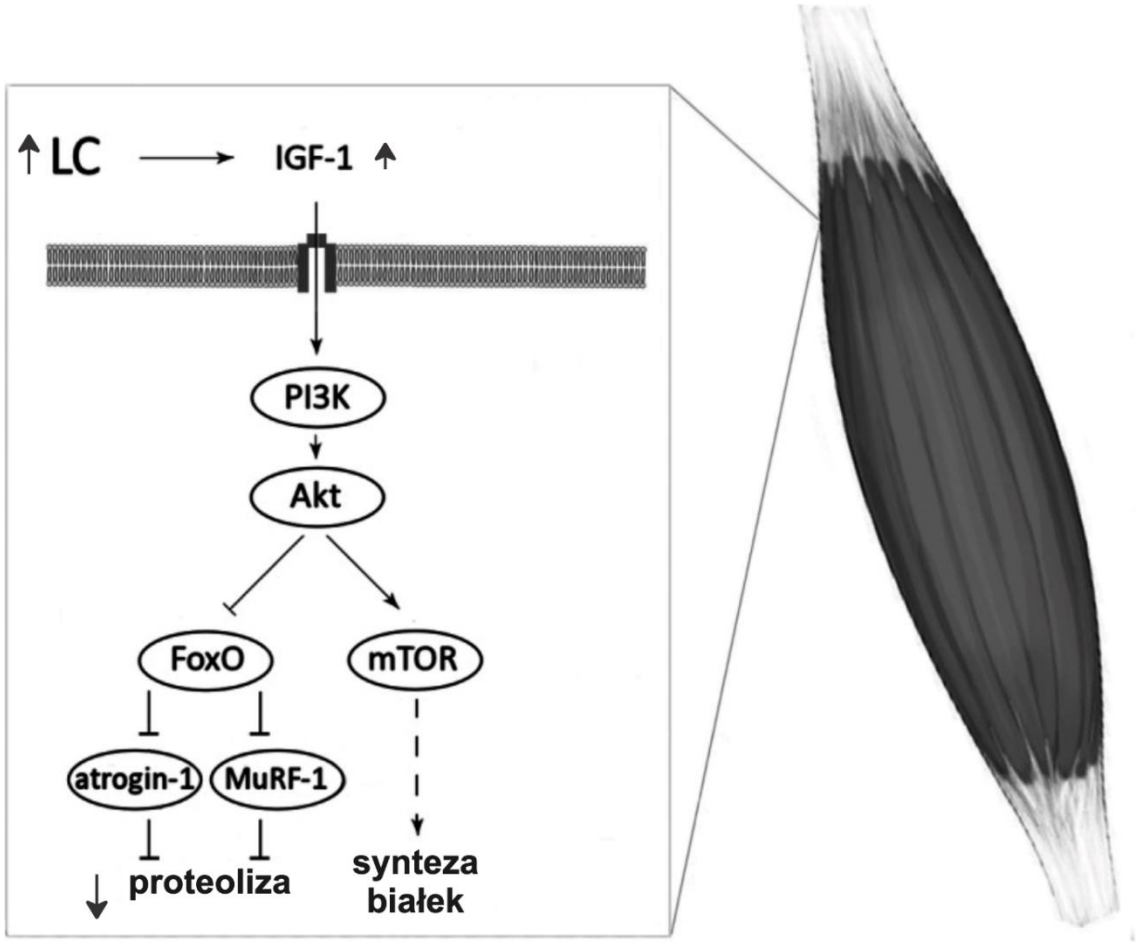
5. Wprowadzenie

Podeszły wiek wiąże się z postępującą utratą sprawności fizycznej, której bezpośrednią przyczyną jest mimowolna utrata masy i siły mięśniowej, jak i zanik funkcji tkanki mięśniowej [1]. Pomimo utraty masy mięśniowej w populacji osób starszych nie obserwuje się spadku masy ciała, zmienia się bowiem skład poszczególnych jego komponentów, a dokładniej stosunek tkanki tłuszczowej do beztłuszczowej masy ciała. Tkanka tłuszczowa, obok funkcji magazynującej, pełni również funkcję wydzielniczą [2]. Odpowiedzialna jest za produkcję i uwalnianie do krwiobiegu wielu bioaktywnych cząsteczek, zwanych adipokinami, w tym m.in. czynników prozapalnych, takich jak interleukina 6 (ang. *interleukin-6*; IL-6), czynnik martwicy guza (ang. *tumor necrosis factor*; TNF-α), czy białko ostrej fazy, inaczej białko C-reaktywne (ang. *C-reactive protein*; CRP) [3]. Procentowy wzrost tłuszczowej masy ciała może być jedną, obok innych przyczyn, podwyższonego stężenia krążących w organizmie czynników prozapalnych, odnotowywanych w starszym wieku. Mowa tu o wspomnianych wcześniej: TNF-α, IL-6, CRP i podwyższonej liczbie neutrofili [4]. Liczne badania naukowe wskazują na to, że cytokiny odgrywają dużą rolę w metabolizmie białek mięśni szkieletowych [5]. Znanych jest kilka mechanizmów biochemicznych, które potwierdzają negatywny wpływ czynników prozapalnych na tkankę mięśniową [6]. IL-6 i TNF- α hamują syntezę białek [7] oraz zwiększają ich proteolizę [5, 8]. Pośrednim skutkiem oddziaływania TNF-α na metabolizm białek mięśniowych może być również jego zdolność do blokowania działania insuliny, która wpływa pozytywnie na syntezę tychże białek oraz ochronnie na procesy ich rozkładu [9, 10].

L-karnityna jest niskocząsteczkowym, azotowym związkiem, którego główną rolą jest transport długołańcuchowych kwasów tłuszczowych z cytoplazmy do macierzy mitochondrialnej [11]. Związek ten wykazuje również działanie przeciwzapalne [12]. Potwierdzono to w badaniach naukowych z wykorzystaniem różnych modeli eksperymentalnych, m.in.: w starzeniu [13], przy zwłóknieniu wątroby [14], czy przy kacheksji nowotworowej [15]. Badania Lee i in. [16] wykazały, że 12 tygodniowa suplementacja L-karnityną w ilości 1g/doba zmniejsza stan zapalny u osób z chorobą wieńcową. Meta-analiza porównująca 44 randomizowane badania kliniczne wykazała, że suplementacja L-karnityną związana jest m.in. z obniżeniem poziomów CRP, IL-6 i TNF-α [17].

Co więcej, L-karnityna może wpływać na równowagę białek mięśniowych, dzięki aktywacji kinazy białkowej treoninowo-serynowej (ang. *mammalian target of rapamycin kinase;* mTOR), poprzez podnoszenie stężenia insulinopodobnego czynnika wzrostu (ang. *insulin-like growth factor 1*; IGF-1) [18]. Szlak sygnałowy IGF-1 – PI3K (kinaza 3-

fosfoinozytydu; ang. *phosphoinositide-3-kinase*) – Akt (kinaza białkowa B; ang. *protein kinase B*) – mTOR zarówno przyspiesza proces syntezy białek, jak i hamuje proces proteolizy (Ryc.1) [19, 20, 21].



Rycina 1. Związek pomiędzy suplementacją L-karnityną a regulacją szlaków metabolicznych zaangażowanych w równowagę białek mięśniowych. L-karnityna (LC); insulinopodobny czynnik wzrostu-1 (IGF-1); kinaza 3-fosfoinozytydu (PI3K); kinaza białkowa B (Akt); kinaza białkowa treoninowo-serynowa (mTOR); białka FOX (FoxO); specyficzne dla mięśni białko RING finger 1 (MuRF-1); muscle atrophy F-box (atrogin-1) [21].

IGF-1 jest jednym z najlepiej poznanych czynników wzrostu; w badaniach na ludziach wykazano, że koreluje dodatnio z parametrami sprawności tlenowej, wytrzymałością mięśni czy niższym poziomem tkanki tłuszczowej [22]. Badanie Keller i in. [23] przeprowadzone na modelu zwierzęcym wykazało, że L-karnityna zwiększa poziom krążącego IGF-1 w osoczu, przyczyniając się do spowolnienia katabolizmu białek w mięśniach szkieletowych. Co ważne, poziom IGF-1 zmniejsza się wraz z wiekiem, średnio o 10% co dekadę [24].

Analiza próbek mięśni ludzi wskazuje na istotną redukcję L-karnityny oraz acetylo-l-karnityny wraz z wiekiem [25]. Zależność ta postrzegana jest jako potencjalna przyczyna sarkopenii, oraz osłabienia mięśni i szybszego męczenia obserwowanych w procesie starzenia [26].

W badaniach Wall i in. [27] wykazano, że zawartość karnityny całkowitej (ang. *total carnitine*; TC) w mięśniach można regulować poprzez interwencję dietetyczną. W tym samym eksperymencie dowiedziono, że suplementacja L-karnityną wpływa na metabolizm energetyczny mięśni, a mianowicie zwiększa zużycie lipidów w celu uzyskania energii podczas wysiłku o niskiej intensywności, oszczędzając tym samym zmagazynowany w mięśniach glikogen. Efekt ten zaobserwowano po podawaniu dwa razy dziennie 2g L-karnityny w połączeniu z 80g węglowodanów (ang. *carbohydrates;* CHO) przez okres 24 tygodni. Do protokołu suplementacji włączono CHO, ponieważ transport L-karnityny do komórek mięśniowych jest insulino-zależny [28].

Chcąc uzyskać podobny efekt, czyli zwiększyć zawartość TC w mięśniach, suplementację L-karnityną należy uzupełnić o związek regulujący poziom insuliny we krwi. Innym szeroko stosowanym składnikiem, który znany jest ze swoich właściwości stymulujących wydzielanie insuliny przez komórki beta trzustki jest leucyna [29].

Leucyna jest egzogennym aminokwasem rozgałęzionym, jednym z najefektywniej wpływających na syntezę białek mięśniowych [30]. Wykazano, że połączenie wysiłku siłowego wraz z suplementacją aminokwasami egzogennymi, w tym przede wszystkim leucyną uaktywnia szlak sygnałowy dla mTOR, który związany jest z syntezą białek mięśniowych [31]. Wykorzystanie tego związku jako transportera L-karnityny do komórek mięśniowych dodatkowo może wzmocnić działanie stymulujące produkcję białek mięśniowych.

Odpowiednio dobrana aktywność fizyczna również może pozytywnie wpłynąć na funkcje tkanki mięśniowej, zapobiegając a nawet odwracając proces utraty masy mięśniowej [32]. W przypadku wzrostu syntezy białek mięśniowych u starszych ludzi, idealnym rozwiązaniem okazuje się zastosowanie treningu siłowego [33]. Wzrost syntezy białek mięśniowych przy treningu oporowym (ang. *resistance exercise training*; RET) może być regulowany przez hormony i czynniki wzrostu, w tym przez wspominany już IGF-1 czy miostatynę.

Miostatyna jest negatywnym czynnikiem wzrostu i odpowiada za degradację białek mięśniowych [34]. Badania przekrojowe pokazały, że poziom miostatyny rośnie wraz z

wiekiem (zarówno u kobiet jak i u mężczyzn), a jej poziom jest odwrotnie skorelowany z masą mięśniową i beztłuszczową masą ciała [35]. Dodatkowo badania prowadzone na osobach starszych wykazały, że wzrost tego białka jest związany z niższą siłą mięśni u kobiet [36]. Aktywność miostatyny jest blokowana w macierzy pozakomórkowej poprzez bogaty w leucynę proteoglikan – dekorynę [37], która wydzielana jest w odpowiedzi na aktywność fizyczną [38, 39]. Co ciekawe badania Wiloughby i in. [39] wskazują, że pojedyncza jednostka RET powoduje znaczny wzrost dekoryny, która jest w stanie związać, a następnie zmniejszyć ilość miostatyny w surowicy, mimo jej wzrostu w mięśniach szkieletowych po treningu.

Patrząc na powyższe, trening oporowy w połączeniu z interwencją żywieniową stanowi lepszy bodziec dla utrzymania siły i masy mięśniowej niż sam trening.

Jak się okazuje, nie bez znaczenia w procesie utraty tkanki mięśniowej wraz z wiekiem może pozostawać rola mikrobioty jelitowej [40], która to zaangażowana jest w metabolizm L-karnityny do krążącego N-tlenku trimetyloaminy (ang. *trimethylamine-N-oxide;* TMAO) [41]. Długotrwała suplementacja L-karnityną podnosi poziom TMAO w osoczu na czczo u ludzi [42]. Z kolei badania in vitro wskazują, że TMAO może zwiększać syntezę białek [43] lub modulować aktywność ATPazy miozynowej [44, 45].

Podsumowując, masa mięśni szkieletowych zależy od tempa syntezy i degradacji białek. U ludzi w podeszłym wieku dysproporcja między syntezą nowych białek mięśniowych, a ich degradacją jest bardzo wysoka. Suplementacja L-karnityną, tym bardziej w połączeniu z systematycznym treningiem siłowym, może skutecznie przeciwdziałać opisanym zmianom. Toteż podjęcie zaproponowanego badania, które ma na celu wskazanie najskuteczniejszej i wieloaspektowej interwencji, wydaję się być wysoko zasadne.

6. Cele rozprawy doktorskiej

Cele badawcze:

1. Ocena wpływu 24 tygodniowej suplementacji L-karnityną na stężenie markerów stanu zapalnego oraz czynników wzrostu we krwi i wpływ tych zmian na siłę mięśni szkieletowych oraz skład ciała.
2. Określenie efektu połączenia suplementacji L-karnityną i leucyną wraz z treningiem oporowym na siłę i hipertrofię mięśni szkieletowych, a także związane z nimi zmiany w poziomie IGF-1, dekoryny, miostatyny oraz TMAO.

7. Materiały i Metody

7.1 Konstrukcja przeprowadzonych badań

Na cały proces badawczy składają się dwa eksperymenty, przeprowadzone w okresie między grudniem 2015 r. a majem 2018 r., opisane kolejno w pierwszej i drugiej publikacji. Trzecia publikacja – przegląd systematyczny, został opublikowany w 2020 roku. Przegląd powstał w celu usystematyzowania wiedzy na temat wykorzystania L-karnityny w suplementacji u ludzi, celowanej na zwiększenie siły i masy mięśniowej.

Pierwszy etap badań, opisany w publikacji *„L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function."* trwał od grudnia 2015 do czerwca 2016 r. Ochotników losowo przydzielono do jednej z dwóch grup – suplementowanej L-karnityną w ilości 1500 mg/doba (LC) i kontrolnej (CON), przyjmującej placebo. Obie grupy przyjmowały kapsułki przez okres 6 miesięcy, ze wskazaniem odstawienia innych suplementów diety i nie modyfikowania swojego dotychczasowego stylu życia.

W czasie trwania eksperymentu badani zostali poddani testom trzykrotnie - przed rozpoczęciem, po 12 tygodniach i po zakończeniu 24 tygodniowej procedury suplementacji. Badanie obejmowało: analizę składu ciała (przy użyciu metody spektroskopowej bio-impedancji – InBody), pomiar siły prostowników i zginaczy kolana (przy pomocy platformy dynamometrycznej Biodex System 4 Pro) oraz oznaczenie poziomu czynników prozapalnych, stężenie wolnej karnityny oraz IGF-1 we krwi żylnej.

Drugi etap badań, opisany w publikacji *„L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women."* miał miejsce od października 2017 do maja 2018 r. W tym etapie osoby badane zostały podzielone losowo na trzy grupy – dwie grupy suplementowane: 1000 mg L-karnityny w połączeniu z 3000 mg leucyny (LC+L), lub 4000 mg leucyny (L) oraz grupę kontrolną (CON; nieprzyjmującą suplementów). Wszystkie grupy przez okres suplementacji, tj. 6 miesięcy były poddawane regularnemu treningowi siłowemu (opisany poniżej).

Przed rozpoczęciem i po 24 tygodniach procesu treningowego i suplementacji osobom badanym zostały wykonane testy. Badania obejmowały te same pomiary, co w przypadku etapu pierwszego, dodatkowo zostały rozszerzone o wolumetrię uda, wykonaną za pomocą rezonansu magnetycznego - skaner 1,5 Tesli Magnetom Aera (Siemens) oraz o pomiary 1RM (ang. *one repetitium maximum*, test jednego maksymalnego powtórzenia). Analiza próbek krwi obejmowała ocenę stężenia całkowitej karnityny, dekoryny, miostatyny, IGF-1 oraz TMAO.

Badania posiadają zgodę Komisji Bioetycznej przy Okręgowej Izbie Lekarskiej w Gdańsku – NKBBN/354/2012, NKBBN/354-304/2015 oraz NKBBN/354-201/2017, a wszystkie osoby włączone do badań zostały poinformowane o celach i przebiegu badania oraz podpisały świadomą zgodę na udział w projekcie.

7.2 Charakterystyka grupy badanej

Do pierwszego etapu badań zgłosiło się 42 ochotników, z czego po wstępnym badaniu przesiewowym, obejmującym wywiad, badanie krwi oraz konsultacje kardiologiczną do eksperymentu zakwalifikowano 28 osób (26 kobiet i 2 mężczyzn). Osoby niezakwalifikowane zrezygnowały z uczestnictwa bądź nie spełniały kryteriów włączenia. Kryteriami do udziału w badaniu były: wiek 65 – 70 lat, abstynencja nikotynowa, ogólny dobry stan zdrowia (z udziału wykluczały choroby układu krążenia, choroby wątroby i nerek, zaburzenia żołądkowo – jelitowe, w tym wrzody żołądka i nadżerki, choroby nowotworowe, cukrzyca, choroby układu ruchu i inne ciężkie choroby przewlekłe). Osoby zakwalifikowane losowo przydzielono do grupy suplementowanej L-karnityną (LC; n=14; wiek: 67.8 ± 2.3 lat), lub do grupy przyjmującej placebo (CON; n=14; wiek: 66.4 ± 1.3 lat). Ostatecznie pierwszy etap badań ukończyło 20 kobiet.

Do drugiego etapu badań zgłosiło się 100 osób, z których po badaniu przesiewowym (wywiad, badania krwi, konsultacja kardiologiczna) do procesu badawczego zostało zakwalifikowanych 60 kobiet w wieku 65-70 lat, w wywiadzie bez widocznych zaburzeń poznawczych. Kryteriami wykluczającymi były: nikotynizm, choroby układu krążenia, choroby wątroby i nerek, zaburzenia żołądkowo – jelitowe, w tym wrzody żołądka i nadżerki, choroby nowotworowe, cukrzyca, choroby układu ruchu i inne ciężkie choroby przewlekłe oraz jakiekolwiek przeciwskazania do badania rezonansem magnetycznym. Dodatkowo ochotniczki musiały przedstawić zaświadczenie od lekarza o braku przeciwwskazań do wykonywania ćwiczeń siłowych. Badane zostały losowo przydzieleni do grupy LC + L (n=20; wiek: 67.8 ± 2.7 lat), przyjmującej kapsułki z L-karnityną i leucyną, do grupy L (n=20; wiek: 67.8 ± 2.3 lat) suplementowanej czystą leucyną oraz grupy kontrolnej (n=20; wiek: 66.8 ± 3.3 lat). Ostatecznie procedurę badawczą ukończyło 37 osób.

7.3 Procedura treningowa

Zastosowany trening obejmowała wyłącznie etap 2 eksperymentu. Okres treningowy trwał przez 6 miesięcy, obejmował 48 jednostek treningowych, które odbywały się regularnie 2 razy w tygodniu na siłowni. Protokół ćwiczeń siłowych został przygotowany na podstawie planu

treningowego stosowanego już wcześniej w badaniach klinicznych [46]. Jest to program ułożony zgodnie z wytycznymi dla osób starszych, przedstawionymi przez Amerykańskie Kolegium Medycyny Sportowej (ang. *American College of Sports Medicine*; ACSP) i Amerykańskie Stowarzyszenie Kondycji Siłowej (ang. *National Strength and Conditioning Association;* NSCA).

Na kompletny plan treningowy składało się 6 ćwiczeń wykonywanych na przyrządach: horyzontalne wypychanie nóg na maszynie, wyprost nóg na maszynie siedząc, ćwiczenia na mięśnie piersiowe większe i mniejsze, ściąganie drążka wyciągu górnego do klatki w siadzie podchwytem, przyciąganie linki wyciągu dolnego w siadzie, wyciskanie ciężaru na klatkę piersiową siedząc. Horyzontalne wypychanie nóg na maszynie oraz wyprost nóg na maszynie siedząc były wykonywane na każdej sesji treningowej. Ćwiczenia angażujące górne partie ciała były wykonywane na przemian: podczas pierwszego treningu w tygodniu - ściąganie drążka wyciągu górnego do klatki w siadzie podchwytem oraz wyciskanie ciężaru na klatkę piersiową siedząc, natomiast podczas drugiego treningu w tygodniu - ćwiczenia na mięśnie piersiowe większe i mniejsze oraz przyciąganie linki wyciągu dolnego w siadzie. Na każdej sesji treningowej badane wykonywały 4 ćwiczenia.

Tydzień przed rozpoczęciem treningów każdy z uczestników zapoznał się z planem treningowym i wykonał test 1RM, dla każdego z przewidzianych ćwiczeń. Uzyskane wyniki były punktem odniesienia do zadawanej intensywności podczas treningów. Przez pierwsze dwa tygodnie badani wykonywali 3 serie po 10-12 powtórzeń dla każdego ćwiczenia przy 65% maksymalnej intensywności. Po dwóch tygodniach adaptacji intensywność została podniesiona do 80% 1RM, a ćwiczenia wykonywane były w 3 seriach po 6-8 powtórzeń. Test 1RM powtarzany był średnio co 6 tygodni w celu weryfikacji postępów oraz ustalania kolejnych poziomów zadawanych obciążeń.

Każda sesja treningowa była poprzedzana 10 minutową rozgrzewką na bieżni (marsz), a kończona spokojną jazdą na ergometrze rowerowym. Wszystkie ćwiczenia wykonywane były pod opieką instruktora. Do badania zostały włączone osoby, które ukończyły co najmniej 80% sesji treningowych.

7.4 Metody i narzędzia badawcze

W badaniach zastosowano poniższe metody i narzędzia badawcze:

7.4.1 Pomiary antropometryczne

Do oceny masy ciała (ang. *body mass;* BM) oraz analizy komponentów składu ciała, takich jak beztłuszczowa masa ciała (ang. *fat free mass;* FFM) oraz masa mięśni szkieletowych (ang. *skeletal muscle mass*; SMM) użyto metody spektroskopowej bioimpedancji posługując się urządzeniem InBody 720 (Biospace, Korea). Urządzenie mierzy impedancję z kończyn oraz z tułowia poprzez 8-polarną elektrodę dotykową. Uczestnicy byli poddawani badaniu w pozycji stojącej, boso, ubrani w bieliznę, pozostając na czczo.

7.4.2 Pomiary siły mięśni

Pomiary siły mięśni dotyczyły zginaczy (eksperyment pierwszy) i prostowników (eksperyment pierwszy i drugi) stawu kolanowego nogi dominującej, obejmowały zarówno ocenę izokinetyczną jak i izometryczną (w drugim eksperymencie). Badanie zostało wykonane przy użyciu platformy dynamometrycznej Biodex System 4 Pro, (Biodex Medical Systems, New York). Przed testem badani zostali unieruchomieni w sposób izolujący ruch w danym stawie, wykluczając wspomaganie go innymi częściami ciała. Pomiar izokinetyczny był wykonywany przy prędkości kątowej 60°/s w pięciu próbach. Natomiast test izometryczny był wykonywany przy zgięciu kolana w 90°. W teście izometrycznym badani wykonywali po trzy powtórzenia maksymalnego skurczu prostowników stawu kolanowego (trwającego 4s), każdorazowo z 20 s spoczynkiem między powtórzeniami. Szczytowy moment obrotowy mierzony był przez wykonanie maksymalnego skurczu izometrycznego (ang. *maximum voluntary contraction;* MVC) podczas wyprostu kolana. Badanie zostało poprzedzone krótką rozgrzewką na ergometrze rowerowym.

7.4.3 Pomiar przekroju poprzecznego mięśnia

Pomiar wolumetryczny mięśnia wykonywany był przy użyciu rezonansu magnetycznego 1.5T Siemens MAGNETOM Aera (Siemens, Monachium, Niemcy) w Zakładzie Radiologii Uniwersyteckiego Centrum Klinicznego w Gdańsku. Badanie obejmowało obszar od stawu kolanowego do końca stawu biodrowego nogi dominującej.

Protokół badania obejmował skanowanie uda w płaszczyźnie czołowej, strzałkowej i poprzecznej, w obrazach T1 i T2-zalenych, w sekwencjach Turbo Spin Echo oraz VIBE Dixon.

Po zebraniu danych, u każdego z uczestników został wyznaczony obszar na 2/3 wysokości kości udowej, który jest właściwym dla pomiaru maksymalnej siły prostowników kolana. W

wyznaczonym obszarze został zmierzony przekrój poprzeczny mięśnia uda (CSA TM; *cross section area of total muscle*) oraz przez główne prostowniki kolana (CSA KE; *cross section area of knee extensors*), takie jak mięsień poprzeczny boczny (łac. *vastus lateralis*), mięsień obszerny pośredni (łac. *vastus intermedius)* i mięsień obszerny przyśrodkowy (łac. *vastus medialis*). Uzyskane skany analizowane były za pomocą oprogramowania OsiriX Lite (Pixmeo SARL, Bernex, Szwajcaria), przez dwóch niezależnych badaczy (główny badacz oraz radiolog). Między indywidualny współczynnik zmienności analizy wynosił 1.3%.

7.4.4 Analiza próbek krwi oraz oznaczenia biochemiczne

Do określenia stężenia wolnej karnityny, całkowitej karnityny oraz TMAO została użyta metoda wysokosprawnej chromatografii cieczowej sprzężonej z detektorem mas (UPLC/MS/MS), natomiast do oznaczenia IL-6, CRP, TNF-α, IGF-1, dekoryny oraz miostatyny zastosowana została metoda immunoenzymatyczna ELISA, przy użyciu gotowych zestawów z firm R&D (R&D Systems, Minneapolis, MN, USA) i Cloud-Clone Corp. (Cloud-Clone Corp., Houston, TX, USA).

7.4.5 Metoda przeprowadzenia przeglądu systematycznego

Literatura do pracy przeglądowej została zebrana poprzez bazy danych: MEDLINE (przez wyszukiwarkę PubMed) oraz Web of Science. Wyszukiwanie dotyczyło prac opublikowanych do lutego 2020 roku i zawierających niniejsze kombinacje terminów: “carnitine supplementation” lub “carnitine treatment” w połączeniu z “exercise”, “training”, “athletic performance”, “muscle strength”, “muscle fatigue”, “muscle damage”, “muscle recovery”, “muscle synthesis” lub “proteolysis”. Wstępne wyszukiwanie przyniosło wynik 1024 publikacji (po usunięciu duplikatów), które zostały poddane dalszej selekcji.

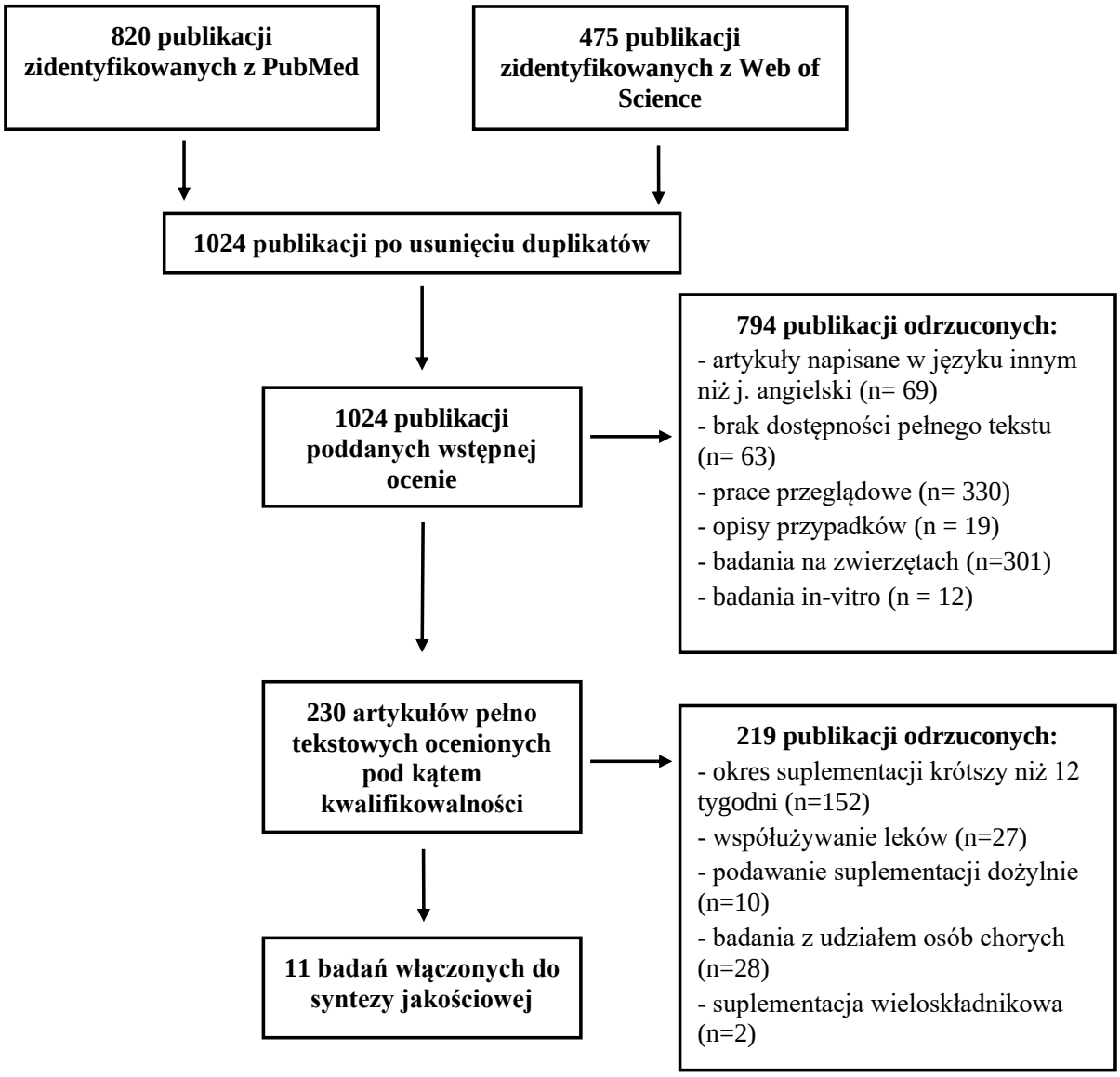
W celu klaryfikacji kryteriów włączenia badań do przeglądu został zastosowany model PICOS, który odpowiadając na cele badawcze niniejszej pracy został zdefiniowany w następujący sposób: „P” – uczestnicy (ang. *participants*) – zdrowi ludzie; „I” – interwencja (ang. *intervention*) – doustna suplementacja L-karnityną; „C” – porównanie (ang. *comparision*) – pomiędzy prowadzoną suplementacją a placebo; "O" – efekty względem, których oceniana będzie efektywność interwencji (ang. *outcomes*) – zmienne dotyczące mięśni; „S" – projekt badania (ang. study design) - randomizowane badania kontrolowane, nierandomizowane badania kontrolowane, nierandomizowane badania niekontrolowane.

Drugi etap weryfikacji zebranych publikacji przebiegał z analizą streszczeń, pośród których zostały wykluczone prace przeglądowe, opisy przypadków, badania prowadzone na

zwierzętach, badania in-vitro oraz wszystkie prace, które były sporządzone w języku innym niż język angielski i nie były dostępne w formacie pełno tekstowym. Odrzucenie prac, które nie spełniało powyższych warunków pozwoliło włączyć do dalszej analizy 230 artykułów.

Ostatni etap kwalifikacji polegał na analizie pełnych manuskryptów i odrzuceniu prac, które nie spełniały kryteriów włączenia, takich jak: a) grupa badana – ludzie zdrowi; b) suplementacja co najmniej przez 12 tygodni; c) suplementacja doustna; d) brak współużywania leków; e) brak suplementacji wieloskładnikowej. Ostatecznie powyższe kryteria włączenia spełniło 11 publikacji, które zostały włączone do analizy jakościowej w pracy (Ryc. 2).

Proces selekcji prac i zbierania danych przeprowadzany był niezależnie przez dwóch autorów.



Rycina 2. Schemat wyszukiwania i wyboru artykułów włączonych do przeglądu systematycznego.

8. Najważniejsze wyniki opublikowane w pracach zawartych w cyklu

8.1 Najważniejsze wyniki opublikowane w pracy pt.: *L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function.*

Niniejsze badanie ukończyło 20 kobiet (grupa LC n=11; średni wiek: 67.8 ± 2.3; grupa CON n=9; średni wiek: 66.4 ± 1.3).

Dwadzieścia cztery tygodnie suplementacji L-karnityną istotnie wpłynęły na wzrost stężenia wolnej karnityny we krwi, podnosząc poziom wyjściowy 41.1 ± 6.4 µmol/L o 22 ± 9%. Podobnego wzrostu nie zaobserwowano w grupie przyjmującej placebo, gdzie wartość wyjściowa 39.5 ± 3.7 µmol/L wzrosła jedynie o 10 ± 11%, a skorygowany efekt dla obu grup wynosił 13% ± 5.8% (średnia; przedział ufności, ang. *confidence limit*; CL 90%), dając tym samym możliwość wnioskowania o wysoce umiarkowanym efekcie. Niemniej jednak wzrost wolnej karnityny w grupie LC nie wpłynął na zmiany stężenia pozostałych badanych wskaźników. Wartości mierzone dla IL-6 i IGF-1 charakteryzował niewielki spadek w obu grupach (w grupie LC odpowiednio o –11 ± 42% i o −6 ± 28% ; w grupie CON odpowiednio −10 ± 23% i −10 ± 12%), a skorygowany efekt został zinterpretowany jako nieznaczący. Podobnie w przypadku wartości zmierzonych dla TNF-α, zaobserwowany minimalny wzrost (LC o 24 ± 82%; CON o 14 ± 70%) przyniósł skorygowany efekt dla obu grup 9.0%; ± 50% (średnia; CL 90%) i został zinterpretowany jako nieznaczący. W przypadku mierzonego CRP zaobserwowany skorygowany efekt (21% ± 37%; średnia; CL 90%), został zinterpretowany jako niejasny, gdzie wartości wyjściowe w grupie LC wskazywały minimalne tendencje wzrostowe (o 8 ± 68%),a w grupie CON spadkowe (o −6 ± 15%).

Zastosowany w badaniu protokół suplementacji L-karnityną nie wpłynął istotnie na zmianę BM, ani też poszczególnych jej komponentów: FFM i SMM. Różnica skorygowanych średnich zmian w BM w grupie suplementowanej L-karnityną i placebo w odniesieniu do wartości wyjściowych wynosiła 0.5% ± 1.9% (średnia; CL 90%). Natomiast dla FFM była to wartość 0.7% ± 2.6%, a dla SMM 1.2% ± 3.2%. Podobnie w przypadku mierzonej siły mięśni uda w dominującej kończynie dolnej nie zaobserwowano istotnych zmian w żadnym z parametrów z wykonywanych testów izokinetycznych. Zarówno dla pomiarów pracy całkowitej (ang. *total work;* TW) i średniej mocy (ang. *average power;* AP) skorygowany efekt dla obu grup oceniony został jako nieznaczący; przyjmując odpowiednio wartość TW podczas wyprostu

(5.6% ± 7.1%; średnia ± CL90%) i zgięcia (−2.9% ± 13%), natomiast AP podczas wyprostu (1.4% ± 6.8%), a zginania (−7.1% ± 9.7%).

8.2 Najważniejsze wyniki opublikowane w pracy pt.: *L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women*.

Niniejsze badanie ukończyło 37 kobiet (grupa LC n=12; średni wiek: 68.0 ± 2.7; grupa L n=13; średni wiek: 67.9 ± 2.1; grupa CON n=12; średni wiek: 65.8 ± 2.6).

Dwadzieścia cztery tygodnie RET wykonywanego przez badanych dwa razy w tygodniu przyniosło statystycznie istotne zmiany w hipertrofii mięśni, mierzonych jako przekrój poprzeczny mięśni uda (CSA TM; p=0.005) jak i jako przekrój poprzeczny przez główne prostowniki stawu kolanowego (czyli mięsień poprzeczny boczny, mięsień obszerny pośredni i mięsień obszerny przyśrodkowy; CSA KE; p=0.006). Przeprowadzony protokół treningowy spowodował również istotny wzrost w średniej mocy (p<0.001), pracy całkowitej (p<0.001) oraz wzrost w szczytowych wartościach maksymalnego momentu siły (p<0.001) w testach siły izokinetycznej prostowników stawu kolanowego. Podobne zmiany zostały zaobserwowane w szczytowych wartościach maksymalnego momentu siły (p=0.009) w teście izometrycznym prostowników stawu kolanowego. Niemniej jednak, protokół suplementacji w grupach LC+L i L nie wpłynął istotnie na poziom zaobserwowanych zmian w żadnych z wymienionych parametrów w analizie międzygrupowej [47].

Prowadzona z treningiem suplementacja L-karnityną zwiększyła istotnie poziom TC w osoczu w grupie LC+L (9.9 ± 6.3 µmol/L; p= 0.009), której nie zaobserwowano w grupie L (0.4 ± 9.1 µmol/L), ani CON (2.3 ± 6.6 µmol/L). Suplementacja L-karnityną wpłynęła istotnie również na poziom krążącego TMAO w grupie LC+L (p<0.001). W grupie L i CON nie zaobserwowano zmian w stężeniu TMAO po zastosowanej interwencji (odpowiednio: p=0.959 i p=0.866). Sam trening ani trening wspomagany suplementacją w żadnej z grup nie wpłynął istotnie na poziom krążącego IGF-1 (% zmiana wartości przed i po interwencji w grupach: LC+L = 11.3% ± 36.3; L = 6.8% ± 19.9; CON = 1.6% ± 17.8), a relacja między grupami nie była istotna statystycznie (p=0.757). Podobnie brak statystycznie istotnych różnic widoczny był w stężeniu miostatyny (p=0.619). Zaobserwowana tendencja zmian w stężeniu dekoryny (p=0.075), przyczyniła się do wykonania dodatkowej analizy grup suplementowanych łącznie

(LC+L i L). U osób suplementowanych leucyną zaobserwowano statystycznie istotny wzrost stężenia dekoryny (przed 8.2 ± 1.5µg/L, po 8.9 ± 1.5µg/L; p=0.012), jednak wartości po interwencji nie różniły się istotnie statystycznie w porównaniu do grupy CON (p=0.231).

Przeprowadzone analizy korelacji wykazały, że podwyższony poziom TC w osoczu pozytywnie korelował ze zmianą poziomu TMAO (rho=0.595, p<0.001). Nie zaobserwowano natomiast żadnych istotnych korelacji między parametrami siły (MVC PT, PT, TW, AP) i przekroju poprzecznego mięśni (CSA TM i CSA KE) a zmianami w poziomie krążących markerów we krwi (IGF-1, TMAO, TC).

8.3 Najważniejsze wyniki opublikowane w pracy pt.: *The bright and the dark sides of L-carnitine supplementation: a systematic review*.

Niniejszy przegląd systematyczny został przygotowany w celu usystematyzowania wiedzy na temat wpływu długotrwałej suplementacji L-karnityną na metabolizm mięśniowy osób zdrowych.

Do analizy jakościowej włączono jedenaście publikacji (wydanych w latach 2002 – 2020), opisujących badania z udziałem zdrowych dorosłych, poddawanych doustnej suplementacji przynajmniej przez okres 12 tygodni. Co istotne były to prace, w których badani nie przyjmowali suplementów wieloskładnikowych, ani innych leków. Każda z prac została przeanalizowana pod kątem podawanej dawki i długości stosowanej suplementacji oraz uzyskanego efektu głównego.

W wybranych publikacjach, badani byli suplementowani L-karnityną w dawce od 1g do 4,5g na dobę przez okres 12 lub 24 tygodni. W trzech spośród włączonych badań suplementację łączono z węglowodanami (CHO) w ilości 80g/doba [27, 48, 49], a w jednym badaniu z leucyną 3g/doba [50].

Zebrane dane pozwoliły wyciągnąć wspólne wnioski, iż 12 – tygodniowa suplementacja samą L-karnityną nie zwiększa poziomu TC w mięśniach i tym samym nie wpływa na metabolizm mięśniowy [51, 52]. Jedynie u wegetarian zaobserwowano wzrost poziomu TC w mięśniach po 12 tygodniowej suplementacji bez CHO [51]. Nie miało to jednak wpływu na metabolizm mięśni szkieletowych, ani poziom fosfokreatyny, mleczanu czy glikogenu mięśniowego [51]. Istotny wzrost TC w mięśniach obserwuje się dopiero po połączeniu jej z

CHO; efekty te zostały zaobserwowane po 12 [49] i 24 tygodniach suplementacji [27]. Zaobserwowane zmiany wynikają z faktu, iż stężenie L-karnityny w mięśniach jest wyższe niż w osoczu, dlatego konieczny jest aktywny transport karnityny [53], który staje się możliwy dzięki zastosowaniu kontrolowanej hiperinsulinemii [28], np. przez stosowanie L-karnityny wraz z cukrami prostymi. W przytoczonych badaniach [27, 49] interwencja ta zwiększyła poziom TC w mięśniach odpowiednio o 20 i 21% i wpłynęła na metabolizm wysiłkowy [27], poprawiając wydolność [27] i wydatek energetyczny, jednak nie zmieniając składu ciała [49] (co może być związane ze zwiększoną podażą CHO). Co ciekawe wpływ na ekspresję genów związanych z metabolizmem kwasów tłuszczowych i karnityny zauważono zarówno po 12 tygodniach suplementacji w połączeniu z CHO [49] jak i samą L-karnityną w ilości 2g/dziennie [54].

W kontekście wpływu samej suplementacji L-karnityną na wzrost masy mięśniowej zauważono po 24 tygodniach podawania L-karnityny w grupie stulatków, jednocześnie obserwując zwiększenie tolerancji na wysiłek fizyczny [55]. Niemniej jednak nie potwierdziły tego badania przeprowadzone na grupie zdrowych kobiet po 65 roku życia [56], co wiązać można chociażby z wiekiem osób badanych i ich stanem zdrowia.

Ważnym aspektem w kontekście suplementacji L-karnityną pozostaje jej wpływ na metabolizm TMAO. Długoterminowe spożywanie L-karnityny powoduje wzrost TMAO w osoczu na czczo [57], a już trzymiesięczna suplementacja 1500mg/dobę powoduje jego 10-krotny wzrost, w porównaniu do wartości sprzed suplementacją [42, 58]. W literaturze pozostają badania, które łączą podwyższony poziom tego związku ze zwiększonym ryzykiem zdarzeń sercowo-naczyniowych [59, 60]. Niemniej jednak rozbieżność wyników badań nie pozwala jednoznacznie stwierdzić czy TMAO jest związkiem aterogennym wpływającym na rozwój chorób sercowo-naczyniowych, czy jedynie markerem zmian z nimi związanych [61].

Podsumowując, zastosowanie odpowiedniego protokołu suplementacyjnego, czyli połączenia L-karnityny ze związkiem regulującym poziom insuliny we krwi, może wpływać na zmiany metaboliczne oraz na usprawnienia związane z siłą i masą mięśni. Co ważne, z uwagi na niską biodostępność L-karnityny suplementacja powinna być stosowana co najmniej przez okres 3 miesięcy. Patrząc na wyniki badań własnych, lepszą strategią wydaje się połączenie suplementacji L-karnityną z CHO niż leucyną. Być może leucyna nie stwarza wystarczających warunków do tego, aby umożliwić transport L-karnityny do mięśni szkieletowych.

9. Wnioski z cyklu publikacji

Uzyskane wyniki z zaprezentowanych eksperymentów pozwoliły na wyciągnięcie następujących wniosków:

1. Suplementacja L-karnityną w ilości 1500mg/na dobę nie wpływa na poziom czynników prozapalnych we krwi (takich jak CRP, IL-6, TNF-a) ani czynników wzrostu (IGF-1) u zdrowych kobiet w starszym wieku.
2. Dwudziestoczterotygodniowa suplementacja L-karnityną (1500mg/na dobę) nie wpływa również na masę i skład ciała oraz siłę mięśni zdrowych kobiet po 65 roku życia.
3. Trening oporowy przeprowadzany dwa razy w tygodniu przez okres dwudziestu czterech tygodni zwiększa hipertrofię mięśni uda oraz siłę mięśni w grupie kobiet po 65 roku życia.
4. Suplementacja L-karnityną w połączeniu z leucyną (1000mg + 3000mg) zwiększa ilość całkowitej karnityny we krwi oraz poziom krążącego TMAO. Zmiany te nie wpływają na efektywność treningu siłowego w grupie starszych kobiet.
5. Dwudziestoczterotygodniowa suplementacja leucyną zwiększa poziom krążącej dekoryny we krwi, jednak nie wpływa to na poziom miostatyny, ani zwiększenie efektywności treningu oporowego w kontekście hipertrofii i poprawy siły mięśniowej w badanej grupie kobiet.

10. Piśmiennictwo

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L-Carnitine Supplementation in Older Women. A Pilot Study on Aging Skeletal Muscle Mass and Function

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Abstract: Skeletal muscle wasting, associated with aging, may be regulated by the inflammatory cytokines as well as by insulin-like growth factor 1 (IGF-1). L-carnitine possesses anti-inflammatory properties and increases plasma IGF-1 concentration, leading to the regulation of the genes responsible for protein catabolism and anabolism. The purpose of the present study was to evaluate the effect of a 24-week L-carnitine supplementation on serum inflammatory markers, IGF-1, body composition and skeletal muscle strength in healthy human subjects over 65 years of age. Women between 65 and 70 years of age were supplemented for 24 weeks with either 1500 mg L-carnitine-L-tartrate or an isonitrogenous placebo per day in a double-blind fashion. Before and after the supplementation protocol, body mass and composition, as well as knee extensor and flexor muscle strength were determined. In the blood samples, free carnitine, interleukin-6, tumor necrosis factor-α, C-reactive protein and IGF-1 were determined. A marked increase in free plasma carnitine concentration was observed due to L-carnitine supplementation. No substantial changes in other parameters were noted. In the current study, supplementation for 24 weeks affected neither the skeletal muscle strength nor circulating markers in healthy women over 65 years of age. Positive and negative aspects of L-carnitine supplementation need to be clarified.

Keywords: sarcopenia; cytokines; body composition; muscle strength

1. Introduction

Aging is accompanied by a progressive change in the ratio between fat and lean body mass (BM). Fat mass, in particular visceral adipose tissue, increases, whereas fat free mass (FFM) declines [1]. Adipose tissue itself produces and releases a number of cytokines, such as interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and other bioactive molecules [2]. In fact, even healthy aging results in slight elevations of circulating proinflammatory mediators, corresponding to a chronic low-grade inflammatory profile [3].

A body of literature has demonstrated that inflammatory cytokines activate many of the molecular pathways involved in skeletal muscle wasting, leading to an imbalance between protein synthesis and degradation [4,5]. High doses of TNF-α lead to reduced muscle cell differentiation in human and mouse muscle cells [6,7] and cause myotube (in vitro) and myofibre (in vivo) atrophy in animals [8,9],

suggesting a predominant role for pro-inflammatory cytokine in reduced muscle regeneration, and thus a contributor to atrophy. Moreover, higher levels of IL-6 and TNF-α have been related with lower muscle strength and lower muscle mass in a cross-sectional study [10]. Cesari et al. [11], found associations of high levels of C-reactive protein (CRP) and IL-6 with poorer physical performance and muscle strength in elderly people. Longitudinal studies have shown elevated inflammatory markers as a predictor of increased incidence of mobility limitation [12] or the risk of muscle strength loss [13]. Skeletal muscle signaling pathways, which regulate anabolic and catabolic processes, may also be activated by insulin-like growth factor 1 (IGF-1) [4]. In cross-sectional studies of the European population recruited in five different countries, it has been demonstrated that circulating levels of IGF-1 decrease with age in both men and women [14].

L-carnitine is a low-molecular, nitrogenous compound, the main role of which is transporting long-chain fatty acids from the cytoplasm into the mitochondrial matrix [15]. It also possesses anti-inflammatory properties [16]. L-carnitine attenuates inflammatory changes in various experimental models: aging [17], liver fibrosis [18] or cancer cachexia [19]. Lee et al. [20] demonstrated that L-carnitine supplementation at a dose of 1000 mg/day for 12 weeks reduces the inflammatory status in the patients with coronary artery disease. The animal studies have indicated that L-carnitine suppresses the genes responsible for protein catabolism [21] and up-regulates the main drivers of protein anabolism [22] in skeletal muscle. Moreover, the activation of the signaling pathway is mediated by increased IGF-1 plasma concentration [22]. Furthermore, analysis of muscle samples of healthy humans of various ages shows a drastic reduction of L-carnitine and acetyl carnitine in the older subjects with a strong reverse correlation between age and L-carnitine levels [23]. Therefore, we hypothesized that L-carnitine supplementation for 24 weeks would affect the level of serum inflammatory markers, IGF-1, body composition and skeletal muscle strength in healthy human subjects over 65 years of age.

2. Materials and Methods

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Independent Bioethics Commission for Research at Medical University of Gdansk Ethics (NKBBN/354-304/2015).

2.1. Subjects

Forty-two subjects replied to the advertisements in the local newspaper at the University of Third Age and at the Senior Activity Center, and volunteered to participate in the study. Subjects with cancer, cardiovascular disease, gastrointestinal disease, liver, and renal diseases were excluded from the study. After the initial screening, 28 were included in the study and were randomly assigned to either an L-carnitine or a placebo supplementation group (Figure 1).

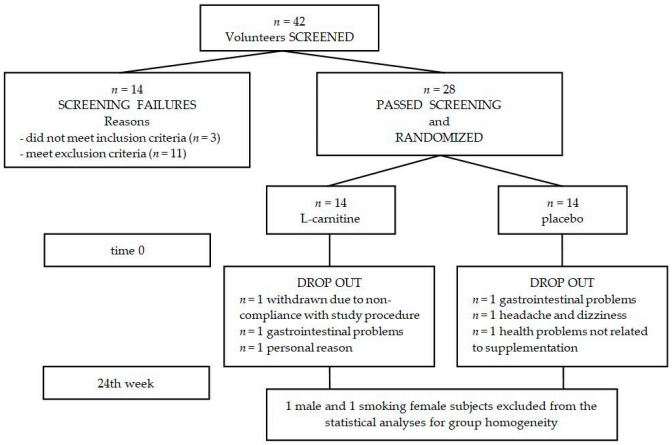

Figure 1. Disposition of study participants. A total of 42 participants were screened and 28 passed screening. The remaining 28 participants were enrolled in the study (14 in each group), but 22 completed the study while adhering to study protocols. One male subject and one smoking female subject were excluded from the statistical analyses for group homogeneity.

2.2. Study Procedure

Subjects were supplemented for 24 weeks with either 1500 mg L-carnitine-L-tartrate or a isonitrogenous placebo per day in a double-blind fashion. Supplements were kindly provided by Trec Nutrition Ltd. (Gdynia, Poland) and put inside identical gelatin capsules. Subjects were instructed to consume capsules daily after their main meal during the study period, because of the insulin-dependent L-carnitine transport into the skeletal muscle cell [24,25]. Before the start and after completion of the supplementation protocol, subjects arrived at the laboratory in the fasted state. Following weighting and blood sampling, standard light breakfasts were provided. Then, the set of laboratory tests were performed.

2.3. Anthropometric Measurements

Body mass and composition were estimated using a bioelectrical impedance analyzer, (InBody720, InBody Co., Ltd., Seoul, Korea). The participants had emptied their bladders and bowels prior to the measurement. The analyses were performed in the position recommended by the manufacturer's guidelines and the subjects clad only underwear [26]. The InBody720 measures impedance of five segments of the body (each arm, each leg, trunk) at frequencies of 1, 5, 50, 250, 500, and 1000 kHz through the 8-polar tactile-electrode. Based on the impedance, FFM and skeletal muscle mass (SMM) were calculated.

2.4. Blood Sampling

Blood samples were drawn from a participant's antecubital vein, maintaining a sterile field and using BD Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) with serum-separator or ethylenediaminetetraacetic acid (EDTA). After collection, the samples were centrifuged at 2000× *g* at 4 °C for 10 min. Aliquots were stored at −80 °C for later analyses.

2.5. Biochemical Determination

Plasma free carnitine was determined by the ultra-high-performance liquid chromatography-mass spectrometry (UPLC/MS/MS) method as previously described [27]. Serum IL-6, TNF-α, CRP and IGF-1 concentrations were determined by the enzyme immunoassay method using commercially available kits (IL-6, cat. no. HS600B; TNF-α, cat. no. HSTA00D; total IGF-1, cat. no. DG100—R&D Systems, Minneapolis, MN, USA; CRP, cat. no. SEA821Hu—Cloud-Clone Corp., Houston, TX, USA).

2.6. Skeletal Muscle Strength Test

Knee extensor and flexor muscle strength were determined by a Biodex System 4 Pro™ dynamometer (Biodex Medical Systems, Inc., Shirley, NY, USA). After a warm-up, subjects were positioned according to the manufacturer's manual (seated with arms hanging along the body, hands holding the lateral handles, and strap stabilization of trunk, hip, and tested thigh, with the knee flexed at 90°) [28]. The testing range of motion was 80° and was set from 10–90° of knee flexion (with 0° = full voluntary extension). The seat position was adjusted for the leg length of each tested person. The dominant leg was examined using isokinetics tests. The isokinetic knee flexion and extension was measured at angular velocities of 60°/s [29]. All the measurements were normalized by the lean mass of the working leg.

2.7. Nutritional and Physical Activity Habits

Nutritional intake patterns, especially red meat, have a great impact on total body carnitine content [30]. Therefore, to assess the frequency of meat consumption, a specific survey was designed in a way that was both simple to complete and easily comprehensible. The survey used quantitative research methods to identify six "frequency consumption groups" [31]:

F0—never,
F1—occasionally,
F2—several times per year,
F3—several times per month,
F4—2–5 times per week,
F5—6–7 times per week.

The short form of the International Physical Activity Questionnaire (IPAQ) was used for the assessment of habitual physical activity. The following metabolic equivalent (MET) values were used for the analysis of IPAQ: walking = 3.3 METs, moderate physical activity = 4.0 METs, vigorous physical activity = 8.0 METs [32]. The total physical activity of the subjects was classified as low, moderate or high. Subject characteristics are presented in Table 1.

Table 1. Basic characteristics of the participants.

Variables	Placebo		L-Carnitine	
	Mean ± SD (Standard Deviation)		Mean ± SD	
Age (years)	66.4 ± 1.3		67.8 ± 2.3	
Height (cm)	162 ± 5.3		159 ± 5.4	
BMI (kg/m^2)	26.5 ± 4.4		27.5 ± 4.5	
	n	%	*n*	%
Education level				
Primary	0	0	0	0
Secondary	2	22.2	4	36.4
High	7	77.8	7	63.6
Physical activity				
Low	0	0	0	0
Moderate	4	44.4	6	54.5
High	5	55.6	5	45.5
	median	range	median	range
Meat consumption				
Poultry	F4	F3–F4	F4	F0–F4
Pork	F2	F0–F3	F3	F0–F4
Beef	F1	F0–F3	F2	F0–F4
Fish	F3	F1–F4	F2	F1–F4
Lamb	F0	F0–F3	F0	F0–F3
Venison	F0	F0–F3	F0	F0–F1
Horseflesh	F0	F0–F2	F0	F0–F1

2.8. Statistical Analyses

Basic anthropometric characteristics of the subjects were evaluated as mean ± SD. For educational and physical activity levels, a percentage of the total tested was used, while a frequency of meat consumption was presented as median and range. Changes in both groups across the supplementation time and ratios as well as changes in other measurements were analyzed with a Microsoft Excel (Microsoft Office Home and Student 2007, Version 12.0.6612.1000, Microsoft, Redmond, WA, USA) spreadsheet for the analysis of parallel-group controlled trials [33]. For this, effects were interpreted

using magnitude-based inferences [34]. All data were log-transformed for analysis to reduce bias arising from non-uniformity of error; means of change scores in the placebo and L-carnitine groups, standard deviations of change scores, and effects (variations of change in both the means and their confidence limits (CL)) were back-transformed to percent units. Mean changes and effects were adjusted to the overall mean baseline value of the placebo and L-carnitine groups, by including the baseline value as a covariate in the analysis. Magnitudes of the effects were evaluated with the log-transformed data by standardizing the deviation of the baseline values of the placebo and L-carnitine groups. Threshold values for assessing magnitudes of standardized effects were 0.20, 0.60, 1.2 and 2.0 for small, moderate, large and very large respectively. Uncertainty in the effects was expressed as 90% CL and as probabilities that the true value of the effect was beneficial, trivial or harmful. These probabilities are not presented quantitatively but were used to make qualitative probabilistic clinical inferences about effects in preference to a statistical inference based on a null-hypothesis significance test [34]. The effect was deemed unclear when the chance of benefit was sufficiently high to warrant the use of the treatment but the risk of harm was unacceptable. Such unclear effects were identified as those with an odds ratio of benefit to harm of <66, a ratio that corresponds to an effect that is borderline possibly beneficial (25% chance of benefit) and borderline most unlikely harmful (0.5% risk of harm). All other effects were deemed clinically clear and the likelihood of the true effect as being trivial, beneficial or harmful was expressed with the following scale: 25–75%, possibly; 75–95%, likely; 95–99.5%, very likely; >99.5%, most likely. To maintain an overall error rate of <5% to declare one or more changes as having opposite magnitudes (a substantial decrease instead of an increase, and vice versa), the effects were also evaluated as beneficial or harmful with a threshold of 1%, equivalent to the consideration of the overlap of substantial values with a 98% confidence interval (CI).

3. Results

In the study, participants were all non-smoking, non-obese, non-vegetarian, physically active women in the age range of 65 to 70 years. The ratio of percent changes in BM at baseline and across a supplementation period in placebo and L-carnitine groups was most likely trivial (0.5%; ±1.9%; mean; 90% CL). The effects were clear, which indicates that supplementation did not affect BM. Ratios of percent changes in BM components (FFM and SMM) were likely trivial (clear effects, 0.7%; ±2.6%; 1.2%; ±3.2%, respectively) (Table 2).

Table 2. Baseline and changes in the measures across a supplementation period in both groups.

Variables	Group	Baseline Mean ± SD	Observed Change Mean ± SD	Adjusted Change [a] Mean ± SD	Adjusted Effect [b]	
					Mean; CL	Inference
BM (kg)	placebo	69.7 ± 12.1	−0.3 ± 2.8%	−0.3 ± 2.1%	0.5%; ±1.9%	trivial †
	L-carnitine	69.8 ± 12.9	0.2 ± 2.9%	0.2 ± 2.9%		
FFM (kg)	placebo	45.8 ± 6.7	−1.6 ± 5.2%	−1.1 ± 3.7%	0.7%; ±2.6%	trivial *
	L-carnitine	43.9 ± 4.6	0.0 ± 3.5%	−0.4 ± 2.8%		
SMM (kg)	placebo	24.9 ± 4.1	−1.9 ± 6.4%	−1.3 ± 4.4%	1.2%; ±3.2%	trivial *
	L-carnitine	23.9 ± 2.7	−0.2 ± 3.8%	−0.1 ± 3.2%		

CL, 90% confidence limit; † most likely, * possible. [a] Adjusted to the overall mean of both groups at baseline. [b] Adjusted mean change in the L-carnitine group minus the adjusted mean change in the placebo group. BM: body mass; FFM: fat free mass; SMM: skeletal muscle mass.

Baseline measures of circulating parameters, their percent changes across a supplementation period in both groups, and ratios of the changes are presented in Table 3.

Table 3. Baseline and changes across the supplementation period in both groups and ratios of changes as effects.

Variables	Group	Baseline Mean ± SD	Observed Change Mean ± SD	Adjusted Change [a] Mean ± SD	Adjusted Effect [b]	
					Mean; CL	Inference
free carnitine (µmol/L)	placebo	39.5 ± 3.7	10 ± 11%	8 ± 6%	13%; ±5.8%	moderate †
	L-carnitine	41.1 ± 6.4	22 ± 9%	22 ± 8%		
CRP (mg/L)	placebo	1.8 ± 0.8	−6 ± 15%	−4.7 ± 15%	21%; ±37%	unclear
	L-carnitine	2.6 ± 1.1	8 ± 68%	16 ± 65%		
IL-6 (ng/L)	placebo	1.8 ± 0.7	−10 ± 23%	−13 ± 20%	4.9%; ±22%	trivial *
	L-carnitine	2.2 ± 1.1	−11 ± 42%	−8.2 ± 32%		
TNF (ng/L)	placebo	0.56 ± 0.26	14 ± 70%	12 ± 68%	9.0%; ±50%	trivial *
	L-carnitine	0.58 ± 0.32	24 ± 82%	28 ± 38%		
IGF-1 (µg/L)	placebo	78 ± 19	−10 ± 12%	−10 ± 13%	1.8%; ±16%	trivial *
	L-carnitine	69 ± 15	−6 ± 28%	−8 ± 28%		

CL, 90% confidence limit; † most likely; * possible, underlined effect is also clear at the 0.5% level (98% confidence interval). [a] Adjusted to the overall mean of both groups at baseline. [b] Adjusted mean change in the L-carnitine group minus the adjusted mean change in the placebo group. CRP: C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor-alpha; IGF-1: insulin-like growth factor 1.

A marked increase in free plasma carnitine concentration was observed due to L-carnitine supplementation. Other ratios of changes, with the exception of unclear change in CRP, were possibly trivial (Table 3).

No substantial changes in the isokinetic measures were noted in response to the supplementation period (Table 4).

Table 4. Isokinetic measures for the dominant leg at baseline, and changes across a supplementation period in both groups.

Variables	Group	Baseline Mean ± SD	Observed Change Mean ± SD	Adjusted Change [a] Mean ± SD	Adjusted Effect [b]	
					Mean; CL	Inference
TW extension (J/kg)	placebo	76 ± 15	7.4 ± 26%	5.7 ± 8.3%	5.6%; ±7.1%	trivial *
	L-carnitine	78 ± 11	11 ± 13%	12 ± 9.5%		
TW flexion (J/kg)	placebo	43 ± 17	9.4 ± 40%	13 ± 13%	−2.9%; ±13%	trivial *
	L-carnitine	36 ± 9	14 ± 34%	9.7 ± 23%		
APT extension (Nm/kg)	placebo	12.0 ± 2.6	4.7 ± 20%	1.9 ± 9.7%	3.0%; ±9.0%	unclear
	L-carnitine	12.8 ± 2.3	2.7 ± 18%	5.0 ± 13%		
APT flexion (Nm/kg)	placebo	6.8 ± 2.6	4.9 ± 26%	7.0 ± 7.8%	−4.2%; ±9.0%	trivial *
	L-carnitine	5.9 ± 1.1	5.0 ± 22%	2.6 ± 16%		
AP extension (W/kg)	placebo	8.3 ± 2.0	6.3 ± 25%	3.9 ± 6.2%	1.4%; ±6.8%	trivial †
	L-carnitine	8.6 ± 1.4	3.3 ± 18%	5.4 ± 11%		
AP flexion (W/kg)	placebo	4.4 ± 1.7	10 ± 40%	14 ± 12%	−7.1%; ±9.7%	trivial †
	L-carnitine	3.9 ± 1.4	12 ± 83%	5.8 ± 17%		

CL, 90% confidence limit; * possible, † likely. [a] Adjusted to the overall mean of both groups at baseline. [b] Adjusted mean change in the L-carnitine group minus the adjusted mean change in the placebo group. TW: total work; APT: average peak torque; AP: average power.

4. Discussion

Systemic L-carnitine depletion has been described in aging, and is characterized by fatigue, muscle wasting, and geriatric frailty [35]. In the current study, we have evaluated the effect of L-carnitine supplementation on muscle mass, strength, and selected blood markers in older women at risk for sarcopenia.

In a recent meta-analysis, Pooyandjoo et al. [36] calculated that subjects supplemented with L-carnitine lost significantly more weight (−1.33 kg), compared with the control group. This article was later criticized [37] for mixing studies using L-carnitine alone or in association with other

factors (i.e., pharmacological therapy-sibutramine [38] or orlistat [39] and changes in lifestyle–diet intervention [40]). Inclusion of the studies using treatments consisting exclusively of L-carnitine indicated no modification in body weight [37]. Twenty-four weeks of L-carnitine supplementation, without changing nutritional or physical activity habits, did not influence our participants' body weight. Interestingly, participants supplemented with L-carnitine maintained a similar SMM from baseline to 24 weeks, while the placebo group showed a decline in SMM within the population range—an approximate annual rate of 1 to 2% [41]. However, due to the variability within the groups themselves, the effect between the two groups was trivial. In a previous study, Malaguarnera et al. [42] supplemented the diet of centenarians with 2 g of L-carnitine per day. Six months of intervention induced a significant increase in FFM in comparison with pre-treatment values, and in comparison with post-treatment values in the placebo group [42]. However, the mechanism has not been proposed.

Muscle wasting in sarcopenia populations is associated with a shift from muscle protein synthesis to muscle protein degradation. The mechanism of muscle wasting may involve low-grade inflammation [4,5]. It has been proposed that circulating cytokines and CRP negatively affect muscle mass during aging [10,11]. Meta-analysis of human studies suggests potential beneficial effects of L-carnitine in lowering the circulating CRP level [43]. However, the magnitude of significant effect of L-carnitine intervention is based on the results of the trials with an initial CRP level > 5 mg/L [40,44,45]. Be that as it may, L-carnitine treatment does not reveal the anti-inflammatory properties in low-grade inflammation conditions [46,47]. No alterations in serum CRP, IL-6 and IL-10 have been reported in obese women following an eight-week supplementation of 2 g L-carnitine per day [47]. Similarly, in our study, the elevation of plasma-free L-carnitine concentration after a 24-week supplementation period did not affect the circulating proteins and was not accompanied by the anti-inflammatory effect.

The muscle protein synthesis and degradation rate is multifactorial and includes various signaling pathways [48,49]. The animal studies show that supplementation of L-carnitine elevates plasma IGF-1 concentration, despite various doses consumed daily: from ~1 mg/kg body weight in sows [50], to ~100 mg/kg body weight in rats [22]. The binding of IGF-1 to its receptor triggers the activation of the protein synthesis pathway phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) [51], which is up-regulated during hypertrophy and down-regulated during muscle atrophy [52]. The IGF-1/PI3K/Akt signaling pathway is not only capable of activating anabolic pathways, but also of suppressing catabolic pathways, due to an inactivation of a specific transcription factor family called forkhead box-O transcription factors (FOXO) [53]. L-carnitine supplementation may affect both of these pathways in rat skeletal muscle [22]. Human studies show a decrease in circulating IGF-1 with aging [14], which is in agreement with our observation. However, L-carnitine caused a trivial attenuation in plasma IGF-1 level decline compared to the placebo.

Recent human aging studies present the increase in skeletal muscle mass and strength due to the mTOR pathway activation, following an eight-week supplementation of L-carnitine when combined with creatine, L-leucine and vitamin D [54]. However, these effects are not present in the group supplemented only by the same dose of L-carnitine without any additional supplements [54]. Therefore, it cannot be ruled out that other supplements affect sarcopenia by their own properties; creatine delays muscle atrophy and improves strength in aging [55], L-leucine increases the muscle protein fractional synthesis rate in the elderly individuals [56], and vitamin D improves muscle strength in people $\geq$ 65 years of age [57]. A similar dose of L-carnitine was used in our study, without any additional supplements, but for a prolonged period of time and did not significantly affect muscle mass and function.

The quadriceps muscle strength declines in aging women in correlation to the muscle cross sectional area [58]. Therefore, in order to obtain better relative values, we normalized total performed work, peak torque and power per lean mass of the working leg. The longitudinal study shows losses of 11.8% per decade in women's knee extensor strength and 17.4% per decade in flexor strength [59]. It is noteworthy that in men, these values showed no reduction in muscle strength parameters. The lack

of changes in muscle strength may have been influenced by physical activity, since the group of participants was characterized by relatively high physical activity.

5. Conclusions

L-carnitine is a popular nutritional supplement, which may be beneficial in some pathological conditions characterized by chronic systemic inflammation and muscle wasting [60]. In the current study, supplementation for 24 weeks did not affect either the skeletal muscle strength or circulating markers in healthy women over 65 years of age. Positive and negative aspects of L-carnitine supplementation need to be clarified, especially considering its metabolite trimethylamino-*N*-oxide, which may be associated with the etiology of some diseases [61].

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Original Research

L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women

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Abstract

OBJECTIVES: To evaluate the effect of L-carnitine (LC) in combination with leucine supplementation on muscle strength and muscle hypertrophy in aged women participating in a resistance exercise training (RET) program.

DESIGN/SETTING/PARTICIPANTS: Thirty-seven out of sixty (38.3% dropout) healthy women aged 60-75 years (mean 67.6 ± 0.7 years) completed the intervention in one of three groups. One of the supplemented groups received 1 g of L-carnitine-L-tartrate in combination with 3 g of L-leucine per day (LC+L group; n = 12), and the second supplemented group received 4 g of L-leucine per day (L group; n = 13). The control group (CON group; n = 12) received no supplementation.

INTERVENTION: All three groups completed the same RET protocol involving exercise sessions twice per week for 24 weeks.

MEASUREMENTS: Before and after the experiment, participants performed isometric and isokinetic muscle strength testing on the Biodex dynamometer. The cross-sectional areas of the major knee extensors and total thigh muscles were assessed using magnetic resonance imaging. Fasting serum levels of insulin-like growth factor-1 (IGF-1), myostatin and decorin, and plasma levels of total carnitine (TC) and trimethylamine-N-oxide (TMAO) levels were measured.

RESULTS: The 24-week RET significantly increased muscle strength and muscle volume, but the group and time interactions were not significant for the muscle variables analyzed. Plasma total carnitine increased only in the LC+L group ($p = 0.009$). LC supplementation also caused a significant increase in plasma TMAO, which was higher after the intervention in the LC+L group than in the L ($p < 0.001$), and CON ($p = 0.005$) groups. The intervention did not change plasma TMAO concentration in the L ($p = 0.959$) and CON ($p = 0.866$) groups. After the intervention serum decorin level was higher than before in both supplemented groups combined ($p = 0.012$), still not significantly different to post intervention CON ($p = 0.231$). No changes in serum IGF-1 and myostatin concentrations and no links between the changes in blood markers and muscle function or muscle volume were observed.

CONCLUSIONS: LC combined with leucine or leucine alone does not appear to improve the effectiveness of RET.

Key words: Sarcopenia, trimethylamine-N-oxide, insulin-like growth factor-1, myostatin, decorin, exercise training, muscle cross-sectional area.

Introduction

Age-related changes in anabolic and catabolic processes are associated with progressive loss of muscle mass, strength, and function (1). Exercise training is an intervention that can prevent or even reverse the muscle-wasting process (2), and the greatest effect of exercise on this process in older adults results from resistance exercise training (RET) (3). RET-accelerated skeletal muscle hypertrophy is controlled by hormones and growth factors (4). Protein synthesis is induced by insulin-like growth factor-1 (IGF-1) and inhibited by myostatin. Myostatin activity is suppressed by a small leucine-rich proteoglycan, decorin (5), which is secreted in response to exercise (6).

RET in combination with nutritional intervention provides a better stimulus for maintaining muscle strength and mass than RET alone (7). Leucine is an important regulator of protein synthesis (8). In short-term studies, leucine intake stimulates muscle protein synthesis (9). However, longer studies of older adults, supplemented with leucine for 3 months (10) or 6 months (11) have not confirmed the effects of supplementation on muscle strength or mass. This discrepancy might be attributable to the fact that the other factors, such as growth factors and hormones, satellite cells and neuromuscular factors may be required for the translation of acute increases in protein synthesis into chronic increases in muscle mass (12).

Leucine has also been reported to increase insulin levels (13), and insulin is required for the transport of L-carnitine (LC) into muscles (14). In addition, animal studies have shown that the metabolic pathways involved in muscle protein balance can be upregulated by LC supplementation (15, 16), possibly through its ability to elevate the circulating level of IGF-1, a potential promotor of muscle protein balance (17). A recent study reported increases in muscle mass and strength in older adults consuming LC mixed with leucine, creatine, and vitamin D for 8 weeks (18). Whether similar results can be obtained with LC combined with leucine during the course of an RET protocol remains unknown.

A crucial role in the development of muscle loss during aging may play gut microbiota (19), also involved in the metabolism of orally administered LC to circulating

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trimethylamine-N-oxide (TMAO) (20). In vitro studies indicate that TMAO can increase protein synthesis (21), or modulate myosin ATPase activity (22, 23). Interestingly, TMAO has been shown to be taken up by human skeletal muscles (24), and prolonged LC supplementation elevates circulating TMAO levels in healthy aged women (25).

The main purpose of our study was to examine the effects of the combination of LC and leucine on muscle volume and strength in healthy aged women undertaking RET twice a week for 24 weeks. We measured circulating IGF-1, myostatin, decorin, and TMAO levels to identify potential confounding factors of muscle cross-sectional area (CSA) and function in aging. We hypothesized that LC combined with leucine supplementation would alter peripheral factors that would be manifested as skeletal muscle changes in older women. We also hypothesized that an increased TMAO level induced by LC treatment would affect the force production over time.

Materials and Methods

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the Independent Bioethics Commission for Research at the Medical University of Gdansk (NKBBN/354-201/2017) and was registered in the ClinicalTrials.gov Registry (NCT03907592). All participants were informed about the procedures, risks, and expected outcomes before starting the experimental procedure and gave their written informed consent for participation. Partial results of this study have been reported previously (26).

Sample size

The sample size calculation was carried out by G*Power 3. This study was designed to detect a moderate effect size (f = 0.3) for muscle mass, muscle strength, and physical performance. Using the analysis of variance (ANOVA) for repeated measures, within-between interaction, setting the α-error to 0.05, the power to 85% and 3 groups, the minimal sample size was estimated at 36. Considering at least 20% dropout rate (27), a total of 60 participants were recruited for this trial.

Participants

Participants were recruited through local advertisements between April and June 2017. Volunteers with a chronic disease (such as cardiovascular disease, liver or kidney disease, gastrointestinal disorder, including stomach ulcer or erosions, cancer, diabetes, disease of the musculoskeletal system, and other severe chronic diseases), with metal body implants, or who smoked were excluded. A short questionnaire was used for the assessment of habitual physical activity. Additional activities were converted into metabolic equivalents (METs). Volunteers with low and moderate physical activity and without a professional sports history were included in the study. All included participants presented a physician's certificate indicating a lack of contradictions to strength training. Sixty women aged 60-75 years (mean 67.6 ± 0.7 years) were examined at the beginning of the study protocol (Fig. 1). Height was measured to the nearest 0.1 cm with a portable stadiometer. Weight was measured using a bioelectrical impedance analyzer (InBody720, InBody Co., Ltd., Seoul, Korea). Body mass index was calculated by dividing weight (kg) by height squared (m^2). The characteristics of the subjects enrolled are shown in Table S1.

Experimental design and study procedure

After the initial screening, participants were randomly assigned to one of two supplemented groups, LC in combination with leucine (LC+L group; n = 20), or leucine alone (L group; n = 20) or control group (CON group; n = 20). Because of LC poor bioavailability, LC intake should be combined with insulinogenic compound, and the supplementation protocol should take about 100 days to increase muscle carnitine content by ~ 10% (28). In our study, leucine was used as an insulinogenic compound (13), which could potentially improve LC transport to skeletal muscles. Therefore, the participants were supplemented with either 1 g of L-carnitine-L-tartrate and 3 g of leucine per day (LC+L group) or 4 g of leucine per day (L group) for 24 weeks in a double-blind fashion. The supplements were encapsulated in identical gelatin capsules, and the supplement packages were coded so that neither the investigators nor the participants were aware of the contents until completion of the analysis. The participants received the packages in separate portions every 2 weeks and were instructed to consume the supplements once a day with their main meal. Adherence to the supplementation protocol was based on information about the unused supplements. In parallel, the CON group participated in the RET but did not receive the supplements.

During the week before starting the training protocol, and the week following the last training session, all participants performed the series of tests described below (Fig. S1). Fasting blood samples were obtained and, after a standardized breakfast, strength tests were performed. A magnetic resonance imaging (MRI) scan was performed on a separate day, but no earlier than 2 days after muscle strength testing.

RET protocol

The training sessions were held in groups of up to 12 people twice a week in a commercial gym. Each participant attended on Mondays and Wednesdays, or on Tuesdays and Thursdays. Each class was conducted by professional coaches. Over the 24 weeks, each participant had participated in 48 training sessions, each lasting 45-60 min.

The RET protocol was based on a previously described procedure (29). Each training session started with a 10 min warm-up on a treadmill (walking), and participants then performed three sets of four exercises: leg press, leg extension,

shoulder press or horizontal row, and chest press or lateral pulldown. The leg press and leg extension were performed at every training session, but the shoulder press and lateral pulldown were performed only on Monday or Tuesday, and horizontal row and chest press only on Wednesday or Thursday. Each session ended with a 10 min cooldown on a cycle ergometer.

A one-repetition maximum test (1RM) was performed, according to National Strength and Conditioning Association guidelines (30), before and then every 6 weeks of the training protocol. In total each participant performed 1RM five times. During the first 2 weeks, the workload was set at 65% of 1RM for each exercise, and the exercise was performed in three sets of 10-12 repetitions. After 2 weeks, the workload was increased to 80% of 1RM, and each exercise was performed in three sets of 6-8 repetitions. All three groups completed the same RET protocol.

Skeletal muscle strength

All strength tests were performed on the Biodex System 4 Pro dynamometer (Biodex Medical Systems, Inc., Shirley, NY, USA). Before the start of each testing session, the Biodex was calibrated according to the manufacturer's specifications. Before testing, the participant performed a 5 min warm-up at 50 W on a mechanically braked cycle ergometer (Monark, Vansbro, Sweden). The test started with an isometric test at a 90° knee angle, followed by an isokinetic test at 60°/s for the dominant leg. Participants were stabilized with two shoulder straps, a waist strap, and a thigh strap. The rotational axis of the knee was aligned with the center of the dynamometer shaft. Adjustments were made to the length of the knee attachment to ensure that the ankle strap was proximal to the lateral and medial malleoli and comfortable for the participant. Gravity correction was used for all trials. Verbal encouragement was provided during all tests (31). Peak torque was measured by performing maximum voluntary contractions (MVC) during isometric knee extension. The test comprised a maximum 4 s knee extensor isometric contraction, which was repeated three times separated by a 20 s recovery. To assess muscle isokinetic strength, the participant completed five repetitions at a speed of 60°/s (32). During concentric isokinetic leg extension, the total work of the five repetitions was recorded (33).

Cross-sectional area

The dominant leg was analyzed using a 1.5 T Siemens MAGNETOM Aera MRI scanner (Siemens, Munich, Germany) with the body 18 and spine 32 coils and Auto Coil Select mode on. The study protocol included, the following sequences: T1-weighted turbo spin echo coronal (voxel 1.8 x 1.8 x 4 mm, FOV 250 x 200 mm, TE 17 ms, TR 500 ms, NSA 2), T2 space transverse (voxel 0.8 x 0.8 x 3 mm, TE 96 ms, TR 1600 ms, NSA 1.4) and T1 VIBE Dixon (voxel 0.7 x 0.7 x 2.5 mm, TE 2.39 and 4.77 ms, TR 6.89 ms, NSA 1). The examination covered the area from the knee joint level to the end of the hip joint, and all sequences were performed twice and then combined into one composite image. The Dixon sequence comprised in-phase and out-of-phase images and reconstructed water-only and fat-only images.

After all data collection, the 2/3 upper femur height, as specified for measuring the maximal strength of the knee extensors (34), was determined by an experienced radiologist. Subsequently, the CSAs of the total thigh muscle (CSA TM) and of vastus lateralis, vastus intermedius, and vastus medialis, as the main knee extensors (CSA KE), were measured using an OsiriX Life (Pixmeo SARL, Bernex, Switzerland). All evaluations were performed by two independent investigators who were blinded to the intervention. The interindividual coefficient of variation of the analysis was 1.3%.

Blood collection and analysis

Fasting blood samples were taken from the antecubital vein into BD Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). After collection, the samples were centrifuged at 2000 g at 4°C for 10 min, and aliquots were stored at -80°C for later analyses. Plasma TMAO concentration was measured as described previously (35). For total carnitine (TC), 5 µL of the sample (plasma, calibration points) was transferred into a 1.5 mL test tube, then 200 µL of acetonitrile containing the internal standard was added for protein precipitation, and 100 µL of 1 M KOH in methanol was added to hydrolyze acylcarnitines. The solution was incubated at 50°C for 60 min, and 100 µl of 1 M HCl in methanol was added to neutralize the mixture (36). Samples were centrifuged for 2 min at 14000 rpm and injected into liquid chromatography-mass spectrometry (LC-MS/MS) system in the Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics, Polish Academy of Sciences (Warsaw, Poland). Serum IGF-1, myostatin, and decorin concentrations were measured using commercially available enzyme immunoassay kits (total IGF-1, cat. #DG100, myostatin, cat. #DGDF80, decorin cat. #DY143 and #DY008; R&D Systems, Minneapolis, MN, USA).

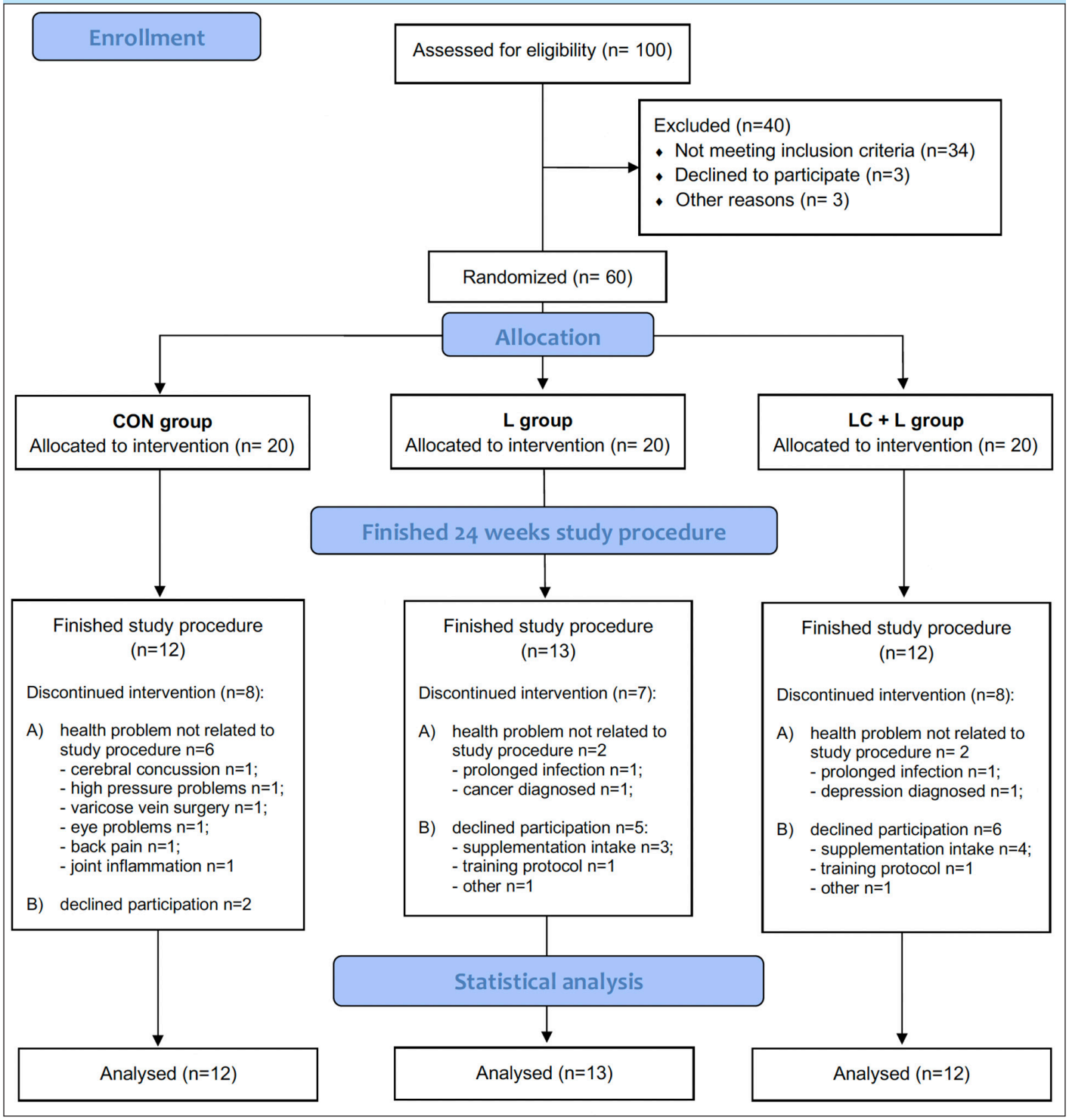
Diet

Three-day food records were self-reported for two weekdays and one weekend day at the beginning of the study. Participants were instructed to note the amounts of food and beverages consumed. The diet was analyzed in terms of the amount of energy, protein, carbohydrates (CHO), and fat consumed.

Statistical analysis

Participants included in the statistical analyses completed a minimum of 80% of the training sessions. All calculations were performed using Statistica 13.1 software (Dell Inc., Tulsa, OK, USA). The normality of the data distribution was established using the Shapiro-Wilk test. Repeated-measures analysis of variance (ANOVA) was used for normally distributed data, and the Friedman repeated-measures ANOVA by ranks was performed for nonnormally distributed data. The Kruskal-Wallis ANOVA was used to compare groups at the same time

Figure 1. Flow chart of participant recruitment and participation in the study



point. Correlations between the changes in absolute values from before to after the RET intervention were calculated using Pearson and Spearman correlation tests for normally and nonnormally distributed data, respectively. A probability level of $p < 0.05$ was considered to be significant. All data are expressed as mean ± SD, unless otherwise stated.

Results

The study protocol was completed by 37 participants (Fig. 1). Despite the high dropout (38.3%), the characteristics of the analyzed subjects did not differ between the groups (Table 1). The direct comparison between subjects who completed the study protocol and those who dropout the study also indicated no differences (Table S2).

Table 1. Baseline characteristics and dietary composition of the participants

	CON (n = 12)	L (n = 13)	LC+L (n = 12)	p
Age (years)	65.8 ± 2.6	67.9 ± 2.1	68.0 ± 2.7	0.067
Weight (kg)	70.6 ± 11.5	68.9 ± 13.2	73.0 ± 14.2	0.743
Height (cm)	162.8 ± 6.3	158.9 ± 4.6	159.5 ± 5.5	0.176
BMI	26.6 ± 3.9	27.2 ± 4.7	28.7 ± 5.7	0.551
METs	2.6 ± 1.5	3.1 ± 1.1	3.3 ± 1.6	0.769
Diet				
Energy (MJ/d)	6.5 ± 2.0	6.6 ± 0.9	7.0 ± 1.8	0.742
CHO (g/kg/d)	2.6 ± 1.3	2.9 ± 1.0	2.7 ± 1.2	0.743
Protein (g/kg/d)	1.0 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.852
Fat (g/kg/d)	0.9 ± 0.4	0.9 ± 0.3	1.0 ± 0.5	0.821

CON: control group; L: leucine supplemented group; LC+L: L-carnitine and leucine supplemented group; BMI: Body mass index; METs: Metabolic equivalents of additional daily physical activity; CHO: carbohydrates

Table 2. Measurements of isometric and isokinetic strength and cross-sectional area in the dominant leg before and after the RET protocol

	CON (n = 12)			L (n = 13)			LC+L (n = 12)			p group x time
	pre	post	% change	pre	post	% change	pre	post	% change	
Isometric										
Peak Torque (Nm)	153 ± 31	159 ± 39	4.0 ± 17.8	144 ± 34	157 ± 51	7.9 ± 13.1	154 ± 30	170 ± 39	11.4 ± 24.3	0.663
Isokinetic										
Peak Torque (Nm)	127 ± 18	134 ± 30	5.2 ± 17.3	117 ± 29	127 ± 34	8.8 ± 9.5	124 ± 19	135 ± 20	9.4 ± 12.4	0.814
Average power (W)	53 ± 11	56 ± 12	8.5 ± 27.8	48 ± 10	57 ± 16	19.5 ± 19.2	51 ± 11	61 ± 10	19.8 ± 15.9	0.209
Total work (J)	483 ± 91	529 ± 116	10.9 ± 26.0	478 ± 87	512 ± 135	6.8 ± 16.4	484 ± 106	557 ± 87	17.6 ± 19.9	0.509
Cross-sectional area										
Thight muscles (cm^2)	114 ± 15	116 ± 16	2.4 ± 4.8	112 ± 18	114 ± 18	2.0 ± 3.7	113 ± 13	116 ± 13	2.5 ± 5.3	0.966
Vastus muscles (cm^2)	38.3 ± 4.7	39.3 ± 5.2	2.9 ± 8.7	36.9 ± 8.3	37.7 ± 7.9	2.5 ± 5.1	36.6 ± 5.7	38.5 ± 5.8	5.6 ± 7.0	0.567

CON: control group; L: leucine supplemented group; LC+L: L-carnitine and leucine supplemented group;- % change: percent changes between pre- and post- intervention values

Table 3. Serum biomarkers before and after the RET protocol, with calculated percent changes between pre- and post- intervention values

	CON (n = 12)			L (n = 13)			LC+L (n = 12)			p group x time
	pre	post	% change	pre	post	% change	pre	post	% change	
IGF-1 (µg/L)	92 ± 28	93 ± 29	1.6 ± 17.8	83 ± 22	88 ± 28	6.8 ± 19.9	87 ± 27	92 ± 25	11.3 ± 36.3	0.757
Myostatin (µg/L)	3.2 ± 1.2	3.3 ± 1.2	5.1 ± 24.4	4.3 ± 1.8	4.2 ± 1.6	2.2 ± 24.4	3.4 ± 1.6	3.7 ± 1.6	12.2 ± 27.5	0.619
Decorin (µg/L)	8.1 ± 1.3	7.9 ± 1.9	-2.5 ± 15.5	8.4 ± 1.4	9.2 ± 1.8	9.4 ± 11.1	8.0 ± 1.6	8.7 ± 1.3	9.9 ± 16.9	0.075

CON: control group; L: leucine supplemented group; LC+L: L-carnitine and leucine supplemented group; % change: percent changes between pre- and post- intervention values

Effect of the RET intervention on muscle strength and CSA

RET caused significant increases in isometric peak torque ($p = 0.009$), isokinetic peak torque ($p < 0.001$), average power ($p < 0.001$), total work ($p < 0.001$) (Table 2). RET also had a significant effect on muscle hypertrophy, as measured by CSA TM ($p = 0.005$) and CSA KE ($p = 0.006$). However, the group and time interactions were not significant for the muscle variables analyzed (Table 2).

Changes in blood markers

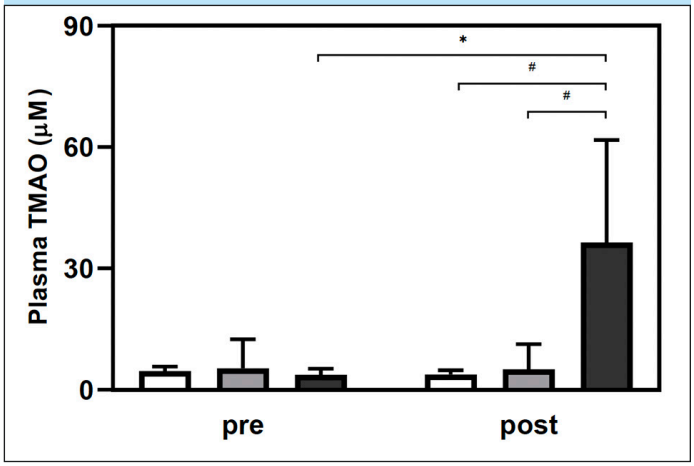
TMAO level increased only in the LC+L group ($p < 0.001$) and was higher after the intervention in the LC+L group than in the L ($p < 0.001$), and CON ($p = 0.005$) groups. The intervention did not change plasma TMAO concentration in the L ($p = 0.959$) and CON ($p = 0.866$) groups (Fig. 2). LC supplementation also caused a significant increase in plasma TC (CON 2.3 ± 6.6, L 0.4 ± 9.1, LC+L 9.9 ± 6.3 μmol/L; $p = 0.009$). No differences were observed in circulating

IGF-1 (group p = 0.790, time p = 0.190, group*time p = 0.757), myostatin (group p = 0.255, time p = 0.508, group*time p = 0.619), and decorin (group p = 0.380, time p = 0.030, group*time p = 0.075) levels (Table 3). After the intervention serum decorin level was higher than before in both supplemented groups combined (p = 0.012), still not significantly different to post intervention CON (p = 0.231). Other parameters did not change significantly (Table S3).

Correlations between skeletal muscle parameters and circulating markers

The elevation in plasma TC level positively correlated with the change in plasma TMAO level (rho = 0.595, p < 0.001; Table S4). However, no other significant correlations between the changes in circulating markers and muscle function or muscle volume were noted (Table S4). In addition, the change in muscle volume from before to after the RET did not correlate with improvements in strength test variables (Table S4).

Figure 2. Plasma trimethylamine-N-oxide (TMAO) presented as means (± SD) in CON (white bars), L (gray bars), and LC+L (black bars) groups before and after 24 weeks of the RET



*p < 0.001, compared with before the intervention in the same group; #p < 0.005, compared between groups at the same time.

Discussion

The main findings of this study were that LC combined with leucine or leucine alone does not improve the effectiveness of RET; the increase in circulating TC level was associated with the increase in TMAO level; the changes in circulating markers did not correlate with muscle function or volume; supplemented groups analyzed together had higher decorin levels.

LC supplementation has been proposed as a potential promotor of muscle protein balance (37). The suggested mechanism involves activation of the mammalian target of rapamycin kinase (mTOR) pathway in skeletal muscle (for review see (38)). Activation of the mTOR signaling pathway, which is associated with increases in muscle mass and strength, was observed after 8 weeks of supplementation with LC in combination with leucine, creatine, and vitamin D without additional physical activity (18). Leucine is an important regulator of skeletal muscle anabolism (8, 39). However, long-term studies of older adults supplemented with leucine for 6 months (11), even when combined with strength training twice a week (40), have not produced positive effects on muscle mass or strength. Similarly, we observed no additional increase in muscle volume or function in the group supplemented with leucine alone (L group) or with leucine in combination with LC (LC+L group) after the 24 weeks of RET. Considering these observations, it cannot be ruled out that previous effects (18) may have been related to supplementation with creatine, which delays muscle atrophy and improves strength during aging (41, 42), or to vitamin D, which can improve muscle strength in people aged ≥65 years (43). Especially that supplementation with a mixture containing creatine and vitamin D, among others, for 12 weeks without additional physical activity improved muscle strength and muscle power in healthy elderly humans (44).

Circulating IGF-1 has been suggested as a potential mechanism underlying the beneficial effects of LC supplementation on skeletal muscle protein turnover (37, 38). An elevation in circulating IGF-1 level after LC supplementation was observed in animal (15, 17) and clinical (45) studies. LC supplementation for 10 weeks in prefrail older people (46), and for 24 weeks in healthy older women (47) did not affect circulating IGF-1 levels. Despite improvements of strength and skeletal muscle hypertrophy following RET (3) evidence to support a role of circulating IGF-1 is inconsistent. Increased IGF-1 level was reported in older people following 12 months (48) and 8 months (49) of RET. By contrast, no change in serum IGF-1 was found in older people after 6 months of RET (50, 51), even in those supplemented with 20.7 g of protein (3 g leucine, >10 g of essential amino acids), 9.3 g of CHO, 3 g of fat, and vitamins and minerals (51). Similarly, in our study, RET alone and RET with supplementation did not change blood IGF-1 levels. In addition, we observed no effect of LC on thigh muscle volume following 24 weeks of RET combined with leucine supplementation. Correspondingly, a higher LC dose combined with an insulinogenic beverage (44.4 g CHO, 13.8 g protein) and moderate-intensity cycling over 25 weeks did not affect lean body mass in older men (52). By contrast, 2 g of LC per day for 6 months increased fat-free mass in centenarians (53). The contradictory results may be related to the age of the participants. Accelerated skeletal muscle mass loss is observed in humans aged ≥80 years, and is strongly associated with a decrease in serum IGF-1 level (54). Therefore, LC supplementation does not appear to affect fasting serum IGF-1 level in people aged <80 years (46, 47) but may be effective in centenarians. Importantly, in longitudinal studies, older people may not ingest sufficient protein (55) or energy (56). Although energy intake and dietary composition did not differ between groups in our study, a protein intake ~1.0g/kg/day may limit the extent of adaptation to RET (57).

Myostatin is a negative regulator of skeletal muscle growth (58) and may be downregulated by decorin (5). Increased muscle decorin expression correlates with improvement in leg press performance (6). Acute resistance exercise elevates

plasma decorin at the end of the session, although 120 min after the end of exercise it returns to baseline level (6) and resting plasma decorin level is not affected by 5 weeks exercise intervention (59). Similarly, in our study, decorin level did not change after 24 weeks of RET in the CON group but increased significantly in both supplemented groups. However, we found no associations between the differences in circulating decorin levels and changes in muscle volume and function, and no effects of RET or supplementation on myostatin levels. The lack of association between exercise performance and circulating mediators may indicate that exercise increases muscle strength by predominately locally derived mediators rather than circulating factors (60).

The recent meta-analysis of 37 randomized controlled trails indicated that LC supplementation might affect body weight and composition, with a dose of 2 g LC per day providing the maximum effect in adults (61). In fact, LC supplementation (2g/day) in combination with a resistance training program (4 days/week) applied to healthy men (age range 18-40 years), for 9 weeks caused statistically significant improvements in bench and leg presses. There were no differences between the supplemented and placebo groups, but the number of repetitions and lifting volume increased in the LC group compared to baseline values (62). In the present study, the applied daily LC dose was lower than previously reporting enhancement in muscle strength of healthy humans (18, 62), but the total amount of LC consumed was higher due to prolonged period of supplementation. Importantly, LC needs insulin for transportation into muscle (14). Muscle carnitine level increases only when LC is coingested with a large amount of CHO to induce an insulin response (63), and the supplementation protocol should take minimum 100 days (28). Leucine has been reported to increase insulin level (13), and we assumed that 24 weeks of LC supplementation in combination with leucine could improve LC transport into muscle. The increase in insulin levels in our study may have been low compared with a previous study on carnitine supplementation with CHO to induce release of insulin (63). Indeed, the same supplementation protocol did not change skeletal muscle TC level in young participants (unpublished), suggesting that muscle TC was not affected also in the present study.

Prolonged LC treatment elevates fasting plasma TMAO level (25), and TMAO can modulate myosin ATPase activity (22, 23). We hypothesized that LC supplementation would affect muscle strength by increasing circulating TMAO level and modifying ATPase activity. Erickson et al. (64) recently reported a correlation between fasting TMAO level and aerobic capacity in older sedentary adults with obesity. However, we did not observe any significant associations between plasma TMAO level and muscle function parameters. Erickson and colleagues (64) also reported that 12 weeks of exercise training combined with a hypocaloric diet induced a percentage reduction (but not in the absolute level) in fasting TMAO concentration. The similar fasting TMAO levels in the CON and L groups after 24 weeks of RET in the present study suggest a minimal role of exercise in the reduction in plasma TMAO concentration. Nutritional intervention seems to be more important factor for modifying plasma TMAO level. The correlation observed between the increases in plasma TC and TMAO concentrations may be because LC is a substrate for TMAO production (20).

In light of current findings, it should be noted that LC combined with leucine or leucine alone supplementation does not improve the effectiveness of RET in healthy aged women, at least in studied doses. In addition, LC supplementation elevates plasma TMAO level. Studies have shown that TMAO is a risk factor for the development of noncommunicable diseases, but whether it affects the health status of people without any chronic diseases is widely discussed (65-67), and needs further research.

Our study has several limitations. First, we did not evaluate the markers in skeletal muscle. Second, the high dropout and small sample size may have limited the statistical power and the ability to identify differences between groups. Third, CON was not provided a placebo supplement, and was involved only in the training protocol, which made this study partially blinded. Finally, self-reported data were used to monitor dietary intake only once. Given the increase in physical activity levels by the participants, the energy intake may have also increased, which could have resulted in changes in the dietary macronutrient composition.

Conclusions

LC combined with leucine or leucine alone supplementation does not improve the efficacy of RET in healthy aged women. Leucine supplementation elevated circulating decorin level, but this increase was not associated with serum myostatin level. LC supplementation increased plasma TMAO level. There are no links between changes in circulating markers and muscle function or volume. Further research using combined nutritional and exercise interventions, as well as the creation of targeted recommendations for elderly populations, would be important for personalized medicine.

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Erratum to: L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women.

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REVIEW

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The bright and the dark sides of L-carnitine supplementation: a systematic review

Angelika K. Sawicka[1], Gianluca Renzi[2] and Robert A. Olek[3*]

Abstract

Background: L-carnitine (LC) is used as a supplement by recreationally-active, competitive and highly trained athletes. This systematic review aims to evaluate the effect of prolonged LC supplementation on metabolism and metabolic modifications.

Methods: A literature search was conducted in the MEDLINE (via PubMed) and Web of Science databases from the inception up February 2020. Eligibility criteria included studies on healthy human subjects, treated for at least 12 weeks with LC administered orally, with no drugs or any other multi-ingredient supplements co-ingestion.

Results: The initial search retrieved 1024 articles, and a total of 11 studies were finally included after applying inclusion and exclusion criteria. All the selected studies were conducted with healthy human subjects, with supplemented dose ranging from 1 g to 4 g per day for either 12 or 24 weeks. LC supplementation, in combination with carbohydrates (CHO) effectively elevated total carnitine content in skeletal muscle. Twenty-four-weeks of LC supplementation did not affect muscle strength in healthy aged women, but significantly increased muscle mass, improved physical effort tolerance and cognitive function in centenarians. LC supplementation was also noted to induce an increase of fasting plasma trimethylamine-N-oxide (TMAO) levels, which was not associated with modification of determined inflammatory nor oxidative stress markers.

Conclusion: Prolonged LC supplementation in specific conditions may affect physical performance. On the other hand, LC supplementation elevates fasting plasma TMAO, compound supposed to be pro-atherogenic. Therefore, additional studies focusing on long-term supplementation and its longitudinal effect on the cardiovascular system are needed.

Keywords: Insulin-like growth factor-1, Protein kinase B, Mammalian target of rapamycin, Forkhead box O, MuRF-1, Atrogin-1, Trimethylamine-N-oxide

Background

The main function of L-carnitine (LC) is the transport of long-chain fatty acids into the mitochondrial matrix for their conversion in energy, via β-oxidation process [1]. Moreover, LC by the reaction with acetyl-CoA and maintaining the acetyl-CoA/CoA ratio in the cell regulates pyruvate dehydrogenase activity [2]. LC also plays an important role in the regulation of metabolic pathways involved in skeletal muscle protein balance: proteolysis and protein synthesis [3]. Furthermore, LC acts as anti-oxidant and anti-inflammatory compound [3]; thus, it may attenuate the exercise-induced muscle damage.

The opinion that LC supplementation does not change metabolism is based mostly on short-term supplementation protocols [4]. Recent studies demonstrate that prolonged supplementation, especially in combination with carbohydrates (CHO), may increase muscle total carnitine (TC) content in skeletal muscle [5–7]. Therefore, LC supplementation in specific conditions may affect physical performance. On the other hand, LC has been proposed as the red meat nutrient responsible for atherosclerosis promotion [8]. As a potential link between

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red meat consumption and the increasing risk of cardiovascular disease, trimethylamine-N-oxide (TMAO) has been indicated [8]. Since LC is still used by the athletes [9, 10], the aim of this systematic review is to evaluate the effect of prolonged LC supplementation on metabolism/metabolic changes in healthy human subjects.

Methods

Eligibility criteria

The PICOS strategy was defined as follows: "P" (participants) human subjects, "I" (interventions) oral LC treatment, "C" (comparisons) between supplementation and placebo, supplementation and control, or pre- and post-supplementation, "O" (outcomes) muscle variables, and "S" (study design) randomized controlled trials, non-randomized controlled trials, non-randomized non-controlled trials.

Studies with the following criteria were excluded: described in languages other than English, articles without full-text availability, reviews and case reports. Subsequently, the following eligibility criteria were applied: a) healthy human subjects; b) supplementation at least for 12 weeks; c) oral LC administration; d) no drugs co-ingestion; e) no multi-ingredients supplementation.

Information sources and search

The literature was explored using the MEDLINE (via PubMed) and Web of Science databases, including all articles published from the inception up February 2020. The search was conducted using the terms: "carnitine supplementation" or "carnitine treatment" in combination with "exercise", "training", "athletic performance", "muscle strength", "muscle fatigue", "muscle damage", "muscle recovery", "muscle synthesis" or "proteolysis".

Study selection

Firstly, studies were assessed by title verification between databases (duplicates were removed). The second assessment performed by abstracts analysis, excluded studies in a language other than English, studies with lack of full text, reviews, case reports, animal studies and in-vitro studies. The last step was performed by analysis of full manuscripts based on the described above eligibility criteria.

Data collection process

The following information was compiled for each study: authors, year of publication, type of study, length of supplementation, a dose of supplementation and main effect. Lastly, the thematic analysis was carried out, to synthesize and interpret all the data that appeared from the included publications. The process of selecting papers, data collection as well as the quality assessment was performed independently by two authors (A.S., G.R.), and all disagreements were resolved by the discussion with the third author (R.O).

Results

Study selection

By the above-described search strategy, 1295 publications were identified. After the first selection, adjusted by duplicates, persisted 1024 articles. Of these, 794 were excluded after abstracts screening and identified articles in languages other than English, lack of full text or being review articles, case reports, animal or in-vitro studies. The full texts of 230 articles were screened by eligibility criteria. Finally, to the qualitative analysis were accepted 11 studies performed on healthy human subjects, treated for at least 12 weeks with LC administered orally, with no drugs or any other multi-ingredient supplements co-ingestion (Fig. 1).

Description of the included studies

Table 1 provides details and results of the 11 studies reviewed. Selected studies were published between 2002 and 2020. In the selected studies, participants were supplemented in a dose ranging from 1 g to 4,5 g per day for either 12 or 24 weeks, mostly by L-carnitine-L-tartrate (LCLT). In three studies, supplementations were combined with carbohydrates (CHO) [5–7], and in one with L-leucine [18].

Muscle carnitine content was not affected following 12 weeks of LC supplementation alone [11, 12]. On the other hand, LC supplementation in combination with CHO effectively elevated muscle TC after 12 [6] and 24 weeks [5]. Moreover, 12 weeks of supplementation alone [13], or in combination with CHO [6] promote the expression of the genes related to fatty acids and carnitine metabolism.

Twenty-four-weeks of LC supplementation alone did not affect muscle strength in healthy aged women [15], but significantly increased muscle mass, improved physical effort tolerance and cognitive function in centenarians [14].

In two studied groups of healthy aged woman, LC supplementation alone [16, 17], or in combination with L-leucine [18], induced an increase of fasting plasma TMAO levels. However, higher TMAO was not associated with determined inflammatory [16] nor oxidative stress [17] markers. Moreover, despite elevated TMAO, LC supplementation together with resistance training induced positive changes in mitochondrial DNA methylation of platelets [18].

Discussion

The present findings have been debated in the six separate paragraphs, and for a better picture of LC supplementation, other studies were also disputed.

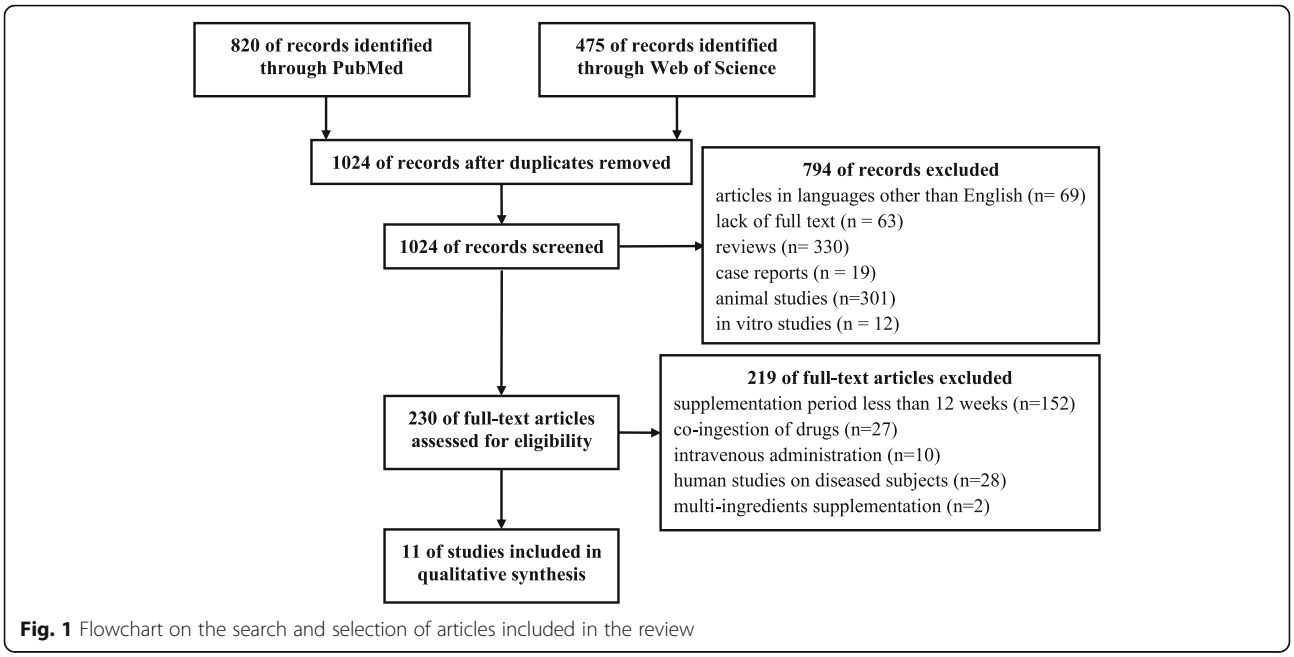

Fig. 1 Flowchart on the search and selection of articles included in the review

"Fat burner"

It has been assumed that LC supplementation, by increasing muscle carnitine content, optimizes fat oxidation and consequently reduces its availability for storage [19]. Nevertheless, the belief that carnitine is a slimming agent has been negated in the middle of 90s [20]. Direct measurements of carnitine in skeletal muscles failed to show any elevation in the muscle carnitine concentration following 14 days of 4 g/day [21], or 6 g/day [22] LC ingestion. These findings implied that LC supplementation was not able to increase fat oxidation and improve exercise performance by the proposed mechanism. Indeed, many original investigations, summarized in later review [4], indicated that LC supplementation lasting up to 4 weeks, neither increase fat oxidation nor improve performance during prolonged exercises.

Since LC concentration in skeletal muscles is higher than that of blood plasma, active uptake of carnitine must take place [23]. Stephens et al. [24] noted that 5 h steady-state hypercarnitinemia (~ 10-fold elevation of plasma carnitine) induced by the intravenous LC infusion does not affect skeletal muscle TC content. On the other hand, similar intervention in combination with controlled hyperinsulinemia (~ 150mIU/L) elevates TC in skeletal muscle by ~ 15% [24, 25]. Moreover, higher serum insulin maintained by the consumption of simple sugars resulted in augmented LC retention in healthy human subjects supplemented by LC for 2 weeks [26]. Based on these results, Authors suggested that oral ingestion of LC, combined with CHO for activation carnitine transport into the muscles, should take ~ 100 days to increase muscle carnitine content by ~ 10% [26]. This assumption has been confirmed in later studies [5–7]. These carefully conducted studies clearly showed that prolonged procedure (for ≥12 weeks) of a daily LC and CHO ingestion induced a raise of skeletal muscle TC levels [5–7], affecting exercise metabolism [5], improving performance [5] and energy expenditure [6], without altering body composition [6]. The lack of body fat stores loss may be explained by the 18% increase in body fat mass associated with CHO supplementation alone, noted in the control group [6].

Nevertheless, 12 weeks of LC supplementation 2 g/day applied without CHO, elevated muscle TC only in vegetarian but not in omnivores [12]. Neither exercise metabolism nor muscle metabolites were modified by augmented TC in vegetarian [12].

Skeletal muscle protein balance regulation

Skeletal muscle mass depends on the rates of protein synthesis and degradation. Elevated protein synthesis and attenuated proteolysis are observed during muscle hypertrophy. Both of these processes are mainly regulated by the signaling pathway: insulin-like growth factor-1 (IGF-1) – phosphoinositide-3-kinase (PI3K) – protein kinase B (Akt) – mammalian target of rapamycin (mTOR). The activation of mTOR leads to phosphorylation and activation of S6 kinases (S6Ks) and hyperphosphorylation of 4E-binding proteins (4E-BPs), resulting in the acceleration of protein synthesis. At the same time, Akt phosphorylates and inactivates forkhead box O (FoxO), thereby inhibit the responsible for proteolysis ubiquitin ligases: muscle-specific RING finger-1 (MuRF-

Table 1 Summary and results of the studies reviewed examining the LC supplementation

Studies	Participants characteristics	Study design	Supplementation dose and period	Main effect
[11]	Moderately trained male subjects ($n = 7$) age 23–25	NRNC	4 g LC/day for 3 months	Increase of TC plasma concentration after the supplementation; No change in muscle TC concentration, mitochondrial enzymes activity, physical performance and muscle fiber composition
[12]	Male vegetarians ($n = 16$) and omnivores (C) ($n = 8$) age 18–40	NRC	2 g LCLT /day for 12 weeks	Increase of TC plasma concentration after the supplementation and muscle TC concentration only in vegetarians; No change in physical performance and muscle metabolism either in omnivores or vegetarians.
[13]	Middle aged untrained male subjects (S $n = 12$; P $n = 12$) age not reported (both groups involved in endurance training; 3x/ week)	RC	2 g LCLT /day for 12 weeks	Increase of TC plasma concentration after the supplementation; Plasma triacylglycerols and free fatty acids not affected by training or supplementation; Training resulted in an increase in the mRNA expression of genes coding proteins involved in long chain fatty acid transport in white blood cells, LC supplementation enhanced the effect on gene expression
[6]	Non-vegetarian, male recreational athletes (S n = 6; P $n = 6$) age 28 ± 2 (S); 25 ± 2 (P)	RC	2 g LCLT + 80 g CHO /day for 12 weeks	Increase in muscle TC concentration after LC supplementation; Upregulation of seventy-three genes relating to fuel metabolism in LC vs. control; Higher exercise energy expenditure after LC supplementation; No change in carnitine palmitolytransferase 1 activity; Body mass and whole-body fat mass increased in control, but did not change in LC supplemented
[5]	Non-smoking, non-vegetarian recreational athletes (S $n = 7$; P $n = 7$) age 26 ± 2	RC	2 g LCLT + 80 g CHO /day for 24 weeks	Increase in muscle TC concentration after LC supplementation; Lower muscle glycogen utilization during low intensity exercise, lower lactate production during high intensity exercise, higher work output during a 30 min 'all-out' exercise performance test in LC supplemented group;
[7]	Healthy, non-vegetarian, untrained males (S $n = 7$; P $n = 7$) age 23 ± 2 (both groups involved in HIIT; 3x/week)	RC	2.25 g LCLT + 80 g CHO /day for 24 weeks	Muscle TC concentration tend to increase after LC supplementation ($p = 0.06$ vs. pre-supplementation); Skeletal muscle adaptations to training not augmented by elevated muscle carnitine availability;
[14]	Centenarians (S $n = 27$; P $n = 27$) age 100–106	RC	2 g LC/day for 24 weeks	Increase of TC plasma concentration after the supplementation; Fat mass reduction, muscle mass elevation, physical effort tolerance and cognitive function improvement in LC supplemented group
[15]	Healthy women (S $n = 11$; P $n = 9$) age 65–70	RC	1.5 g LCLT /day for 24 weeks	Increase of free carnitine plasma concentration after the supplementation; No changes in body composition, skeletal muscle strength and IGF-1 after LC supplementation
[16]	Healthy women (S $n = 11$; P $n = 9$) age 65–70	RC	1.5 g LCLT /day for 24 weeks	Increase of plasma TMAO concentration after the supplementation; No changes in serum C-reactive protein, interleukin-6, tumor necrosis factor-α, L-selectin, P-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and lipid profile after LC supplementation
[17]	Healthy women (S $n = 11$; P $n = 9$) age 65–70	RC	1.5 g LCLT /day for 24 weeks	No changes in plasma GBB or serum ox-LDL, myeloperoxidase, protein carbonyls, homocysteine, and uric acid concentrations
[18]	Healthy aged women (S $n = 12$; P $n = 13$; C $n = 12$) age 67 ± 3 (all groups involved in resistance training 3x/week)	RC	1 g LCLT + 3 g L-leucine/day for 24 weeks	Increase of plasma TMAO concentration after the supplementation; Increase of D-loop methylation in platelets of LC supplemented

Groups: *C* control; *S* supplemented; *P* placebo; Study design: *RC* randomized controlled; *NRC* non-randomized controlled; *NRNC* non-randomized non-controlled; *LCLT* L-carnitine-L-tartrate; *HIIT* high-intensity interval training

1) and muscle atrophy F-box protein (atrogin-1), (for review see [27–29]).

The association between LC supplementation and the regulation of metabolic pathways involved in muscle protein balance have been shown in several animal studies (Fig. 2) [30–35]. Four weeks of LC supplementation in rats increased plasma IGF-1 concentration [33]. Elevated circulating IGF-1 led to an activation of the IGF-1–PI3K–Akt signalling pathway, causing augmented mTOR phosphorylation and higher phospho-FoxO/total FoxO ratio in skeletal muscle of LC supplemented rats [33]. FoxO inactivation attenuated MURF-1 expression in *quadriceps femoris* muscle of supplemented rats (compared to control) [33]. Moreover, LC administrated for 2 weeks suppresses atrogin-1 messenger RNA (mRNA) level in suspended rats' hindlimb [35], and only 7 days of LC administration downregulates MuRF-1 and atrogin-1 mRNAs reducing muscle wasting in a rat model of cancer cachexia [32]. All these findings together might suggest that LC supplementation protect muscle from atrophy, especially in pathophysiological conditions.

In fact, administration of acetyl-L-carnitine 3 g/day for 5 months in HIV-seropositive patients induced ten-fold increase in serum IGF-1 concentration [36]. Conversely, neither 3 weeks LC supplementation in healthy, recreationally weight-trained men [37], nor 24 weeks LC supplementation in aged women [15] did not affect circulating IGF-1 level concentration. Various effects might be due to different IGF-1 levels; significantly lower in the HIV-seropositive patients than in healthy subjects [38]. Additionally, 8 weeks of LC supplementation in healthy older subjects, did not change total and phosphorylated mTOR, S6K and 4E-BP proteins level of *vastus lateralis* muscle [39]. It must be highlighted that rat skeletal muscle TC increases ~ 50–70% following 4 weeks of LC supplementation [33, 34], whereas comparable elevation has never been observed in human studies, even after 24 weeks of supplementation [5, 7].

Body composition

These findings altogether suggest that prolonged LC supplementation might affect body composition in specific conditions.

Obesity

A recent meta-analysis, summarized studies focused on LC supplementation for a prolonged time (median 3 months) [40]. Pooled results demonstrated a significant reduction in weight following LC supplementation, but the subgroups analysis revealed no significant effect of LC on body weight in subjects with body mass index (BMI) below 25 kg/m^2. Therefore, authors suggested that LC supplementation may be effective in obese and overweight subjects. Surprisingly, intervention longer than 24 weeks showed no significant effect on BMI [40].

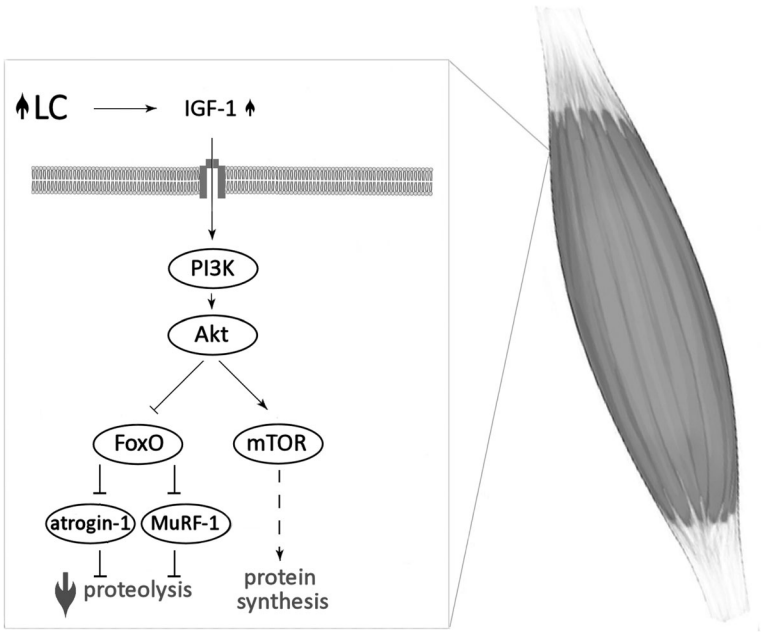

Fig. 2 The association between LC supplementation and the regulation of metabolic pathways involved in muscle protein balance. L-carnitine (LC); insulin-like growth factor-1 (IGF-1); phosphoinositide-3-kinase (PI3K); protein kinase B (Akt); mammalian target of rapamycin (mTOR); forkhead box O (FoxO); muscle-specific RING finger-1 (MuRF-1); muscle atrophy F-box (atrogin-1); increase (↑); decrease (↓); activation (→); inactivation (⊣)

Training

It has been assumed that a combination of LC supplementation with increased energy expenditure may positively affect body composition. However, either with aerobic [41, 42] or resistance [43] training, LC supplementation has not achieved successful endpoint. Six weeks of endurance training (five times per week, 40 min on a bicycle ergometer at 60% maximal oxygen uptake) together with LC supplementation (4 g/day) does not induce a positive effect on fat metabolism in healthy male subjects (% body fat 17.9 ± 2.3 at the beginning of the study) [41]. Similarly, lack of LC effect has been reported in obese women [42]. Eight weeks of supplementation (2 g/day) combined with aerobic training (3 sessions a week) had no significant effects on body weight, BMI and daily dietary intake in obese women [42].

In the recent study, LC supplementation 2 g/day has been applied in combination with a resistance training program (4 days/week) to healthy men (age range 18–40 y.o.), for 9 weeks [43]. Body composition, determined by dual energy X-ray absorptiometry, indicated no significant effect in fat mass and fat-free mass due to supplementation. Moreover, LC administration did not influence bench press results. The number of leg press repetitions and the leg press third set lifting volume increased in the LC group compared to the placebo group [43]. Different LC effect in the limbs may be associated with the higher rates of glycogenolysis during arm exercise at the same relative intensity as leg exercise [44].

Sarcopenia

Aged people have accelerated protein catabolism, which is associated with muscle wasting [45]. LC could increase the amount of protein retention by inhibition of the proteolytic pathway. Six months of LC supplementation augmented fat free mass and reduced total body fat mass in centenarians [14]. Such effect was not observed in elder women (age range 65–70 y.o.) after a similar period of supplementation [15]. The effectiveness of LC supplementation may result from the age-wise distribution of sarcopenia. The prevalence of sarcopenia increased steeply with age, reaching 31.6% in women and 17.4% in men older than 80 years [46]. In subjects below 70 years presarcopenia, but not sarcopenia symptoms were noted [46].

Oxidative imbalance and muscle soreness

Muscle damage may occur during exercise, especially eccentric exercise. In the clearance of damaged tissues assist free radicals produced by neutrophils. Therefore, among other responses to exercise, neutrophils are released into the circulation. While neutrophil-derived reactive oxygen species (ROS) play an important role in breaking down damaged fragments of the muscle tissue, ROS produced in excess may also contribute to oxidative stress (for review see [47, 48].

Based on the assumption that LC may provide cell membranes protection against oxidative stress [49], it has been hypothesized that LC supplementation would mitigate exercise-induced muscle damage and improve post-exercise recovery. Since plasma LC elevates following 2 weeks of supplementation [21, 22], short protocols of supplementation may be considered as effective in attenuating post-exercise muscle soreness. The findings indicated that 3 weeks of LC supplementation, in the amount 2-3 g/day, effectively alleviated pain [50–53]. It has been shown, through magnetic resonance imaging technique that muscle disruption after strenuous exercise was reduced by LC supplementation [37, 51]. This effect was accompanied by a significant reduction in released cytosolic proteins such as myoglobin and creatine kinase [50, 52, 53] as well as attenuation in plasma marker of oxidative stress - malondialdehyde [51, 53, 54]. Furthermore, 9 weeks of LC supplementation in conjunction with resistance training revealed a significant increase of circulating total antioxidant capacity and glutathione peroxidase activity and decrease in malondialdehyde concentration [43].

Risks of TMAO

In 1984 Rebouche et al. [55], showed that rats, orally receiving radiolabeled LC, metabolized it to γ-butyrobetaine (up to 31% of the administered dose, present primary in feces) and TMAO (up to 23% of the administered dose, present primary in urine). On the contrary, these metabolites were not produced by the rats receiving the isotope intravenously and germ-free rats receiving the tracer orally, suggesting that orally ingested LC is in part degraded by the gut's microorganisms [55]. Similar observations were noted in later human studies [56, 57], with the peak serum TMAO observed within hours following oral administration of the tracer [56]. Prolonged LC treatment elevates fasting plasma TMAO [16–18, 58, 59]. Three months of oral LC supplementation in healthy aged women induced ten-fold increase of fasting plasma TMAO, and this level remained elevated for the further 3 months of supplementation [16]. Four months after cessation of LC supplementation, plasma TMAO reached a pre-supplementation concentration, which was stable for the following 8 months [60].

In 2011 Wang et al. [61] suggested TMAO as a pro-atherogenic factor. Since diets high in red meat have been strongly related to heart disease and mortality [62], LC has been proposed as the red meat nutrient responsible for atherosclerosis promotion [8]. As a potential link between red meat consumption and the increasing risk of cardiovascular disease, TMAO has been indicated

[8]. Numerous later studies have shown the association between increased plasma TMAO levels with a higher risk of cardiovascular events [63–66]. The recent meta-analyses indicated that in patients with high TMAO plasma level, the incidence of major adverse cardiovascular events was significantly higher compared with patients with low TMAO levels [67], and that all-cause mortality increased by 7.6% per each 10 μmol/L increment of TMAO [68].

Since red meat is particularly rich in LC [69], dietary intervention in healthy adults, indicated a significant increase in plasma and urine TMAO levels following 4 weeks of the red meat-enriched diet [70]. The rise of plasma TMAO was on average three-fold compared with white meat and non-meat diets [70]. Conversely, habitual consumption of red, processed or white meat did not affect plasma TMAO in German adult population [71]. Similarly, a minor increase in plasma TMAO was observed following red meat and processed meat consumption in European multi-center study [72].

In the previous century, the underlined function of TMAO was the stabilization of proteins against various environmental stress factors, including high hydrostatic pressure [73]. TMAO was shown as widely distributed in sea animals [74], with concentration in the tissue increasing proportionally to the depth of the fishes natural environment [75]. Consequently, fish and seafood nutritional intake has a great impact on TMAO level in the human body [76], significantly elevating also plasma TMAO concentration [72]. Therefore, link between plasma TMAO and the risk of cardiovascular disease [8] seems like a paradox, since more fish in the diet reduces this risk [77].

Not only dietary modification may affect TMAO plasma levels. Due to TMAO excretion in urine [56, 57], in chronic renal disease patients, TMAO elimination from the body fails, causing elevation of its plasma concentration [78]. Therefore, higher plasma TMAO in humans was suggested as a marker of kidney damage [79]. It is worthy to note that cardiovascular disease and kidney disease are closely interrelated [80] and diminished renal function is strongly associated with morbidity and mortality in heart failure patients [81]. Moreover, decreased TMAO urine excretion is associated with high salt dietary intake, increasing plasma TMAO concentration [82].

The relation between TMAO and chronic disease can be ambiguous, involving kidney function [79], disturbed gut-blood barrier [83], or flavin-containing monooxygenase 3 genotype [84]. Thus, whether TMAO is an atherogenic factor responsible for the development and progression of cardiovascular disease, or simply a marker of an underlined pathology, remains unclear [85].

Adverse effects

Carnitine preparations administered orally can occasionally cause heart-burn or dyspepsia [86]. No adverse events associated with LC administration were recorded at a dose 6 g/day for 12 months of supplementation in the patients with acute anterior myocardial infarction [87], or at a dose 1.274 g/day (range 0.3–3 g/day) and duration 348 days (range 93–744 days) in patients with liver cirrhosis [88]. Summarizing the risk associated with LC supplementation Hathcock and Shao [89] indicated that intakes up to 2 g/day are safe for chronic supplementation.

Although the optimal dose of LC supplementation for myocardial infarction is 3 g/day in terms of all-cause mortality [90], even lower LC intake elevates fasting plasma TMAO [16–18, 58, 59], which is ten-fold higher than control after 3 months of supplementation [16, 17]. It is worthy to mention that Bakalov et al. [91] analyzing European Medicine Agency database of suspected adverse drug reaction, noticed 143 cases regarding LC.

Strengths and limitations

The strength of this review is a focus on the period of LC treatment, very important aspect often missed in many articles dealing with this supplement. To date, only few studies have examined the effects of LC supplementation for at least 12 weeks, which is, on the other hand, the main limitation of the current review. This limitation is also magnified by the varied design of the studies available including different supplementation protocols and outcome measures. There is also a high degree of heterogeneity among participants of the analyzed studies. Therefore, the results should be taken with caution, and more research is required before definitive recommendations.

Conclusions

Lasting for several years opinion that LC supplementation does not change metabolism, especially exercise metabolism, is based mostly on short-term supplementation protocols. Nevertheless, LC is still used by elite [9] and sub-elite [10] athletes. Recent studies suggest that LC supplementation may elevate muscle TC content; therefore, modify muscle fuel metabolism and performance during the exercise. Due to insulin-mediated LC transport to the muscle, oral administration regimen should be combined with CHO. Because of LC poor bioavailability, it is likely that the supplementation protocol would take at least 3 months. Shorter period of supplementation may be effective in prevention of exercise-induced muscle damage, but not metabolic changes.

On the other hand, it is also clear that prolonged LC supplementation elevates fasting plasma TMAO [16–18, 58, 59], compound supposed to be pro-atherogenic [61]. Therefore, additional studies focusing on long-term

supplementation and its longitudinal effect on the TMAO metabolism and cardiovascular system are needed.

Abbreviations

LC: L-carnitine; TC: Total carnitine; TMAO: Trimethylamine-N-oxide; CHO: Carbohydrates; IGF-1: Insulin-like growth factor-1; PI3K: Phosphoinositide-3-kinase; Akt: Protein kinase B; mTOR: Mammalian target of rapamycin; S6K: S6 kinase; 4E-BP: 4E-binding protein; FoxO: Forkhead box O; MuRF-1: Muscle-specific RING finger-1; atrogin-1: Muscle atrophy F-box; mRNA: Messenger RNA; BMI: Body mass index; ROS: Reactive oxygen species

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Dace Hartmane, MSc

STATEMENT

As co-author of the paper **"L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function"**, I declare that the percentage and substantial contribution to the preparation, conduct and design of the study and preparation of a publication agree with the data given in the table below:

autor	wkład %	Opis *
Angelika Sawicka, MA	35%	A, B, D, E
Dace Hartmane, MSc	5%	B, D
dr hab. Patrycja Lipińska	5%	C, D
dr n.med. Ewa Wójtowicz	5%	C, D
prof. dr hab. Wiesława Łysiak - Szydłowska	8%	A, D, E
dr hab. Robert Olek	42 %	A, B, C, D, E, F

A - design of study; B - conduct of study; C - statistical analysis; D - interpretation of results; E - preparation of publication; F – fundraising

At the same time, I agree to submit the work mentioned above by Angelika Sawicka, M.A., as part of her doctoral thesis in the form of a thematically consistent collection of articles published in scientific journals.

D. HARTMANE

.....

(co-author's signature)

Gdańsk dnia 6.02.2023

dr hab. Patrycja Lipińska
.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. **„L-carnitine supplementation in older women. A pilot study on aging skeletal muscle mass and function”** oświadczam, iż procentowy i merytoryczny wkład w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji zgadza się z danymi podanymi w poniższej tabeli:

autor	wkład %	Opis *
mgr Angelika K. Sawicka	35%	A, B, D, E
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A – przygotowanie projektu badania; B – przeprowadzenie badań; C – analiza statystyczna; D – interpretacja wyników; E – przygotowanie publikacji; F – pozyskanie funduszy

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez mgr Angelikę Sawicką jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Patrycja Lipińska

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(podpis współautora)

Gdańsk dnia 6.02.2023

dr n. med. Ewa Wójtowicz

.....

(tytuł zawodowy, imię i nazwisko)

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(podpis współautora)

Gdańsk dnia 7.02.2023

prof. dr hab. Wiesława Łysiak-Szydłowska

.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

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OŚWIADCZENIE

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Signed by / Podpisano przez:
Robert Olek
Date / Data: 2023-02-06 09:08

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(podpis współautora)

Gdańsk dnia 6.02.2023

dr Joanna Jaworska

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(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „**L-Carnitine Combined with Leucine Supplementation Does Not Improve the Effectiveness of Progressive Resistance Training in Healthy Aged Women**" oświadczam, iż mój procentowy i merytoryczny wkład w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji zgadza się z danymi podanymi w poniższej tabeli:

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mgr inż. Beata Brzeska	3%	B, D
dr inż. Agnieszka Sabisz	3%	B, D
mgr Emilia Samborowska	3%	B, F
mgr Mariusz Radkiewicz	2%	B
prof. dr hab. Elżbieta Szurowska	1%	F
prof. dr hab. Paweł Winklewski	1%	F
dr hab. Arkadiusz Szarmach	5%	B, D, F
dr hab. Robert Olek	41%	A, B, C, D, E, F

A – przygotowanie projektu badania; B – przeprowadzenie eksperymentu; C – analiza statystyczna; D – interpretacja wyników; E – przygotowanie publikacji; F – pozyskanie funduszy

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(podpis współautora)

Gdańsk, dnia 6.02.2023

mgr inż. Beata Brzeska

.....

(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

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Beata Brzeska

(podpis współautora)

Gdańsk, dnia 6.02.2023

dr inż. Agnieszka Sabisz

.....

(tytuł zawodowy, imię i nazwisko)

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Sabisz

.....

(podpis współautora)

Gdańsk, dnia 6.02.2023

mgr Emilia Samborowska

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(tytuł zawodowy, imię i nazwisko)

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(podpis współautora)

Gdańsk, dnia 6.02.2023

mgr Mariusz Radkiewicz

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(tytuł zawodowy, imię i nazwisko)

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MRadkiewicz

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(podpis współautora)

Gdańsk, dnia 6.02.2023

prof. dr hab. Edyta Szurowska

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(tytuł zawodowy, imię i nazwisko)

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KIEROWNIK
II Zakładu Radiologii
prof. dr hab. med. Edyta Szurowska

.....

(podpis współautora)

Gdańsk, dnia 5.02.2023

prof. dr hab. Paweł Winklewski
.....
(tytuł zawodowy, imię i nazwisko)

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KIEROWNIK
Zakładu Fizjologii Człowieka
Wydział Nauk o Zdrowiu
z Instytutem Medycyny Morskiej i Tropikalnej

prof. dr hab. n. med. Paweł Winklewski
(podpis współautora)

Gdańsk, dnia 6.02.2023

dr hab. n. med. Arkadiusz Szarmach

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(tytuł zawodowy, imię i nazwisko)

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dr hab. n. med. Arkadiusz Szarmach
specjalista radiologii i diagnostyki obrazowej
2561707

.....

(podpis współautora)

Gdańsk dnia 6.02.2023

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(tytuł zawodowy, imię i nazwisko)

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Signed by / Podpisano przez:
Robert Olek
Date / Data: 2023-02-06 09:09

.....

(podpis współautora)

Gdansk, 23/02/07

Gianluca Renzi, M.A.

STATEMENT

As a co-author of the paper entitled: **"The bright and the dark sides of L-carnitine supplementation: a systematic review"**, I declare that my contribution to the preparation, conduct and design of the study and presentation of the work in the form of a publication is: data collection and qualitative thematic analysis of the collected materials.

At the same time, I agree to submit the paper mentioned above by Angelika Sawicka, M.A., as part of her doctoral thesis as a thematically coherent collection of articles published in scientific journals.

I declare that the independent and separable part of the mentioned publication above demonstrates Angelika Sawicka, M.A.'s contribution to developing the concept, data collection, and qualitative thematic analysis of the collected materials.

.....

(signature of co-author)

Gdańsk dnia 6.02.2023

dr hab. Robert Olek

.....
(tytuł zawodowy, imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: **"The bright and the dark sides of L-carnitine supplementation: a systematic review"**, oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to: opracowanie koncepcji, zbieranie danych oraz jakościowa analiza tematyczna zebranych materiałów.

Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez mgr Angelikę Sawicką jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład mgr Angeliki Sawickiej przy opracowaniu koncepcji, zbieraniu danych oraz jakościowej analizie zebranych materiałów.

Signed by / Podpisano przez:
Robert Olek
Date / Data: 2023-02-06 09:07

.....

(podpis współautora)