Immunseneszenz, subklinische Entzündung und Insulinresistenz im Alter – Modulation durch einen gesunden Lebensstil?

DISSERTATION

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Vorwort

Lebensstilbedingte chronische Erkrankungen, wie Diabetes mellitus Typ 2, stellen die führende Ursache für vorzeitigen Tod und langfristige Gesundheitsprobleme in industrialisierten Nationen dar. Angesichts des demographischen Wandels wird ihre Inzidenz und Prävalenz zukünftig noch weiter steigen. Subklinische Entzündungsprozesse spielen eine entscheidende Rolle bei der Entstehung und dem Fortschreiten chronischer Erkrankungen. Das Verständnis der zugrundeliegenden Mechanismen und die Umsetzung von Maßnahmen zur Beeinflussung der subklinischen Entzündung können wichtige Gesundheitsauswirkungen haben und sind somit von hohem therapeutischen Interesse.

Die vorliegende kumulative Dissertation umfasst Ergebnisse aus zwei Studien, welche in internationalen Fachzeitschriften mit peer-review Prozess veröffentlicht wurden. Die erste Studie befasst sich mit der Rolle von terminal differenzierten T-Zellen sowie Metaboliten des Kynurenin-Stoffwechselwegs im Rahmen einer gestörten Insulinsensitivität im Alter. Die zweite Studie prüft die Effekte eines zwölfwöchigen Kraft-Ausdauer-Trainings auf die Parameter, um potentielle (präventiv-)therapeutische Empfehlungen abzuleiten.

Zunächst werden die wissenschaftlichen Hintergründe des Themenkomplexes beschrieben. Im Anschluss werden die Ziele sowie Hypothesen der Studien aufgezeigt. Nach zusammenfassender Beschreibung der Methodik folgen die Publikationen, wie sie in den jeweiligen Journalen veröffentlicht wurden. Diese enthalten eine detaillierte Darstellung der verwendeten Methoden und Materialien, die experimentellen Daten sowie eine ausführliche Diskussion der jeweiligen Ergebnisse und Studienprotokolle. Nach einer zusammenfassenden Diskussion aller Ergebnisse wird die Dissertation mit einem Ausblick auf zukünftige Forschungsfragen abgeschlossen.

Publikationen

Nachfolgende Veröffentlichungen bilden die Grundlage der vorliegenden kumulativen Dissertation:

Boßlau, T. K., Wasserfurth, P., Krüger, B., Reichel, T., Palmowski, J., Nebl, J., Weyh, C., Schenk, A., Joisten, N., Stahl, F., Thoms, S., Gebhardt, K., Hahn, A., & Krüger, K. **(2021)**. Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with $CD8^+$ EMRA Cells in the Elderly. *Cells*, *10*(5), 998.

DOI: 10.3390/cells10050998

Boßlau, T. K., Wasserfurth, P., Reichel, T., Weyh, C., Palmowski, J., Nebl, J., Joisten, N., Belen, S., Schenk, A., Hahn, A., Zimmer, P., & Krüger, K. **(2023)**. 12-week combined strength and endurance exercise attenuates $CD8^+$ T-cell differentiation and affects the kynurenine pathway in the elderly: a randomized controlled trial. *Immunity & ageing : I & A*, *20*(1), 19.

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Abkürzungsverzeichnis

AHR	Arylkohlenwasserstoffrezeptor
ANCOVA	Kovarianzanalyse
BMI	Body-Mass-Index
DGE	Deutsche Gesellschaft für Ernährung
ECLIA	Elektrochemilumineszenz-Immunoassay-Methode
EMRA	Effector Memory Re-expressing CD45RA
Et al.	Et alii
HOMA	Homöostatisches Modell
HPLC	Hochleistungsflüssigkeitschromatographie
IDO	Indolamin-2/3-Dioxygenase
IL	Interleukin
KA	Kynurensäure
KMO	Kynurenin-3-Monooxygenase
KYN	Kynurenin
MS	Massenspektrometer
NMDA	N-Methyl-d-Aspartat
PCR	Polymerasekettenreaktion
QA	Chinolinsäure
TNF	Tumor-Nekrose-Faktor
TRP	Tryptophan
XA	Xanthurensäure
ZNS	Zentrales Nervensystem

Zusammenfassung

CD8⁺ EMRA T-Zellen akkumulieren im alternden Organismus und produzieren eine beträchtliche Menge proinflammatorischer Zytokine, womit sie eine entscheidende Rolle in der Entstehung und Aufrechterhaltung subklinischer Entzündungsprozesse spielen könnten. Metabolite des Kynurenin-Stoffwechselwegs scheinen diese chronischen Entzündungsprozesse zu unterhalten, da sie einerseits im Rahmen eines proinflammatorischen Milieus vermehrt synthetisiert werden und andererseits eine Schlüsselrolle bei der T-Zell-Differenzierung und der Induktion von Entzündungsmediatoren spielen. Subklinische Entzündung zeigt sich insbesondere in übergewichtigen älteren Menschen aggraviert und bedingt die Entstehung chronischer Erkrankungen wie Diabetes mellitus Typ 2.

Das Ziel dieser kumulativen Dissertation war es zunächst im Rahmen einer Querschnittstudie (Boßlau et al., 2021) Zusammenhänge zwischen (übergewichtsassoziierter) subklinischer Entzündung, Veränderungen im Kynurenin-Stoffwechselweg und der Akkumulation terminal differenzierter CD8⁺ EMRA T-Zellen im alternden Menschen zu untersuchen sowie ihre klinische Relevanz hinsichtlich der Entstehung einer gestörten Insulinsensitivität zu überprüfen. In der darauf aufbauenden Längsschnittstudie (Boßlau et al., 2023) sollte anschließend der Einfluss eines zwölfwöchigen Kraft-Ausdauertrainings auf die Parameter analysiert werden.

134 untrainierte Proband*innen im Alter zwischen 50 und 70 Jahren wurden in die Querschnittstudie (Boßlau et al., 2021) eingeschlossen. Ihre anthropometrischen Daten wurden erfasst sowie die prozentuale Verteilung der Subtypen der CD8⁺ T-Zell-Population aus dem peripheren Blut mittels Durchflusszytometrie bestimmt. Außerdem wurden die Konzentrationen der Metabolite des Kynurenin-Stoffwechselwegs mittels einer Hochleistungsflüssigkeitschromatographie (HPLC), gekoppelt mit einem Massenspektrometer (MS), quantifiziert und die Insulinsensitivität anhand des homöostatischen Modells (HOMA) analysiert. Die statistische Auswertung ergab eine signifikante Assoziation zwischen einem erhöhten Anteil zirkulierender CD8⁺ EMRA T-Zellen und einer verminderten Insulinsensitivität. Außerdem korrelierten höhere Konzentrationen von Kynurenin im peripheren Blut mit einer verminderten Insulinsensitivität. Darüber hinaus wurden die CD8⁺ EMRA T-Zellen als auch die Kynurenin-Konzentration als Mediatorvariablen in der übergewichtsinduzierten Reduktion der Insulinsensitivität ermittelt. Dies wurde durch die Tatsache gestützt, dass insbesondere die Subgruppe der Probanden mit einem als fettleibig eingestuften BMI (> 30 kg/m²) einen höheren Anteil an CD8⁺ EMRA T-Zellen sowie erhöhte Kynurenin-Konzentrationen aufwies.

Im Anschluss an die Querschnittstudie wurden die Proband*innen im Rahmen der Längsschnittstudie (Boßlau et al., 2023) randomisiert für 12 Wochen auf eine Kontroll- (n=25) oder Interventionsgruppe (n=53) verteilt. Die Proband*innen der Interventionsgruppe absolvierten zweimal wöchentlich ein

kombiniertes Kraft-Ausdauer-Training in kooperierenden Fitnesseinrichtungen unter standardisierten Bedingungen. Außerdem wurde die Interventionsgruppe nochmals unterteilt. Eine Hälfte absolvierte lediglich das Trainingsprogramm (n=29), während die andere Hälfte zusätzlich eine Ernährungsintervention, gemäß Leitlinie der Deutschen Gesellschaft für Ernährung (DGE), erhielt (n=24). Im Anschluss an die Interventionsphase wurden die Zielparameter analog zur Querschnittstudie erhoben und hinsichtlich Zeit- und Gruppenunterschieden statistisch überprüft. Es konnte gezeigt werden, dass sich das 12-wöchige kombinierte Kraft-Ausdauertraining positiv auf den BMI sowie die Insulinsensitivität auswirkte und die progressive Akkumulation von $CD8^+$ EMRA-Zellen, verglichen mit der Kontrollgruppe, reduzierte. Darüber hinaus beeinflusste das Training den Kynurenin-Stoffwechselweg, indem es die Konzentration der neuroprotektiven Kynurensäure im peripheren Blut erhöhte. Die begleitende Ernährungsintervention erbrachte keinen zusätzlichen Benefit.

Die Erkenntnis, dass $CD8^+$ EMRA T-Zellen sowie Metabolite des Kynurenin-Stoffwechselwegs einen Beitrag in der Pathophysiologie einer reduzierten Insulinsensitivität spielen könnten, ist aus zweierlei Gründen wichtig. Zum einen könnten Analysen dieser Parameter einen prädiktiven Marker für eine beginnende Insulinresistenz darstellen. Zum anderen wäre es wichtig, potentielle molekulare Wechselwirkungen zu identifizieren, wie $CD8^+$ EMRA T-Zellen oder Kynurenin-Metabolite mit insulinsensitiven Organen interagieren. Somit könnten möglicherweise neue Targets in der Behandlung und Prävention des Diabetes mellitus Typ 2 aufgedeckt werden. Ein kombiniertes Kraft-Ausdauer-Training erscheint geeignet, um den Kynurenin-Stoffwechselweg zu rebalancieren und die progressive Zunahme des $CD8^+$ EMRA T-Zell-Pools im Alter zu vermindern. Es bedarf jedoch weiterer Forschungsarbeiten, welche die Effektivität unterschiedlicher Trainingsregime miteinander vergleichen.

Abstract

$CD8^+$ EMRA T cells accumulate in the aging organism and produce a considerable amount of proinflammatory cytokines, thus they might play a crucial role in the development and maintenance of subclinical inflammatory processes. Metabolites of the kynurenine pathway appear to maintain these chronic inflammatory processes, as they are synthesized more abundantly in the context of a proinflammatory milieu, on the one hand, and play a key role in T-cell differentiation and induction of inflammatory mediators, on the other. Subclinical inflammation is particularly aggravated in obese elderly and conditions the development of chronic diseases such as type 2 diabetes mellitus.

The aim of this cumulative dissertation was first to investigate in a cross-sectional study (Boßlau et al., 2021) correlations between (overweight-associated) subclinical inflammation, alterations in the kynurenine metabolic pathway and the accumulation of terminally differentiated $CD8^+$ EMRA T-cells in aging humans and to examine their clinical relevance with regard to the development of impaired insulin sensitivity. The subsequent longitudinal study (Boßlau et al., 2023) was designed to analyze the influence of a twelve-week strength-endurance training on the parameters.

134 untrained subjects aged between 50 and 70 years were included in the cross-sectional study (Boßlau et al., 2021). Their anthropometric data were recorded and the percentage distribution of the subtypes of the $CD8^+$ T-cell population was determined from peripheral blood by flow cytometry. In addition, the concentrations of metabolites of the kynurenine pathway were quantified by high-performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS), and insulin sensitivity was analyzed using the homeostatic model (HOMA). Statistical analysis revealed a significant association between increased levels of circulating $CD8^+$ EMRA T-cells and decreased insulin sensitivity. In addition, higher concentrations of kynurenine in peripheral blood correlated with decreased insulin sensitivity. Moreover, $CD8^+$ EMRA T-cells as well as kynurenine concentration were identified as mediator variables in the obesity-induced reduction of insulin sensitivity. This was supported by the fact that in particular the subgroup of subjects with a BMI classified as obese (> 30 kg/m^2) had a higher proportion of $CD8^+$ EMRA T-cells as well as increased kynurenine concentrations.

Following the cross-sectional study, subjects were randomly assigned to a control (n=25) or intervention (n=53) group for 12 weeks in the longitudinal study (Boßlau et al., 2023). Subjects in the intervention group completed combined strength-endurance training twice weekly at cooperating fitness facilities under standardized conditions. In addition, the intervention group was subdivided again. One half completed only the training program (n=29), while the other half additionally received a nutrition intervention according to the guidelines of the German Nutrition Society (DGE) (n=24). Following the intervention phase, the target parameters were surveyed analogously to the cross-

sectional study and statistically tested with regard to time and group differences. It was shown that the 12-week combined strength-endurance training had a positive effect on BMI as well as insulin sensitivity and reduced the progressive accumulation of $CD8^+$ EMRA T-cells, compared to the control group. In addition, exercise training affected the kynurenine metabolic pathway by increasing the concentration of neuroprotective kynurenic acid in peripheral blood. The concomitant nutritional intervention provided no additional benefit.

The finding that $CD8^+$ EMRA T cells as well as metabolites of the kynurenine pathway may play a role in the pathophysiology of reduced insulin sensitivity is important for two reasons. First, analyses of these parameters could provide a predictive marker for the onset of insulin resistance. Second, it would be important to identify potential molecular interactions of how $CD8^+$ EMRA T cells or kynurenine metabolites interact with insulin-sensitive organs. Thus, new targets in the treatment and prevention of type 2 diabetes mellitus could potentially be uncovered. Combined strength-endurance training appears to be suitable to rebalance the kynurenine metabolic pathway and to reduce the progressive increase of the $CD8^+$ EMRA T-cell pool in aging. However, further research comparing the effectiveness of different training regimens is needed.

Wissenschaftlicher Hintergrund

Das adaptive Immunsystem des alternden Menschen unterliegt starken Umstrukturierungs- und Anpassungsprozessen. Neben einer veränderten T-Zell-Funktionalität kommt es zu einer abgewandelten Zusammensetzung der unterschiedlichen T-Zell-Subtypen. Während die Anzahl an zytotoxischen $CD8^+$ Zellen im peripheren Blut zunimmt, kommt es zu einer Abnahme der $CD4^+$ T-Helfer-Zellen. Es resultiert ein vermindertes $CD4^+/CD8^+$ T-Zell-Verhältnis, welches ein Hauptkennzeichen der Immunseneszenz darstellt (Makinodan, 1998). Innerhalb beider Zell-Populationen kommt es außerdem zur progressiven Abnahme der T-Zellen mit naivem Phänotyp. Diese ist das Ergebnis der Thymus-Involution, welche nur teilweise durch die Proliferation von existierenden naiven T-Zellen in der Körperperipherie kompensiert werden kann (Goronzy et al., 2015; Jacomet et al., 2015). Funktionell resultiert ein vermindertes T-Zell-Rezeptor-Repertoire und letztlich eine eingeschränkte Reaktivität gegenüber neu dargebotenen Pathogenen. Im Gegenzug akkumulieren terminal differenzierte (seneszente) T-Zell-Subtypen, was als Resultat wiederholter T-Zell-Stimulation, beispielsweise durch chronische Entzündungen und latente Virusinfektionen, zu sehen ist (Goronzy und Weyand, 2017).

$CD8^+$ EMRA T-Zellen (effector memory T-cells re-expressing CD45RA) sind eine heterogene Zellgruppe, welche anhand ihres Oberflächenexpressionsprofils $CCR7^-/CD45RA^+$ klassifiziert wird (Goronzy & Weyand, 2017). $CD8^+$ EMRA T-Zellen sind mit Zellseneszenz assoziiert, da sie eine reduzierte Proliferationskapazität aufweisen (Geginat et al., 2003), eine erhöhte Aktivierung von Seneszenz-Signalwegen zeigen (Henson et al., 2012) und mit Seneszenzmarkern wie KLRG1 und CD57 assoziiert sind (Koch et al., 2008). Sie sind jedoch nicht funktionell inaktiv, da sie in entzündetes Gewebe einwandern können (Faint et al., 2001; Hertoghs et al., 2010) und eine beträchtliche Menge an entzündungsfördernden Zytokinen produzieren (Callender et al., 2018). Somit steht die beschleunigte T-Zell-Differenzierung möglicherweise in Zusammenhang mit der Entwicklung und Aufrechterhaltung einer chronischen, subklinischen Entzündung im Alter.

Interessanterweise steht der wichtigste Abbauweg von Tryptophan (TRP), der Kynureninweg, in enger Wechselbeziehung mit dem Immunsystem. Einerseits erhöhen lokale und systemische Entzündungsreize die Expression des Schlüsselenzyms Indolamin-2/3-Dioxygenase (IDO) in verschiedenen Geweben, wie z. B. in peripheren mononukleären Blutzellen (Platten et al., 2019; Jones et al., 2015). Andererseits haben sowohl Kynurenin (KYN) als auch einer seiner Metaboliten, die Kynurensäure (KA), selbst immunmodulatorische Wirkungen und können den Arylkohlenwasserstoffrezeptor (AHR) aktivieren, der eine Schlüsselrolle bei der T-Zell-Differenzierung und der Induktion von Entzündungsmediatoren spielt (Mezrich et al., 2010; DiNatale et al., 2010).

Ein direkter Zusammenhang zwischen der Akkumulation von $CD8^+$ EMRA T-Zellen und Veränderungen im Kynurenin-Stoffwechselweg im Rahmen subklinischer Entzündungsprozesse wurde noch nicht überprüft. Ebenso ist die klinische Relevanz der genannten Faktoren nicht abschließend geklärt.

Bekannt ist, dass subklinische Entzündungsprozesse eine entscheidende Rolle im Rahmen altersbedingter Multimorbidität spielen (Franceschi et al., 2007). Auch wurden $CD8^+$ EMRA T-Zellen bereits als Prädiktoren für die kardiovaskuläre Mortalität in älteren Menschen identifiziert (Spyridopoulos et al., 2016), da sie unter anderem eine zytotoxische Aktivität gegenüber Endothel-Plaques zeigen (Nakajima et al., 2002). Die Metabolite des Kynurenin Stoffwechselwegs scheinen hingegen eine modulierende Rolle in der Entstehung neurodegenerativer Erkrankungen zu spielen. Kynurenin selbst kann entweder mittels Kynurenin-Aminotransferasen 1-4 (KAT 1-4) in die neuroprotektive Kynurensäure (KA) oder mittels Kynurenin-3-Monooxygenase (KMO) in Chinolinsäure (QA) umgewandelt werden, die eng mit neuronaler Exzitotoxizität verbunden ist. Im ZNS wirken Kynurensäure und Chinolinsäure als Antagonist bzw. Agonist der N-Methyl-d-Aspartat (NMDA)-Rezeptoraktivierung und vermitteln so entweder neuronalen Schutz oder Exzitotoxizität (Joisten et al., 2020).

Die Rolle von $CD8^+$ EMRA T-Zellen und der Kynurenin-Metabolite in der Entstehung metabolischer Erkrankungen ist im Gegensatz dazu noch nicht abschließend geklärt. Insbesondere übergewichtige Menschen entwickeln im Alter eine Insulinresistenz, wobei neben einer Dysregulation von Adipokinen und Hormonen und einer mitochondrialen Dysfunktion vor allem eine unterschwellige chronische Entzündung als mögliche Mechanismen diskutiert werden (Ahmed et al., 2021). Somit erscheint ein Einfluss der exzessiv Zytokin-produzierenden $CD8^+$ EMRA T-Zellen in der Entstehung eines übergewichtsinduzierten Typ 2 Diabetes mellitus denkbar.

Angesichts der hohen klinischen und gesundheitsökonomischen Relevanz des Typ 2 Diabetes mellitus ist es von zentralem Interesse, tiefergehende Einblicke in die Pathophysiologie der gestörten Insulinsensitivität zu gewinnen. Darüber hinaus gilt es, wirksame Therapieschemata zu etablieren, um einer beginnenden Insulinresistenz im Alter effektiv zu begegnen.

Zielsetzung und Hypothesen

Aufbauend auf dem aktuellen Forschungsstand war es das Ziel der Querschnittstudie von Boßlau et al. (2021), Zusammenhänge zwischen (übergewichtsinduzierter) subklinischer Entzündung, Veränderungen im Kynurenin-Stoffwechselweg und der Akkumulation terminal differenzierter $CD8^+$ EMRA T-Zellen im alternden Menschen zu untersuchen. Zusätzlich sollte die klinische Relevanz der $CD8^+$ EMRA T-Zell-Population sowie der Kynurenin-Metabolite hinsichtlich der Entstehung und Aufrechterhaltung einer gestörten Insulinsensitivität überprüft werden.

In der darauf aufbauenden Längsschnittstudie (Boßlau et al., 2023) wurde untersucht, ob die durch Boßlau et al. (2021) aufgezeigten pathologischen Phänomene und Zusammenhänge reversibel sind und durch ein zwölfwöchiges Kraft-Ausdauertrainings mit oder ohne begleitende Ernährungsintervention beeinflusst werden können.

Entsprechend unserer Studienziele wurden folgende ***Hypothesen*** untersucht:

Boßlau et al. (2021)

Die Anzahl terminal differenzierter $CD8^+$ EMRA T-Zellen ist im peripheren Blut übergewichtiger älterer Personen erhöht. Außerdem sind höhere Level an $CD8^+$ EMRA T-Zellen mit einem aggravierten subklinischen Entzündungsgeschehen, einem dysregulierten Kynurenin-Stoffwechselweg sowie einer erniedrigten Insulinsensitivität assoziiert.

Boßlau et al. (2023)

Ein zwölfwöchiges Kraft-Ausdauertraining (alleine oder in Kombination mit einer Ernährungsintervention) reduziert die Population der $CD8^+$ EMRA T-Zellen im peripheren Blut, rebalanciert den Kynurenin-Stoffwechselweg und erhöht die Insulinsensitivität im alternden Menschen.

Studiendesign und Methodik

Die ethische Legitimation zur Durchführung der Studien im Rahmen der vorliegenden kumulativen Dissertation wurde durch die Ethikkommission der Niedersächsischen Ärztekammer mit Sitz in Hannover erteilt. Zwischen Januar und August 2019 erfolgten die Datenerhebungen und die Studienintervention an der Leibniz Universität Hannover. Basierend auf den Ergebnissen der Querschnittstudie (Boßlau et al., 2021) wurden die Studienziele und Hypothesen der Längsschnittstudie (Boßlau et al., 2023) abgeleitet. Eine Übersicht über den zeitlichen Ablauf der Studien im Rahmen der vorliegenden kumulativen Dissertation ist in Abbildung 1 graphisch dargestellt.

Boßlau et al. (2021)

284 Personen (weiblich/männlich) im Alter zwischen 50 und 70 Jahren wurden gescreent, von denen 134 Personen die Einschlusskriterien der Studie (s. Originalarbeit) erfüllten und als Proband*innen in die Studie eingeschlossen wurden. Sie wurden über die Studienteilnahme aufgeklärt und unterschrieben eine Einverständniserklärung. Alle eingeschlossenen Studienteilnehmer*innen waren während der vorangegangenen zwei Jahre sportlich inaktiv und wiesen eine geringe körperliche Alltagsaktivität auf. Dies wurde mittels Fragebögen kontrolliert (s. Originalarbeit). Ihre anthropometrischen Daten wurden erhoben und es erfolgte eine venöse Nüchternblutentnahme. Das Blut wurde mittels verschiedener laborchemischer Methoden hinsichtlich der relevanten Parameter analysiert. Die Nüchternglukose wurde photometrisch analysiert. Für die Bestimmung von Insulin wurde die Elektrochemilumineszenz-Immunoassay-Methode (ECLIA) verwendet. Die Insulinsensitivität wurde anhand des homöostatischen Modells (HOMA) bewertet: HOMA-IR = Nüchterninsulin (µU/ml) x Nüchternblutglukose (mg/dl) / 405. Die prozentuale Verteilung der Subtypen der $CD8^+$ Zellpopulation wurde mittels Durchflusszytometrie bestimmt. Die Konzentrationen der Metabolite des Kynurenin Stoffwechselwegs wurden mittels einer Hochleistungsflüssigkeitschromatographie (HPLC), gekoppelt mit einem Massenspektrometer (MS), analysiert (s. Originalarbeiten). Die statistische Auswertung erfolgte im ersten Schritt mittels einer ANCOVA, für welche die Proband*innen anhand ihres BMI in drei Gruppen unterteilt wurden (Normalgewicht: 18,5-24,9 kg/m^2, Übergewicht: 25-29,9 kg/m^2, Fettleibigkeit: >30 kg/m^2) und hinsichtlich Gruppenunterschieden analysiert wurden. Im zweiten Schritt erfolgten multiple Regressionsanalysen. Hierbei wurde geprüft, ob die Menge peripherer $CD8^+$ EMRA T-Zellen und/oder die Konzentration der Kynurenin Metaboliten mögliche Prädiktoren für eine abnorme Insulinsensitivität (erhöhter HOMA-IR) sind. Im dritten Schritt wurde eine Mediationsanalyse berechnet, mittels welcher untersucht wurde, ob $CD8^+$ EMRA T-Zellen und/oder Kynurenin Metabolite potentielle Mediatoren einer übergewichtsinduzierten Insulinresistenz sind (s. Originalarbeit).

Boßlau et al. (2023)

Die Proband*innen aus der Querschnittstudie von Boßlau et al. (2021) wurden mittels Fahrradergometer-Untersuchung unter EKG-Kontrolle hinsichtlich ihrer Sporttauglichkeit überprüft und anschließend randomisiert auf eine Kontroll- oder Interventionsgruppe verteilt. Die Proband*innen der Kontrollgruppe (n=25) wurden dazu angehalten, ihren (bewegungsarmen) Alltag unverändert über den Interventionszeitraum von zwölf Wochen beizubehalten. Die Proband*innen der Interventionsgruppe (n=53) absolvierten zweimal wöchentlich ein 60-minütiges kombiniertes Kraft-Ausdauer-Training in kooperierenden Fitnesseinrichtungen unter standardisierten Bedingungen (s. Originalarbeit). Außerdem wurde die Interventionsgruppe nochmals unterteilt. Eine Hälfte absolvierte lediglich das Trainingsprogramm (n=29), während die andere Hälfte zusätzlich eine Ernährungsintervention gemäß Leitlinie der Deutschen Gesellschaft für Ernährung (DGE) erhielt (n=24) (s. Originalarbeit). Die Einhaltung der Vorgaben bezüglich der Studienintervention wurden mittels Telefongesprächen in zweiwöchigem Abstand sowie einem Trainings- und Ernährungstagebuch durch die Studienleitung kontrolliert. Nach der zwölfwöchigen Intervention wurden die Proband*innen erneut im Studienzentrum vorstellig. Es wurde ihnen venöses Blut entnommen, aus welchem analog zur Datenerhebung der Querschnittstudie die relevanten Parameter analysiert wurden. Die statistische Auswertung erfolgte mittels einer ANCOVA mit Messwiederholung. Hierbei wurden die Effekte der zwölfwöchigen Studienintervention auf den HOMA-IR-Wert, die peripheren $CD8^+$ EMRA T-Zellen sowie die Konzentrationen der Kynurenin-Metabolite analysiert und hinsichtlich Zeit- und Gruppenunterschieden überprüft (s. Originalarbeit)

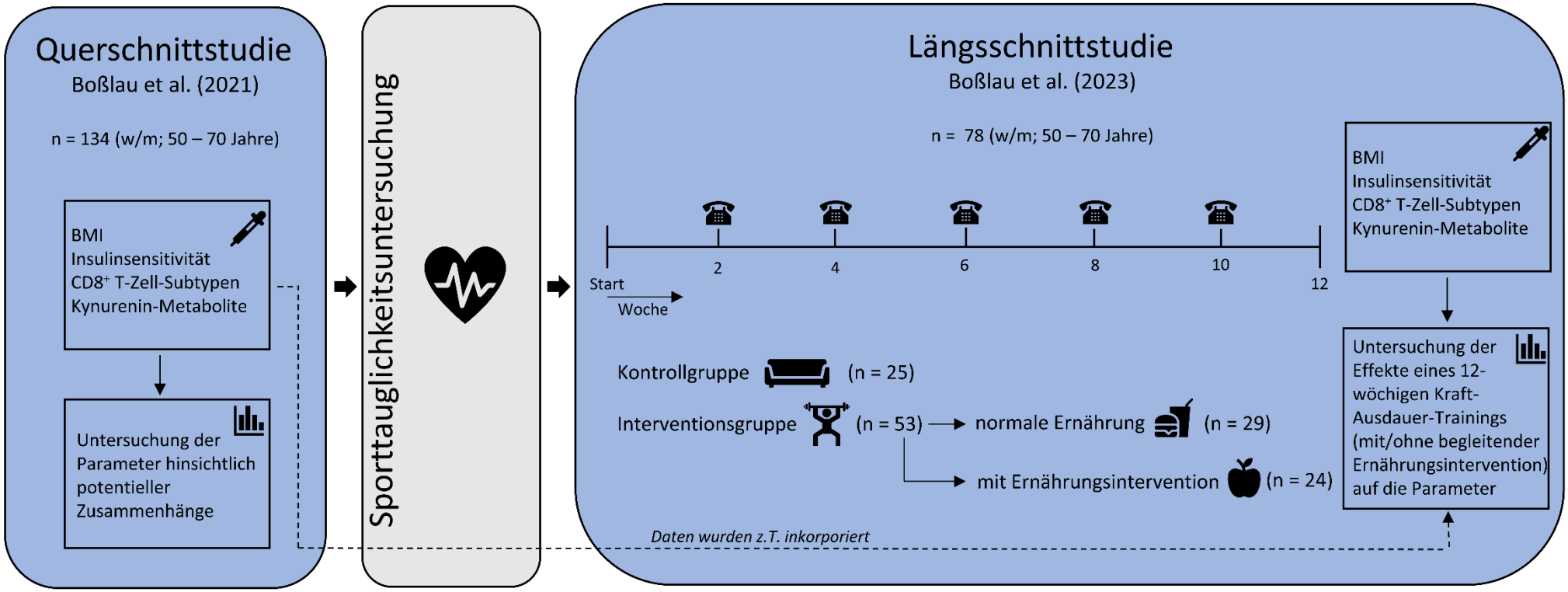

Abbildung 1. Übersicht über den zeitlichen Ablauf der Studien im Rahmen der kumulativen Dissertation.

Publikationen

Die Ergebnisse dieser kumulativen Dissertation wurden in zwei Originalarbeiten veröffentlicht, die in den folgenden Unterkapiteln abgebildet werden.

Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with $CD8^+$ EMRA Cells in the Elderly

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Abdominal Obesity-Related Disturbance of Insulin Sensitivity Is Associated with CD8$^+$ EMRA Cells in the Elderly

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Abstract: Aging and overweight increase the risk of developing type 2 diabetes mellitus. In this cross-sectional study, we aimed to investigate the potential mediating role of T-EMRA cells and inflammatory markers in the development of a decreased insulin sensitivity. A total of 134 healthy older volunteers were recruited (age 59.2 ($_{SD}$ 5.6) years). T cell subpopulations were analyzed by flow cytometry. Furthermore, body composition, HOMA-IR, plasma tryptophan (Trp) metabolites, as well as cytokines and adipokines were determined. Using subgroup and covariance analyses, the influence of BMI on the parameters was evaluated. Moreover, correlation, multiple regression, and mediation analyses were performed. In the subgroup of participants with obesity, an increased proportion of CD8+EMRA cells and elevated concentrations of plasma kynurenine (KYN) were found compared to the lower-weight subgroups. Linear regression analysis revealed that an elevated HOMA-IR could be predicted by a higher proportion of CD8+EMRA cells and KYN levels. A mediation analysis showed a robust indirect effect of the Waist-to-hip ratio on HOMA-IR mediated by CD8+EMRA cells. Thus, the deleterious effects of abdominal obesity on glucose metabolism might be mediated by CD8+EMRA cells in the elderly. Longitudinal studies should validate this assumption and analyze the suitability of CD8+EMRA cells as early predictors of incipient prediabetes.

Keywords: elderly; obesity; T-EMRA cells; kynurenine pathway; insulin resistance

1. Introduction

During aging, the adaptive immune system undergoes a remodeling process characterized by altered T cell subtype composition and changes in major T cell functions. While the number of CD8+ cells generally increases, the proportion of CD4+ cells slightly decreases, resulting in a reduced CD4+/CD8+ T cell ratio [1]. Within both T cell populations, the relative proportion of cells with a naïve phenotype progressively decreases. Homeostatic proliferation of existing naive T cells in the periphery partially compensates for the reduced number of naive T cells [2]. Functionally, the T cell receptor (TCR) repertoire is diminished, resulting in a limited response against emerging pathogens [3]. Accompanying the exhaustion in the naïve T cell pool, an accumulation of terminally differentiated cells occurs. This

is caused by repeated T cell stimulation, e.g., by latent viruses, such as cytomegalovirus (CMV) infection, or by a state of chronic inflammation [4,5].

T effector memory re-expressing CD45RA cells (T-EMRA cells) represent a heterogeneous group of terminally differentiated cells characterized by their surface expression profile CCR7−/CD45RA+. These cells can be further subdivided regarding their expression of CD57 into a functionally viable "young" fraction (CD57−) with proliferative and antiviral capacity and a senescent fraction (CD57+) with extensive functional quiescence that is proliferation incompetent in response to antigen-specific stimulation and susceptible to apoptosis upon T cell activation [6,7]. With age, the number of T-EMRA cells increases, which can be interpreted as a hallmark of immunosenescence [8]. T-EMRA cells are suggested to contribute to various pathological processes by exacerbating inflammation and exhibiting atypical cytotoxic activity towards endogenous structures, such as the vascular endothelium [9,10]. Moreover, T-EMRA cells have been identified as predictors of cardiovascular mortality in the elderly and as risk factors of graft dysfunction in kidney transplant recipients [11,12].

Accelerated T cell differentiation is bi-directionally related to the development of a chronic low-grade inflammation, termed "inflammaging" [13,14]. On the one hand, chronic inflammation is a driver of T cell differentiation by constantly activating existing immune cells. On the other hand, highly differentiated T-EMRA cells represent a pro-inflammatory phenotype and a source of inflammatory factors [10,15]. The concentration of various inflammatory cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α), progressively increases during aging, which contributes to age-associated morbidity and mortality [16]. Overweight superimposed on aging accelerates low-grade inflammation, which might play a leading role not only in the pathogenesis of cardiovascular but also of metabolic diseases, such as diabetes type 2. Accordingly, patients with prediabetes or diabetes show significantly lower insulin sensitivity, increased proportions of terminally differentiated T cells, and higher levels of inflammatory cytokines compared to metabolically healthy individuals [17]. Besides glucose metabolism, the kynurenine (KYN) pathway as the major route of tryptophan degradation represents another popular example of the interplay between metabolism and inflammation. The initial and rate-limiting enzyme indoleamine 2-3-dioxygenase (IDO1) strongly increases in response to elevated proinflammatory cytokine levels [18], leading to a stimulation of the KYN pathway that has been demonstrated in several inflammation-associated pathologies [19,20].

Based on these findings, we speculate that T-EMRA cells, as well as parameters of the kynurenine pathway, may be related to a disturbance of insulin sensitivity in obesity and that this is detectable even in clinically healthy elderly subjects. We explicitly addressed the question: Do increased proportions of T-EMRA cells or activation of the KYN pathway favor an early dysregulation of insulin sensitivity in subjects aged 50–70 without any previous chronic illness? Furthermore, we asked whether T-EMRA cells and changes in Trp metabolites are potential mediators between abdominal obesity and disturbed insulin sensitivity.

2. Materials and Methods

The current work is based on a recent study of our group [21]. Blood samples were obtained from the same subjects. Data regarding anthropometric and physiological characteristics have been incorporated from this publication.

2.1. Study Participants

We enrolled 134 men and women from the general population in Hannover, Germany, between August 2018 and March 2019. Recruitment was conducted widely distributed via advertisements in local newspapers and public notices. The inclusion and exclusion criteria for each participant were determined using a formalized questionnaire. Inclusion criteria for participation were age $\geq$50 and $\leq$70 years, no regular exercise training aside from the daily activities for at least 2 years, and stable body weight ($\pm$5 kg) for at least 6 months.

Exclusion criteria were defined as cardiovascular diseases (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure, cardiac arrhythmia), type 1 and 2 diabetes, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g., ulcers, Crohn's disease, pancreatic insufficiency), immunological diseases (e.g., autoimmune diseases), intake of immunosuppressive drugs or laxatives, intake of supplements containing n3-FAs, smoking, alcohol, drug and/or medicine dependency, pregnancy or lactation, retraction of the consent by the subject, concurrent participation in another clinical study, and participation in a study in the last 30 days. Ethical approval was provided by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany). Following the guidelines of the Declaration of Helsinki, written informed consent was obtained from all participants before participation in the study. Study design and the procedure for the different analyses are shown in Figure 1a.

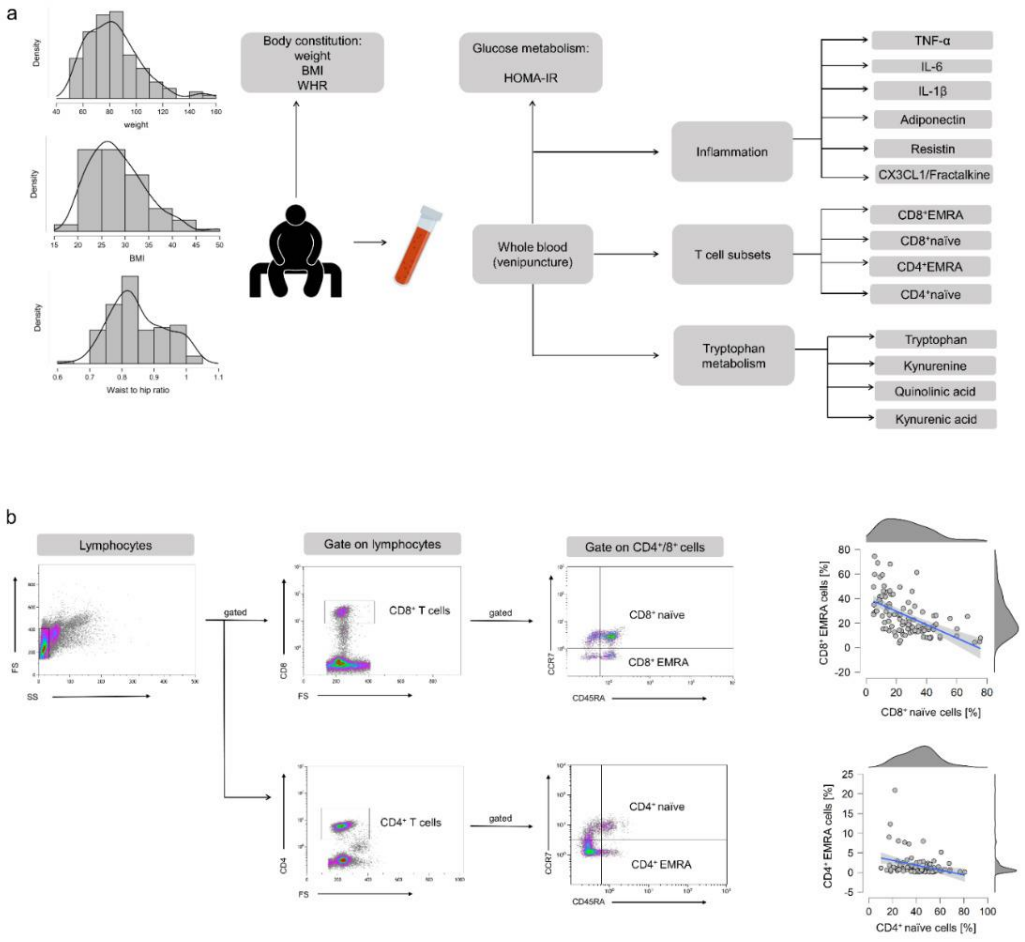

Figure 1. (**a**) Presentation of the study design and the course of analyses. (**b**) Flow cytometric analysis of T cell subpopulations. Lymphocytes were gated using the forward- and sideward scatter. Populations were subdivided into CD4+ T-helper cells and CD8+ cytotoxic T cells. Both cell types were further differentiated into naïve (CD45RA+/CCR7+), central memory (CD45RA−/CCR7+), effector memory (CD45RA−/CCR7−), and effector memory re-expressing CD45RA (CD45RA+/CCR7−) cells. Associations between naïve and EMRA T cells.

2.2. Body Weight and Body Composition

Waist and hip circumferences were measured using a measuring tape in a standing position. Waist-to-hip-ratio (WHR) was calculated. Height was measured with a stadiometer (seca GmbH & Co. KG, Hamburg, Germany) and body weight was measured on a digital scale to the nearest 0.1 kg (seca GmbH & Co. KG, Hamburg, Germany). BMI was calculated by the ratio of weight to the squared height. Body composition was analyzed to a nearest of 0.1 kg using a bioelectrical impedance analyzer (BIA) (Nutriguard M, Data Input Company, Darmstadt, Germany) and the software NutriPlus© 5.4.1 (Data Input Company, Darmstadt, Germany). For the measurements, the participants arrived rested and after an overnight fast ($\geq$10 h). They were instructed to lay down on a stretcher and rest for at least five minutes to ensure a balanced distribution of body fluids before the measurement. During the measurement, participants were instructed to lay still and in a relaxed position with the limbs slightly bend from the torso. All measurements were carried out by the same study personnel.

2.3. Blood Sampling

Blood samples were taken in the morning following a resting period and after an overnight fast ($\geq$10 h) using serum, EDTA, and NaF Glucose tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). Blood was either processed directly or stored at −80 °C in the form of serum and plasma for later analysis.

2.4. Analysis of Glucose Metabolism and Insulin Resistance

Analysis of markers of glucose metabolism was performed by a certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., in Hannover, Germany). Fasting glucose was analyzed photometrically (Beckman Coulter GmbH, Krefeld, Germany). HbA1c was analyzed using high-performance liquid chromatography (HPLC) (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). For the determination of insulin, the electrochemiluminescence immunoassay method (ECLIA) using cobas 801e (Roche Diagnostics GmbH, Mannheim, Germany) was applied. Insulin sensitivity was evaluated using the homeostatic model assessment (HOMA): HOMA-IR = fasting insulin (μU/mL) × fasting blood glucose (mg/dL)/405 [22].

2.5. Analysis of T Cell Subpopulations

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from fresh EDTA whole blood by ficoll density gradient centrifugation. After PBMCs were washed, 1×10^6 cells in 100 μL PBS were stained for 20 min in the dark with 5 μL of different fluorescence-coupled antibodies, respectively (BioLegend Inc., San Diego, CA, USA & ImmunoTools GmbH, Hamburg, Germany). The antibody cocktails were composed as follows. Analysis of CD4+ subtypes: anti-CD4-FITC (clone MEM-241), anti-CD197(CCR7)-PE (clone G043H7), anti-CD45RA-PerCP (clone HI100); Analysis of CD8+ subtypes: anti-CD8-FITC (clone MEM-31), anti-CD197(CCR7)-PE (clone G043H7), anti-CD45RA-PerCP (clone HI100). Percentages of naïve (CD45RA+/CCR7+), central memory (CD45RA-/CCR7+), effector memory (CD45RA-/CCR7−), and EMRA (CD45RA+/CCR7−) CD4+ and CD8+ T cells were quantified by flow cytometer FC 500 using the CXP software (Beckman Coulter, Irving, TX, USA). The gating strategy is shown in Figure 1b and was implemented according to Koch et al. [8]. Besides, CD4+/CD8+ T cell ratio was determined.

2.6. Analysis of Tryptophan Metabolites

Trp and its metabolites KYN, quinolinic acid (QA), and kynurenic acid (KA) were measured via high-performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS). Serum was stored in 50 μL aliquots at −80 °C until analysis. The analysis was performed on a Waters ACQUITY UPLC® system equipped with an ACQUITY UPLC® HSS T3 analytical column coupled to a Xevo® TQ-XS triple quadrupole mass spectrometer (Waters, Eschborn, Germany) as described elsewhere [20].

2.7. Analysis of Plasma Cytokines

Plasma levels of IL-1β, IL1ra, IL-6, IL-15, TNF-α, CX3CL1/fractalkine, adiponectin, and resistin were determined using a human Magnetic Luminex Assay (Bio-Techne, Abingdon, Oxon, UK) and a Magpix Luminex instrument (Luminex Corp, Austin, TX, USA) according to the manufacturer's instructions.

2.8. Analysis of Cytomegalovirus (CMV) Serostatus

Serum anti-CMV immunoglobulin G (IgG) antibodies were detected using a semiquantitative sandwich enzyme-linked immunosorbent assay (ELISA-Viditest anti-CMV IgG, VIDIA, Czech Republic). The procedure followed the manufacturer's instructions. Endpoint optical density was measured by the Emax Plus ELISA reader (Molecular Devices, Sunnyvale, CA, USA).

2.9. Statistical Analysis

First, descriptive statistics were performed for all measured variables and results are given below as mean ± SD. In the next step, we tested all observed variables regarding their distribution features. As most of them did not meet the criterion of a normal distribution, we used Spearman's rank correlation to determine possible relationships between measurements of body composition (as indicated by BMI and WHR), metabolism (as indicated by HOMA-IR and Trp metabolites), CD4+ and CD8+EMRA cells, as well as pro-inflammatory cytokines (e.g., IL-6, TNF-α) and adipokines (adinopectine, resistin) for exploarory purposes. Missing data were addressed using pairwise deletion.

As a preliminary analysis, we further divided our study collective into three BMI subgroups: group 1: 18.5–24.9 kg/m^2 (normal weight); group 2: 25–29.9 kg/m^2 (overweight); group 3: >30 kg/m^2 (obesity). We calculated an ANCOVA for the dependent variable CD8+EMRA cells and Trp metabolite KYN and the independent variable BMI group using age as a covariate to assess the impact of a BMI classified within the obese range (>30) on the proportion of T-EMRA cells and enhanced Trp metabolites.

In the next step, we analyzed the impact of obesity, proportion of T-EMRA cells, and levels of Trp metabolites as potential predictors of abnormal glucose metabolism (indicated by HOMA-IR) using multiple regression analyses. We added several product terms to the model to test for an interaction between abdominal obesity, immune aging, and Trp metabolites. Before multiple regression analysis, we z-standardized all variables. To identify a possible mechanism that underlies the observed relationship between abdominal obesity and disturbed glucose metabolism, we tested the mediation of this relationship by assessing the factors of immune aging, as well as the concentration of tryptophan metabolites. More precisely, we calculated a mediation analysis using bootstrapping with 5000 bootstraps with the predictor variable WHR, the mediators CD8+EMRA cells as well as KYN, and HOMA-IR as the outcome variable. For all statistical analyses as well as data visualization, we used JASP, version 14.0. p values < 0.05 are considered significant.

3. Results

3.1. Baseline Characteristics

After screening for eligibility, 134 participants met the eligibly criteria (Figure 2). Their data are specified as mean ± SD. Of all participants, 72% were female and 28% were male with a mean age of 59.2 ± 5.6 years. Participants were further characterized by a weight of 83.0 ± 20.3 kg, BMI of 28.3 ± 5.8 kg/m^2, a WHR of 0.85 ± 0.09, and a HOMA-IR of 2.72 ± 2.26. The study population can be classified as healthy, but pre-obese. All measured baseline characteristics are summarized in Table 1.

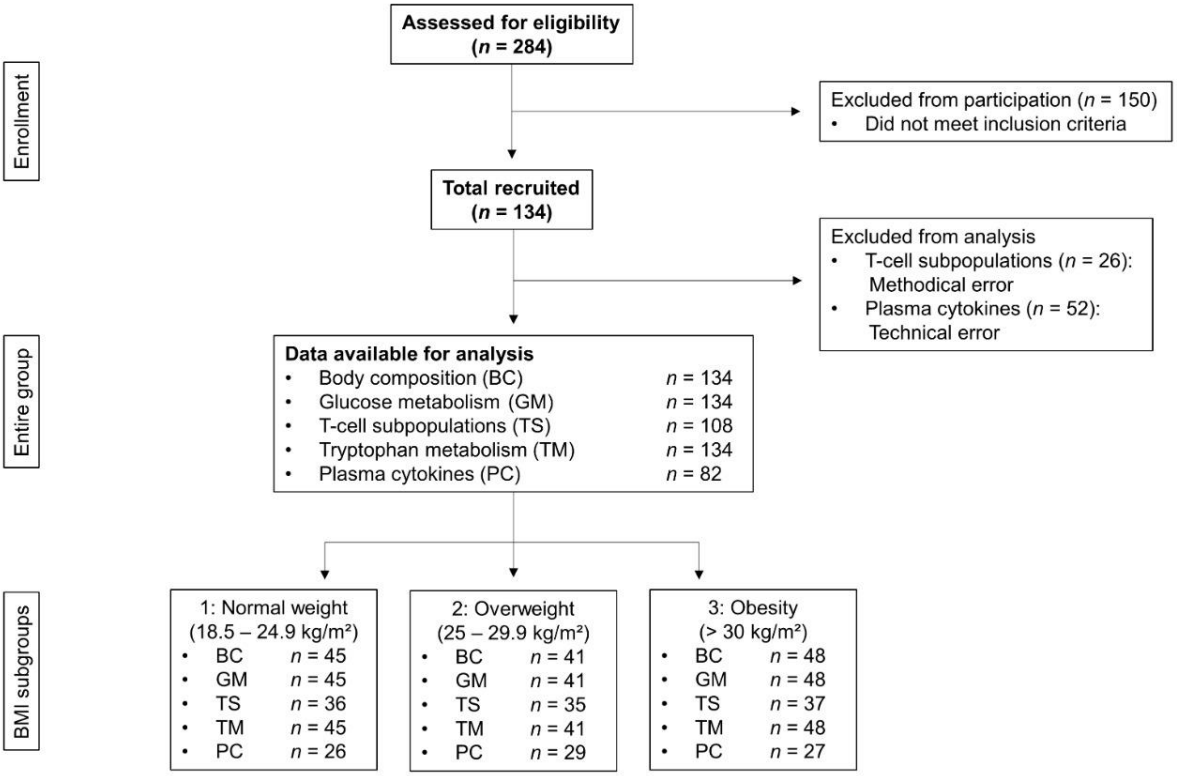

Figure 2. Flow chart of all participants screened and analyzed.

Table 1. Baseline characteristics (mean ± SD) of all participants.

Baseline Characteristics	
Sex [f/m]	[96/38]
Age [years]	59.2 ± 5.6
Height [m]	170.9 ± 8.7
Body weight [kg]	83.0 ± 20.3
BMI [kg/m^2]	28.3 ± 5.8
Waist circumference [cm]	93.30 ± 14.53
Hip circumference [cm]	108.32 ± 12.15
WHR	0.85 ± 0.09
Fasting Glucose [mg/dL]	93.13 ± 16.78
HbA1c [%]	5.44 ± 0.45
Insulin [µU/mL]	11.39 ± 7.96
HOMA-Index	2.72 ± 2.26
CMV positive [Yes/no]	[72/63]

3.2. Associations between Body Composition, Glucose Metabolism, T-EMRA Cells, Trp Metabolites, and Cytokine Status

Proportions of T cell subpopulations and levels of measured cytokines, adipokines, and Trp metabolites of all participants can be found in Supplementary Tables S1–S3.

We examined the Spearman rank correlation between either BMI, WHR, HOMA-IR, CD8+EMRA cells, IL-6, resistin, and KYN separately for each participant. Note that for all

upcoming results, we used the z-standardized variables. The correlation analysis revealed correlations between WHR and CD8+EMRA cells (r = 0.351, $p < 0.001$), IL-6 (r = 0.249, $p = 0.021$), resistin (r = 0.221, $p = 0.046$), KYN (r = 0.207, $p = 0.041$), as well as HOMA-IR (r = 0.447, $p < 0.001$). CD8+EMRA cells were correlated with KYN (r = 0.242, $p < 0.05$), BMI (r = 0.235, $p < 0.05$), and HOMA-IR (r = 0.250, $p < 0.05$). IL-6 was associated with BMI (r = 0.352, $p < 0.001$). Resistin was positively correlated with BMI (r = 0.349, $p < 0.01$). Furthermore, we found a correlation between KYN and BMI (r = 0.355, $p < 0.001$) and HOMA-IR (r = 0.224, $p < 0.05$). All results of the correlation analysis are depicted in Figure 3.

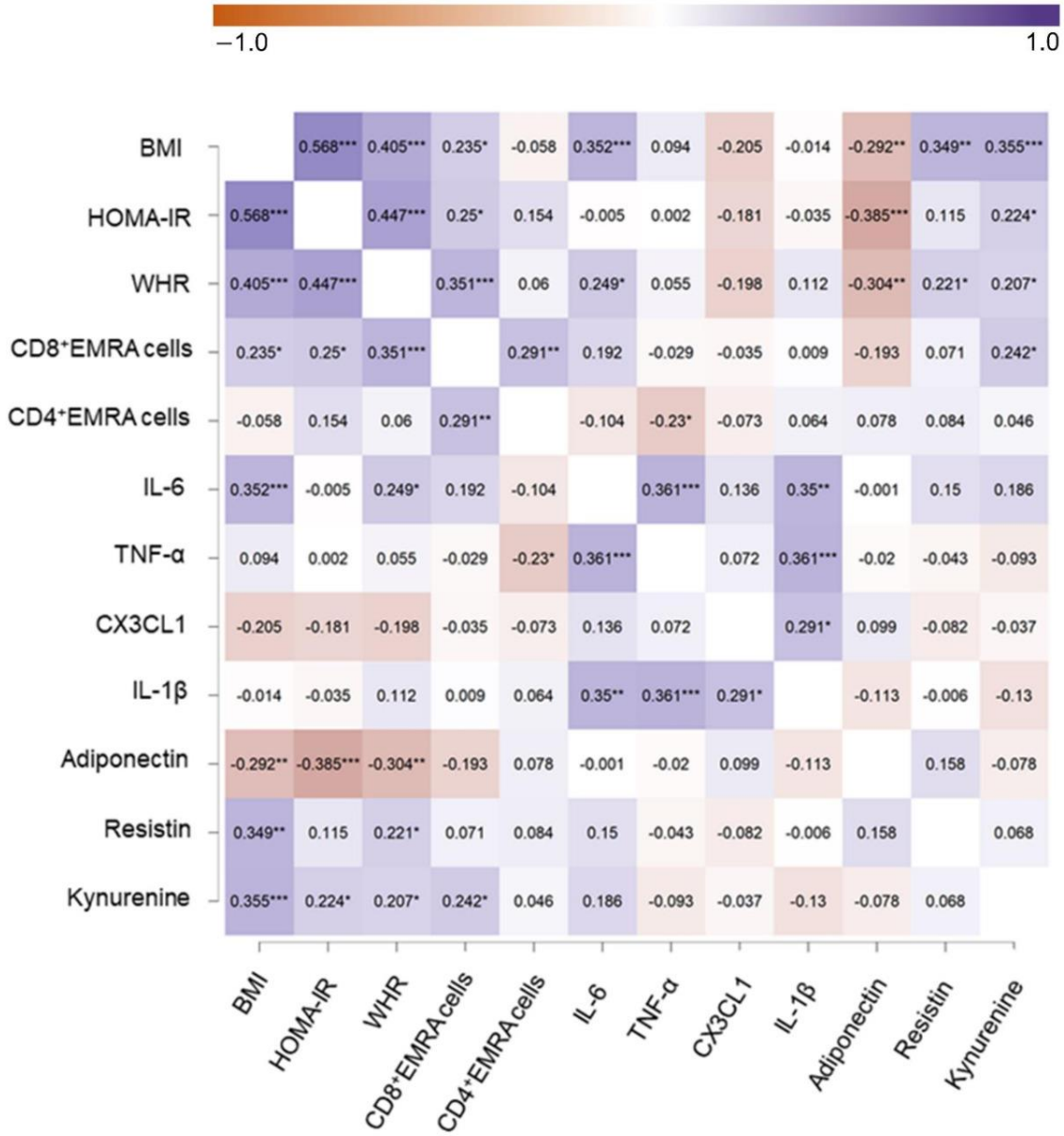

Figure 3. Correlation heat map including BMI, HOMA-IR, WHR, CD8+ and CD4+EMRA cells, IL-6, TNF-α, CX3CL1, adinopectin, resistin, and KYN. Raw p values of Spearman's rank s correlations are presented. * means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$ not corrected for multiple tests.

3.3. Effect of BMI on T-EMRA Cells and Trp Metabolism

As a pre-analysis, we tested whether a BMI classified within the obese range (>30) in particular leads to an increased proportion of T-EMRA cells as well as to enhanced levels of Trp metabolites. The calculated ANCOVAs including age as a covariate revealed a significant main effect of BMI group on the number of CD8+EMRA cells, $F(2, 89) = 3.897$, $p = 0.024$, $\eta p2 = 0.081$, as well as on the level of the Trp metabolite KYN, $F(2, 94) = 6.625$, $p = 0.002$, $\eta p2 = 0.124$. Post-hoc analyses using Bootstrapping indicated that, overall, a BMI > 30 is likely to lead to an increased proportion of CD8+EMRA cells (BMI group 2 vs. 3: $p < 0.05$) as well as to enhanced levels of KYN (BMI group 1 vs. 3 and BMI group 2 vs. 3: $p < 0.05$). Results are depicted in Figure 4a.

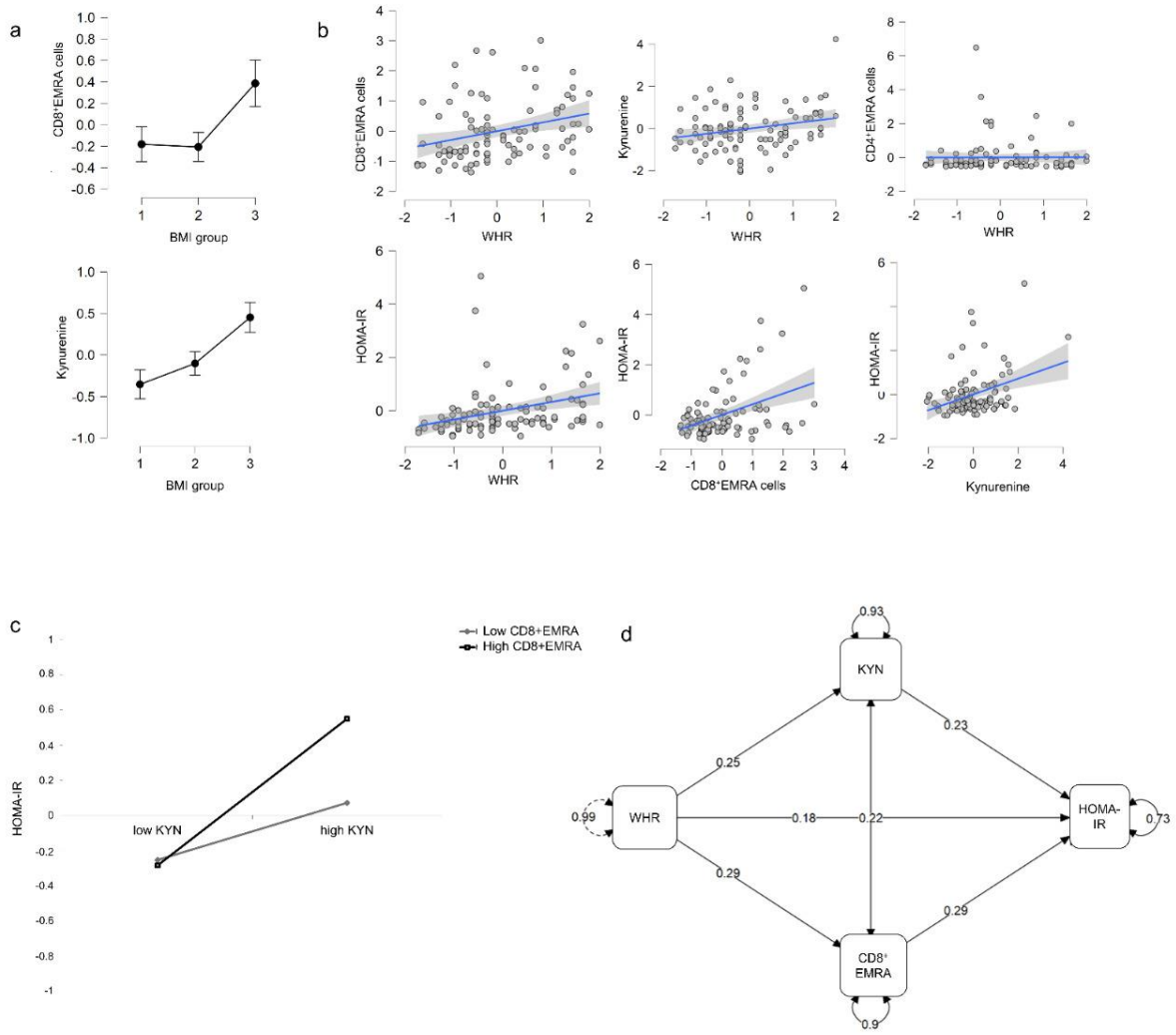

Figure 4. (**a**) Proportion of CD8+EMRA cells and KYN in BMI group 1 (BMI 18.5–24.9 kg/m^2), group 2 (BMI 25–29.9 kg/m^2), and group 3 (BMI > 30 kg/m^2). (**b**) Correlations between WHR and the proportion of CD8+EMRA cells, WHR and KYN, WHR and proportion of CD4+EMRA cells, WHR and HOMA-IR, CD8+EMRA and HOMA-IR, as well as KYN and HOMA-IR. (**c**) Associations between low/high kynurenine levels, levels of CD8+EMRA cells, and HOMA-IR. (**d**) Interaction of WHR on HOMA-IR mediated by CD8+EMRA cells as well as by KYN. Furthermore, a significant covariation between KYN and CD8+EMRA is demonstrated.

Further analyses revealed no main effect of sex, muscle mass, or weekly physical activity level on CD8+EMRA cells and KYN levels. Moreover, no significant interaction effects, for example, between sex and BMI, were found (data not shown).

3.4. Moderation of the Glucose Metabolism by Abdominal Obesity, the Proportion of T-EMRA Cells, and Trp Metabolites

We performed a multiple regression analysis investigating whether WHR, the proportion of T-EMRA cells, and changes of Trp metabolites are predictors of an altered glucose metabolism. Here, the WHR was used because it represents a better indicator of abdominal obesity and related health risks than BMI [23]. Furthermore, we tested whether the proportion of T-EMRA cells and KYN levels moderate the effect of abdominal obesity on the disturbances of glucose metabolism. The performed linear regression analysis revealed that the HOMA-IR could be predicted by the proportion of CD8+EMRA cells and KYN. Results showed that the HOMA-IR increases significantly when CD8+EMRA cells increase ($\beta = 0.302$, $p < 0.01$). Moreover, increased KYN levels correlated significantly with increases in HOMA-IR ($\beta = 0.212$, $p < 0.05$) (Figure 4b). The total variance explained by the model as a whole was adjusted R2 = 0.232, $F(3, 89) = 10.271$, $p < 0.001$. The model that included the product terms did explain little additional variance in the HOMA-IR score, adjusted R2 = 0.276, $F(7, 85) = 6.001$, $p < 0.001$. Results revealed a significant interaction between the proportion of CD8+EMRA cells and KYN ($\beta = 0.277$, $p < 0.01$) reflecting that higher CD8+EMRA cells accompanied by higher KYN levels lead to a stronger increase of HOMA-IR (Figure 4c).

3.5. Abdominal Obesity Affects T-EMRA Cells and Trp Metabolites, Which in Turn Influences HOMA-IR

We further analyzed whether the effect of abdominal obesity on glucose metabolism is mediated by immune senescence and Trp metabolites. A mediation analysis using bootstrapping showed a robust indirect effect of WHR on HOMA-IR mediated by CD8+EMRA cells ($\beta = 0.084$, $p < 0.05$). Additionally, a mediating tendency for KYN ($\beta = 0.058$, $p = 0.077$) was observed. Furthermore, the analysis revealed a significant covariation between KYN and CD8+EMRA cells ($\beta = 0.22$, $p < 0.05$) (Figure 4d).

4. Discussion

Effects of an individual's body composition, namely an increased BMI or abdominal obesity, are well-documented drivers of a dysregulated glucose metabolism [24]. However, potential mediators of this effect remain largely unclear, especially in a population of healthy aged subjects. Our findings show statistical associations between glucose metabolism, levels of Trp metabolites, and proportions of circulating T-EMRA cells. In particular, increased numbers of CD8+EMRA cells and increased levels of KYN could be potential predictors of a dysregulated glucose metabolism, even in clinically as yet unremarkable individuals. The present data also indicated that both the proportion of CD8+EMRA cells and KYN levels might play a mediating role between the increase in abdominal fat and an elevated HOMA-IR. This was supported by the fact that, in particular, the group of subjects with BMI classified as obese had increased KYN levels and CD8+EMRA concentrations.

A methodological limitation of our study in the context of flow cytometric evaluation was that we gated the CD4+ and CD8+ cells separately and not on one plot. CD4+ CD8+ double-positive cells could thus have been captured twice [25]. Although there usually is only a small proportion of double-positive cells, this aspect should be considered in future work. Due to the cross-sectional design, the present study can provide only initial insights into possible relationships but no causal conclusions can yet be drawn with absolute certainty. Nevertheless, the strength of the present work is the large representative human sample in an age-cohort of interest concerning the first occurrence of lifestyle-related diseases. Thus, high generalizability of our results can be assumed. Besides, our preliminary considerations were based on knowledge of biological correlations and

mechanisms in experimental (animal) studies, as described below. This legitimizes our statistical approach, supports our tentative conclusions, and reinforces the utility of follow-up studies in a longitudinal design to validate the assumptions we made here.

4.1. The Role of T-EMRA Cells in Obesity-Related Disturbance of Glucose Homeostasis

Overweight and obesity facilitate the incidence of many internal diseases and various data suggest that this occurs in parallel to the age-related remodeling of the adaptive immune system, particularly due to the accumulation of terminally differentiated T cells [26]. An important driver of these changes are lifestyle factors, such as inactivity and malnutrition, which lead to an increase in visceral adipose tissue (VAT). On one hand, the expansion of VAT favors a dysfunctional metabolic environment, but on the other hand, it accelerates T cell differentiation [27,28]. In this regard, it was previously shown that obesity accelerates thymic involution resulting in a lower naïve T cell production, leading to a proportional increase of terminally differentiated T cells [29]. In parallel, the expansion of visceral adipose tissue accelerates T cell differentiation by the progressive release of pro-inflammatory adipokines and cytokines [30]. A self-reinforcing cycle of progressive T cell differentiation and inflammatory cytokine production occurs because T-EMRA cells themselves secrete a variety of pro-inflammatory factors [10,15].

Our results concretize these data by showing an increased accumulation of T-EMRA cells in aged subjects with obesity and the association between abdominal fat and proportions of CD8+EMRA cells. As described in the introduction, T-EMRA cells are a heterogeneous subset of cells consisting of functional (CD57−) and senescent (CD57+) cells which occur in approximately equal proportions [6]. This further subdivision was not performed in the context of our study. However, results of mouse experiments suggest that mainly the exhausted, senescent T-EMRA cells may be crucial mediators of obesity-associated dysregulated glucose homeostasis.

Yi et al. [31] adoptively transferred senescent CD8+ T cells isolated from the spleens of mice fed a high-fat diet into young mice. Subsequently, the recipients showed aggravation of systemic glucose tolerance and insulin sensitivity compared to control mice. Another causal relationship between T cell aging and metabolic dysfunction was indicated in a study with obese mice. Here, the deletion of senescent cells alleviates the high-fat diet-induced metabolic dysfunction [32]. Cell culture experiments demonstrated a direct interaction between aged senescent CD8+ cells and hepatic insulin sensitivity by suppressing the activity of glycolytic enzymes [31]. However, the detailed molecular interactions between CD8+EMRA cells and metabolic pathways are still unknown. We can only speculate that cellular interactions or secreted molecules reduce tissue insulin sensitivity and possibly disrupt β-cell function. In this context, several inflammatory cytokines, such as IL-6, TNF-α, adiponectin, and resistin, are directly or indirectly involved in the regulation of insulin sensitivity [33,34]. Moreover, IL-6 and resistin have been associated with abdominal obesity on the one hand and an increased number of senescent T cells on the other hand [35]. For T-EMRA cells in general, secretion of TNF-alpha has also been demonstrated [10]. However, in our study, we did not observe a mediating role of any of these cytokines at the systemic level. Thus, follow-up studies should erode this potential mechanism at the tissue or cellular level.

Our finding that CD8+EMRA cells might represent a physiological mediator of disturbed glucose homeostasis is important from two perspectives. First, it would be important to investigate the potential direct molecular interactions, how these immune cells interact with beta cells or insulin-sensing organs, to uncover conceivable preventive and therapeutic targets. On the other hand, analyses of CD8+EMRA cells could represent an early predictive marker of a metabolic shift before subjects show clinically relevant symptoms of metabolic diseases. These assumptions need to be addressed in future (longitudinal) studies. In this context, a more specific subdivision of the T-EMRA cell population concerning additional surface markers, e.g., senescence marker CD57, or functional proper-

ties would be beneficial. This could generate further mechanistic associations and possibly increase the uniqueness of the observed effects.

4.2. KYN as a Mediator between Abdominal Fat and a Disturbed Glucose Metabolism

Our results show associations between Trp metabolite KYN and disturbed glucose metabolism. This finding corresponds with previous studies which found the involvement of tryptophan metabolites in the control of pancreatic hormonal secretion and hepatic glucose production [36]. Increased KYN levels suggest increased activity of IDO1. The turnover rate of this enzyme is dependent on inflammatory signaling. In our work, we could not find a strong correlation between KYN levels and any of the detected cytokines at the systemic level. However, based on our data, we cannot draw any conclusion about possible molecular interactions at the tissue level, for example, in the abdominal adipose store. Enhanced KYN levels in the subgroup with obesity and the association with WHR suggests that there is a mechanistic link to the amount of abdominal fat. Previous studies on this topic found that IDO1 expression and activity are enhanced in adipose tissue during conditions of inflammation [37]. Since chronic inflammation is also favoring T cell differentiation and metabolic disturbances related to overweight, the inflammatory shift in adipose tissue seems to be a precursor for early pathological processes [38]. The positive association between KYN and HOMA-IR corresponds with previous data which found an association between Trp metabolites and insulin resistance. Mechanistically, Trp metabolites have been shown to affect the biosynthesis, release, and activity of insulin [39].

5. Conclusions

Overweight facilitates the incidence of many internal diseases, specifically in those subjects at the end of the BMI spectrum. Especially metabolic pathologies develop slowly and gradually, and such changes can be detected even in individuals clinically assessed as healthy, like in the present study [40].

The increase in body fat in elderly subjects is related to increased CD8+EMRA populations as well as elevated plasma KYN levels and both are in turn associated with pre-clinical metabolic dysregulation. These findings integrate previous data of patients in different disease groups and are more remarkable because we found it in a population of clinically healthy older subjects. It is assumed, that the WHR dependent metabolic changes represent a preliminary stage of incipient pathological processes, which announce themselves especially in the subjects who develop obesity. The study once again highlights the importance of maintaining reliable control over the development of abdominal fat through specific lifestyle factors, especially in older age. At the same time, the data suggest that changes in Trp metabolites and an increase in CD8+EMRA cells may represent early predictors of prediabetes. This needs to be confirmed in longitudinal studies. Furthermore, from a basic science perspective, the interaction of CD8+EMRA cells with metabolic changes related to glucose regulation and Trp metabolites is of particular interest.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/cells10050998/s1, Table S1: T cell subpopulations (mean ± SD) of all participants, Table S2: Cytokine and adipokine levels (mean ± SD) of all participants, Table S3: Tryptophan metabolites (mean ± SD) of all participants.

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Data Availability Statement: The data generated during the current study are available from the corresponding author on reasonable request.

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References

1. Makinodan, T. Studies on the influence of age on immune response to understand the biology of immunosenescence. *Exp. Gerontol.* **1998**, *33*, 27–38. [CrossRef]
2. Goronzy, J.J.; Fang, F.; Cavanagh, M.M.; Qi, Q.; Weyand, C.M. Naive T cell maintenance and function in human aging. *J. Immunol.* **2015**, *194*, 4073–4080. [CrossRef]
3. Jacomet, F.; Cayssials, E.; Basbous, S.; Levescot, A.; Piccirilli, N.; Desmier, D.; Robin, A.; Barra, A.; Giraud, C.; Guilhot, F.; et al. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. *Eur. J. Immunol.* **2015**, *45*, 1926–1933. [CrossRef]
4. Goronzy, J.J.; Weyand, C.M. Successful and Maladaptive T Cell Aging. *Immunity* **2017**, *46*, 364–378. [CrossRef] [PubMed]
5. Wertheimer, A.M.; Bennett, M.S.; Park, B.; Uhrlaub, J.L.; Martinez, C.; Pulko, V.; Currier, N.L.; Nikolich-Žugich, D.; Kaye, J.; Nikolich-Žugich, J. Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. *J. Immunol.* **2014**, *192*, 2143–2155. [CrossRef] [PubMed]
6. Verma, K.; Ogonek, J.; Varanasi, P.R.; Luther, S.; Bünting, I.; Thomay, K.; Behrens, Y.L.; Mischak-Weissinger, E.; Hambach, L. Human CD8+ CD57- TEMRA cells: Too young to be called "old". *PLoS ONE* **2017**, *12*, e0177405. [CrossRef]
7. Brenchley, J.M.; Karandikar, N.J.; Betts, M.R.; Ambrozak, D.R.; Hill, B.J.; Crotty, L.E.; Casazza, J.P.; Kuruppu, J.; Migueles, S.A.; Connors, M.; et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. *Blood* **2003**, *101*, 2711–2720. [CrossRef] [PubMed]
8. Koch, S.; Larbi, A.; Derhovanessian, E.; Ozcelik, D.; Naumova, E.; Pawelec, G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. *Immun. Ageing* **2008**, *25*, 5–6. [CrossRef] [PubMed]
9. Nakajima, T.; Schulte, S.; Warrington, K.J.; Kopecky, S.L.; Frye, R.L.; Goronzy, J.J.; Weyand, C.M. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. *Circulation* **2002**, *105*, 570–575. [CrossRef]
10. Tilly, G.; Doan-Ngoc, T.M.; Yap, M.; Caristan, A.; Jacquemont, L.; Danger, R.; Cadoux, M.; Bruneau, S.; Giral, M.; Guerif, P.; et al. IL-15 Harnesses Pro-inflammatory Function of TEMRA CD8 in Kidney-Transplant Recipients. *Front. Immunol.* **2017**, *8*, 778. [CrossRef]
11. Spyridopoulos, I.; Martin-Ruiz, C.; Hilkens, C.; Yadegarfar, M.E.; Isaacs, J.; Jagger, C.; Kirkwood, T.; von Zglinicki, T. CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: Results from the Newcastle 85+ study. *Aging Cell* **2016**, *15*, 389–392. [CrossRef]
12. Yap, M.; Boeffard, F.; Clave, E.; Pallier, A.; Danger, R.; Giral, M.; Dantal, J.; Foucher, Y.; Guillot-Gueguen, C.; Toubert, A.; et al. Expansion of highly differentiated cytotoxic terminally differentiated effector memory CD8+ T cells in a subset of clinically stable kidney transplant recipients: A potential marker for late graft dysfunction. *J. Am. Soc. Nephrol.* **2014**, *25*, 1856–1868. [CrossRef] [PubMed]
13. Boren, E.; Gershwin, M.E. Inflamm-aging: Autoimmunity, and the immune-risk phenotype. *Autoimmun. Rev.* **2004**, *3*, 401–406. [CrossRef] [PubMed]
14. Franceschi, C. Inflammaging as a major characteristic of old people: Can it be prevented or cured? *Nutr. Rev.* **2007**, *65*, 173–176. [CrossRef]
15. Broadley, I.; Pera, A.; Morrow, G.; Davies, K.A.; Kern, F. Expansions of Cytotoxic CD4+CD28- T Cells Drive Excess Cardiovascular Mortality in Rheumatoid Arthritis and Other Chronic Inflammatory Conditions and Are Triggered by CMV Infection. *Front. Immunol.* **2017**, *8*, 195. [CrossRef] [PubMed]
16. Wikby, A.; Månsson, I.A.; Johansson, B.; Strindhall, J.; Nilsson, S.E. The immune risk profile is associated with age and gender: Findings from three Swedish population studies of individuals 20–100 years of age. *Biogerontology* **2008**, *9*, 299–308. [CrossRef] [PubMed]
17. Bardini, G.; Dicembrini, I.; Cresci, B.; Rotella, C.M. Inflammation markers and metabolic characteristics of subjects with 1-h plasma glucose levels. *Diabetes Care* **2010**, *33*, 411–413. [CrossRef] [PubMed]
18. Baumgartner, R.; Forteza, M.J.; Ketelhuth, D.F.J. The interplay between cytokines and the Kynurenine pathway in inflammation and atherosclerosis. *Cytokine* **2019**, *122*, 154148. [CrossRef]
19. Song, P.; Ramprasath, T.; Wang, H.; Zou, M.H. Abnormal kynurenine pathway of tryptophan catabolism in cardiovascular diseases. *Cell Mol. Life Sci.* **2017**, *74*, 2899–2916. [CrossRef] [PubMed]

20. Joisten, N.; Kummerhoff, F.; Koliamitra, C.; Schenk, A.; Walzik, D.; Hardt, L.; Knoop, A.; Thevis, M.; Kiesl, D.; Metcalfe, A.J.; et al. Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training. *Exerc. Immunol. Rev.* **2020**, *26*, 24–42.
21. Wasserfurth, P.; Nebl, J.; Boßlau, T.K.; Krüger, K.; Hahn, A.; Schuchardt, J.P. Intake of Calanus finmarchicus oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme. *Br. J. Nutr.* **2020**, *125*, 432–439. [CrossRef] [PubMed]
22. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **1985**, *28*, 412–419. [CrossRef]
23. Noble, R.E. Waist-to-hip ratio versus BMI as predictors of cardiac risk in obese adult women. *West. J. Med.* **2001**, *174*, 240–241. [CrossRef] [PubMed]
24. Ryan, A.S. Insulin resistance with aging: Effects of diet and exercise. *Sports Med.* **2000**, *30*, 327–346. [CrossRef] [PubMed]
25. Parel, Y.; Chizzolini, C. CD4+ CD8+ double positive (DP) T cells in health and disease. *Autoimmun. Rev.* **2004**, *3*, 215–220. [CrossRef] [PubMed]
26. Foster, A.D.; Sivarapatna, A.; Gress, R.E. The aging immune system and its relationship with cancer. *Aging Health* **2011**, *7*, 707–718. [CrossRef]
27. Duggal, N.A. Reversing the immune ageing clock: Lifestyle modifications and pharmacological interventions. *Biogerontology* **2018**, *19*, 481–496. [CrossRef] [PubMed]
28. Aguilar, E.G.; Murphy, W.J. Obesity induced T cell dysfunction and implications for cancer immunotherapy. *Curr. Opin. Immunol.* **2018**, *51*, 181–186. [CrossRef]
29. Yang, H.; Youm, Y.H.; Vandanmagsar, B.; Rood, J.; Kumar, K.G.; Butler, A.A.; Dixit, V.D. Obesity accelerates thymic aging. *Blood* **2009**, *114*, 3803–3812. [CrossRef]
30. Shirakawa, K.; Yan, X.; Shinmura, K.; Endo, J.; Kataoka, M.; Katsumata, Y.; Yamamoto, T.; Anzai, A.; Isobe, S.; Yoshida, N.; et al. Obesity accelerates T cell senescence in murine visceral adipose tissue. *J. Clin. Investig.* **2016**, *126*, 4626–4639. [CrossRef]
31. Yi, H.S.; Kim, S.Y.; Kim, J.T.; Lee, Y.S.; Moon, J.S.; Kim, M.; Kang, Y.E.; Joung, K.H.; Lee, J.H.; Kim, H.J.; et al. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. *Cell Death Dis.* **2019**, *10*, 249. [CrossRef] [PubMed]
32. Palmer, A.K.; Xu, M.; Zhu, Y.; Pirtskhalava, T.; Weivoda, M.M.; Hachfeld, C.M.; Prata, L.G.; van Dijk, T.H.; Verkade, E.; Casaclang-Verzosa, G.; et al. Targeting senescent cells alleviates obesity-induced metabolic dysfunction. *Aging Cell* **2019**, *18*, e12950. [CrossRef] [PubMed]
33. Nieto-Vazquez, I.; Fernández-Veledo, S.; Krämer, D.K.; Vila-Bedmar, R.; Garcia-Guerra, L.; Lorenzo, M. Insulin resistance associated to obesity: The link TNF-alpha. *Arch. Physiol. Biochem.* **2008**, *114*, 183–194, Erratum in **2009**, *115*, 117. [CrossRef]
34. Kim, J.H.; Bachmann, R.A.; Chen, J. Interleukin-6 and insulin resistance. *Vitam. Horm.* **2009**, *80*, 613–633. [CrossRef]
35. Dienz, O.; Rincon, M. The effects of IL-6 on CD4 T cell responses. *Clin. Immunol.* **2009**, *130*, 27–33. [CrossRef] [PubMed]
36. Liu, J.J.; Raynal, S.; Bailbé, D.; Gausseres, B.; Carbonne, C.; Autier, V.; Movassat, J.; Kergoat, M.; Portha, B. Expression of the kynurenine pathway enzymes in the pancreatic islet cells. Activation by cytokines and glucolipotoxicity. *Biochim. Biophys. Acta* **2015**, *1852*, 980–991. [CrossRef]
37. Wolowczuk, I.; Hennart, B.; Leloire, A.; Bessede, A.; Soichot, M.; Taront, S.; Caiazzo, R.; Raverdy, V.; Pigeyre, M.; ABOS Consortium; et al. Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: An attempt to maintain immune homeostasis and vascular tone. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2012**, *303*, 135–143. [CrossRef] [PubMed]
38. Batatinha, H.A.P.; Rosa Neto, J.C.; Krüger, K. Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise. *Exerc. Immunol. Rev.* **2019**, *25*, 96–111. [PubMed]
39. Oxenkrug, G. Insulin resistance and dysregulation of tryptophan-kynurenine and kynurenine-nicotinamide adenine dinucleotide metabolic pathways. *Mol. Neurobiol.* **2013**, *48*, 294–301. [CrossRef] [PubMed]
40. McKee, A.; Morley, J.E. Obesity in the Elderly. Available online: https://www.ncbi.nlm.nih.gov/books/NBK532533/ (accessed on 11 December 2020).

12-week combined strength and endurance exercise attenuates $CD8^+$ T-cell differentiation and affects the kynurenine pathway in the elderly: a randomized controlled trial

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RESEARCH Open Access

12-week combined strength and endurance exercise attenuates CD8$^+$ T-cell differentiation and affects the kynurenine pathway in the elderly: a randomized controlled trial

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Abstract

Background Age-related accumulation of highly differentiated CD8$^+$ effector memory re-expressing CD45RA (EMRA) T-cells and disruption of the kynurenine (KYN) pathway are associated with chronic inflammation and the development of insulin resistance.

In this study the aim was to investigate the effects of 12-week combined strength and endurance exercise on CD8$^+$ T-cell differentiation and KYN pathway metabolites. Ninety-six elderly subjects (f/m, aged 50—70) were randomized to a control (CON) or exercise (EX) group. The EX group completed combined strength and endurance training twice weekly for one hour each time at an intensity of 60% of the one-repetition maximum for strength exercises and a perceived exertion of 15/20 for endurance exercises. The EX group was also randomly subdivided into two groups with or without a concomitant balanced diet intervention in order to examine additional effects besides exercise alone. Before and after the intervention phase, the proportions of CD8$^+$ T-cell subsets and levels of KYN pathway metabolites in peripheral blood were determined.

Results The CD8$^+$ EMRA T-cell subsets increased in the CON group but remained almost unchanged in the EX group ($p = .02$). Plasma levels of kynurenic acid (KA) increased in the EX group and decreased in the CON group ($p = .03$). Concomitant nutritional intervention resulted in lower levels of quinolinic acid (QA) compared with exercise alone ($p = .03$). Overall, there was a slight increase in the QA/KA ratio in the CON group, whereas it decreased in the EX group ($p > .05$).

Conclusions Combined strength and endurance training seems to be a suitable approach to attenuate CD8$^+$ T-cell differentiation in the elderly and to redirect the KYN pathway towards KA. The clinical relevance of these effects needs further investigation.

Keywords Ageing, CD8$^+$ EMRA T-Cells, Kynurenine Pathway, Exercise, Nutrition, Insulin Resistance

† Philipp Zimmer and Karsten Krüger shared senior authorship.

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Background

Ageing and persistent viral infections lead to changes within the peripheral $CD8^+$ T-cell compartment. The proportion of naïve cells decreases, reducing the body's ability to target new antigens, resulting in enhanced susceptibility to infection and diminished vaccine efficacy [1]. In parallel, constant cell stimulation leads to an increased proportion of highly differentiated cells, which can be classified as effector memory T-cells re-expressing CD45RA (EMRA T-cells) based on their $CCR7^-/CD45RA^+$ surface expression profile [2–4]. The chemokine receptor CCR7, which enables migration of naïve and central memory T-cells into lymph nodes, is lost in EMRA T-cells [5]. In contrast, CD45RA, an isoform of the common leukocyte antigen found on the surface of naïve T cells and which is also lost after activation, is re-expressed at highly differentiated developmental stages [6]. $CD8^+$ EMRA T-cells are a heterogeneous composition of subsets associated with cell senescence, as they have reduced proliferative capacity [7], exhibit increased activation of senescence signalling pathways [8] and are associated with senescence markers such as KLRG1 and CD57 [4]. However, $CD8^+$ EMRA T-cells are not functionally inactive, as they can migrate into inflamed tissues [9, 10] and produce a substantial amount of pro-inflammatory cytokines, like the senescence-associated secretory phenotype (SASP) seen in non-immune cells [11]. Thus, accelerated T-cell differentiation is possibly related to the development of chronic low-grade inflammation. This could be of high clinical relevance, as "Inflamm-aging" plays a considerable role in the development of age-related multimorbidity, including cancer, dementia, osteoporosis, and metabolic diseases [12].

Interestingly, the main degradation pathway of tryptophan (TRP), the kynurenine (KYN) pathway, interacts with the immune system. On the one hand, local and systemic inflammatory stimuli increase the expression of the initial and rate-limiting enzyme indoleamine 2–3-dioxygenase (IDO) in various tissues, such as peripheral blood mononuclear cells (PBMCs) [13, 14]. On the other hand, both KYN and one of its metabolites, kynurenic acid (KA), have immunomodulatory effects themselves and can activate the aryl hydrocarbon receptor (AHR), which plays a key role in T-cell differentiation and the induction of inflammatory mediators [15, 16].

Boßlau et al. (2021) have recently shown that the proportion of circulating $CD8^+$ EMRA T-cells as well as plasma levels of KYN are associated with abdominal obesity and represent potential predictors of progressive glucose intolerance in the elderly [17]. These findings have been supported by the results of studies using the mouse model [18, 19]. Yi et al. (2019) transferred senescent $CD8^+$ T-cells ($CD44^+CD153^+$) from mice fed a high-fat diet into young recipient mice, which subsequently exhibited worsened systemic glucose tolerance and decreased insulin sensitivity compared with control mice. In addition, the researchers demonstrated that senescent $CD8^+$ T-cells ($CD28^-CD57^+$) are more numerous in human subjects with prediabetes and express a variety of proinflammatory cytokines and cytotoxic substances [18]. Laurans et al. (2018) showed that a high-fat diet in mice increases the expression of IDO in various tissues, thereby directing the metabolism of TRP towards KYN and its downstream products KA and quinolinic acid (QA). However, IDO knockout mice were protected from the obesogenic, inflammatory, and insulin resistance-inducing effects of the diet [19].

Given the high clinical and health economic relevance of metabolic diseases, it is of central interest to verify associations between $CD8^+$ EMRA T-cells, KYN pathway metabolites, and insulin resistance in humans. Furthermore, knowledge of potent therapeutic regimens would be desirable to attenuate progressive $CD8^+$ T-cell differentiation and rebalance the KYN pathway in the elderly, thereby potentially preventing the development of manifest type 2 diabetes mellitus.

Lifestyle modifications such as exercise or a healthy diet are becoming increasingly important in the prevention and treatment of age-associated diseases. Lifelong aerobic exercise has been shown to attenuate immune aging [20]. However, whether resuming exercise at older ages after years of inactivity also has beneficial effects on T-cell differentiation is much less clear. Niemiro et al. (2021) demonstrated that 12 weeks of moderate aerobic endurance training in a population of middle-aged/older women identified as being of high risk of developing breast cancer had a rejuvenating effect on the composition of T-cell subpopulations [21]. The extent to which such effects are transferable to healthy older individuals in the general population remains unclear. It is also unknown whether combined strength and endurance training produces similar effects compared to aerobic exercise programs. The impact of regular physical exercise on the KYN pathway has been analysed by only a few authors so far [22]. These have mainly examined psychiatric and neurodegenerative patient groups, and some of the work has methodological weaknesses so no clear conclusions can yet be drawn for healthy older individuals. Furthermore, no studies have examined potential synergistic effects of exercise and diet interventions on changes in T-cell differentiation and the KYN pathway.

In the present study the aim was to investigate the effects of a 12-week strength-endurance exercise intervention alone or combined with a nutritional intervention on T-cell differentiation, the KYN pathway metabolites and metabolic health. It was hypothesized that regular

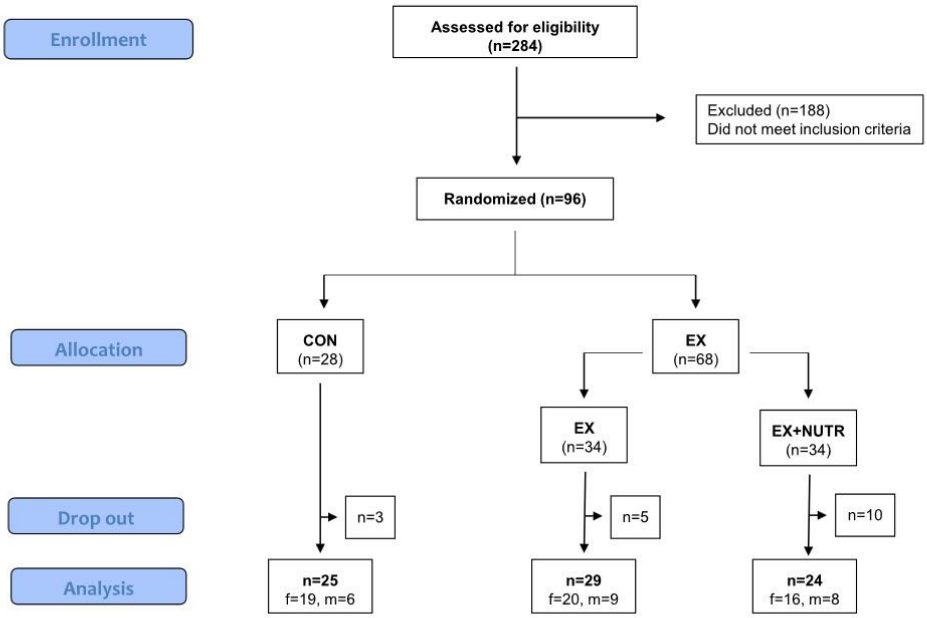
exercise has a 'rejuvenating' effect on the composition of the peripheral $CD8^+$ T-cell pool and reduces the proportion of EMRA subsets. Furthermore, it was hypothesized that there is an influence on the KYN pathway and that a concomitant balanced diet causes additional adaptations compared to exercise training alone.

Results

Baseline characteristics

After screening for eligibility, 96 participants were enrolled in the study, of which 78 were included in the final analysis (Fig. 1A). The reasons for there being dropouts within the intervention groups were due to inadherence to the training and nutrition intervention, whereas in the control group, increased daily activity led to exclusion. A higher dropout rate was recorded in the EX + NUTR group compared to the other groups. Of all participants analysed, 70% were female and 30% were male, with a mean age of 59.8 ± 5.6 years. The study population can be classified as healthy but pre-obese, with a mean weight of 83.9 ± 20.3 kg, a BMI of 28.6 ± 5.8 kg/m^2, and a WHR of 0.85 ± 0.09.

A



B

	CON (*n* = 25)	**EX (*n* = 53)**	***p***	EX (*n* = 29)	EX+NUTR (*n* = 24)	*p*
Sex (f/m)	19/6	36/17	0.674	20/9	16/8	0.783
Age (years)	59.2 ± 5.1	60.5 ± 6.0	0.386	60 ± 6.6	61 ± 5.3	0.564
Height (cm)	169.1 ± 8.1	171 ± 8.6	0.785	170.9 ± 8.7	171.0 ± 8.4	0.648
Body weight (kg)	84.8 ± 22.6	83.3 ± 19.4	0.548	81.9 ± 18.6	85.0 ± 20.3	0.522
BMI (kg/m²)	29.2 ± 6.9	28.1 ± 5.8	0.477	27.8 ± 5.5	28.5 ± 6.2	0.863
WHR	0.85 ± 0.08	0.86 ± 0.09	0.733	0.85 ± 0.09	0.87 ± 0.08	0.798
HOMAi	3.16 ± 3.23	2.73 ± 2.01	0.474	2.39 ± 1.55	3.14 ± 2.43	0.421
IL-6 (pg/ml)	0.95 ± 0.37	1.23 ± 1.36	0.435	0.89 ± 0.39	1.65 ± 1.94	0.163
CD4/CD8 Ratio	3.35 ± 1.62	4.08 ± 2.55	0.234	3.98 ± 2.09	4.2 ± 2.83	0.489
CMV status (+/-)	14/11	30/23	0.474	14/15	16/8	0.179
Total activity (h/wk)	7.8 ± 5.4	5.4 ± 5.3	0.135	6.7 ± 7.7	3.9 ± 2.7	0.082
Sports activity (h/wk)	0.36 ± 1.06	0.35 ± 0.62	0.346	0.34 ± 0.53	0.36 ± 0.73	0.352

Fig. 1 **A** Flow chart of all participants enrolled, randomized, allocated and analysed. **B** Baseline characteristics of participants, separated by study group. Values are given as mean ± SD. Distribution of sexes and CMV status between groups was analysed using Chi-square-test. All other group differences were assessed with Independent Samples T-Tests. Group differences between **CON** and **EX** (bold print) and between EX (normal print) and EX + NUTR were calculated separately and reported as p-values in each case (* $p < .05$; ** $p < .01$; *** $p < .001$). **CON** = control group; **EX** (bold print) = exercise group (with and without nutrition instructions); EX (normal print) = exercise group without nutrition instructions; EX + NUTR = exercise group with nutrition instructions; f = female, m = male; BMI = Body-Mass-Index; WHR = Waist-to-Hip-ratio; HOMAi = Homa-Index, CMV = cytomegalovirus; h/wk = hours per week

At baseline, the groups did not differ in age, body composition, HOMA-IR, IL-6 plasma concentration, CD4/CD8 ratio, CMV status, or activity level (Fig. 1B).

Associations between age, BMI, HOMA-IR, peripheral CD8$^+$ EMRA T-cells, plasma IL-6 concentration and metabolites of KYN pathway

Pearson's correlation of baseline values (t0) shows a significant positive relationship between BMI and HOMA-IR (Fig. 2A). Both BMI and HOMA-IR also correlate positively with IL-6 plasma concentration, proportions of CD8$^+$ EMRA T-cells, as well as plasma levels of KYN and its metabolites QA and KA. The proportion of peripheral CD8$^+$ EMRA T-cells shows a positive association with KYN, QA, and KA. IL-6 plasma concentration also correlates positively with KYN, QA, and KA. Pearson's correlation of changes between the measurement time points (t12-t0) showed no significant correlations, except for the metabolites of the KYN pathway among themselves (data not shown).

Manipulation check: Effects of the study intervention on BMI, HOMA-IR and maximum strength

The manipulation check revealed that BMI was significantly reduced after the intervention in the EX group when compared to the CON group ($F(1, 75) = 4.97$, $p = 0.029$). Within a three-group comparison, BMI decreased slightly more in the EX + NUTR group (exercise group with nutrition instructions) than in the EX ONLY group (exercise group without nutrition instructions), but without statistical significance ($F(2, 74) = 2.49$, $p = 0.09$).

For HOMA-IR, an analysis using rmANCOVA revealed no significant different changes between the CON and EX group ($F(1, 75) = 1.45$, $p = 0.23$). Moreover, there was no statistically significant difference within the three-group comparison ($F(2, 74) = 0.72$, $p = 0.49$). However, HOMA-IR tended to decrease the most in the EX + NUTR group (Fig. 2B). The raw BMI and HOMA-IR data and the line plots of the rmANCOVA analyses are included as Supplementary Material (Table S1, Figure S1).

Maximum strength increased significantly in the EX group for all exercises (Leg Press, Bench Press, Lat Pulldown, Back Extension, Rowing, Abdominal Flexion) at t12 compared to t0 ($p < 0.001$). No maximum strength data were collected in the CON group.

Effects of the study intervention on proportions of peripheral CD8$^+$ T-cell subsets

Between the CON and EX group, no significant different changes were observed for peripheral CD8$^+$ T-cells ($F(1, 60) = 0.36$, $p = 0.85$) or the proportion of naïve ($F(1, 63) = 0.13$, $p = 0.72$), CM ($F(1,63) = 0.79$, $p = 0.39$) and EM subsets ($F(1,63) = 3.13$, $p = 0.08$). In contrast, the proportion of EMRA subsets changed statistically significantly between the CON and EX group ($F(1, 63) = 5.68$, $p = 0.02$), with a greater increase within the CON group (Fig. 3B). Within the three-group comparison, a statistically significant main effect was not seen for EMRA subsets ($F(2, 62) = 2.83$, $p = 0.07$). The raw data of the CD8$^+$ T-cell subsets and the line plots of the rmANCOVA analyses are included as Supplementary Material (Table S2, Figure S1). An exploratory subgroup analysis, with CMV serostatus as an additional between-subject factor, did not reveal any significant 'time x study group x CMV serostatus' interaction effects on the proportions of the CD8$^+$ T-cell subsets. However, a significant between-subjects effect was detected for the proportion of CD8$^+$ EMRA T-cells, which depends on the CMV serostatus (Supplementary Table S3).

Effects of the study intervention on plasma KYN pathway metabolites

For TRP and KYN, no statistically significant interaction effects were observed within the two-group or three-group comparison (Fig. 3C). For QA, the rmANCOVA analysis with the three-group comparison revealed a significant main effect ($F(2, 72) = 4.38$, $p = 0.016$) and a Bonferroni-corrected post-hoc analysis revealed a significant difference between the EX ONLY and EX + NUTR group, with an increase in the EX ONLY group and a decrease in the EX + NUTR group ($p = 0.03$, $M_{Diff} = 0.033$, 95%-CI[0.002, 0.064]). A significant intergroup effect was observed for KA concentrations, with greater values in the EX group and lower values in the CON group after the intervention ($F(1, 73) = 4.79$, $p = 0.032$). The three-group comparison also revealed a significant interaction effect ($F(2, 72) = 3.76$, $p = 0.029$) and a Bonferroni-corrected post-hoc analysis revealed a significant difference between the CON and EX ONLY group ($p = 0.026$, $M_{Diff} = 0.005$, 95%-CI[0.001, 0.02]). For the QA/KA-ratio, no statistically significant difference between groups was observed. However, a slight increase was seen in the CON group (+0.4), whereas it decreased in the EX ONLY (-1.0) and EX + NUTR group (-0.4). The raw data of the KYN pathway metabolites and the line plots of the rmANCOVA analyses are included as Supplementary Material (Table S4, Figure S1). An exploratory subgroup analysis with the CMV serostatus as an additional between-subject factor did not reveal any significant 'time x study group x CMV serostatus' interaction effect on the plasma levels of the KYN pathway metabolites. However, a significant between-subjects effect was detected for KYN, which depends on the CMV serostatus (Supplementary Table S5).

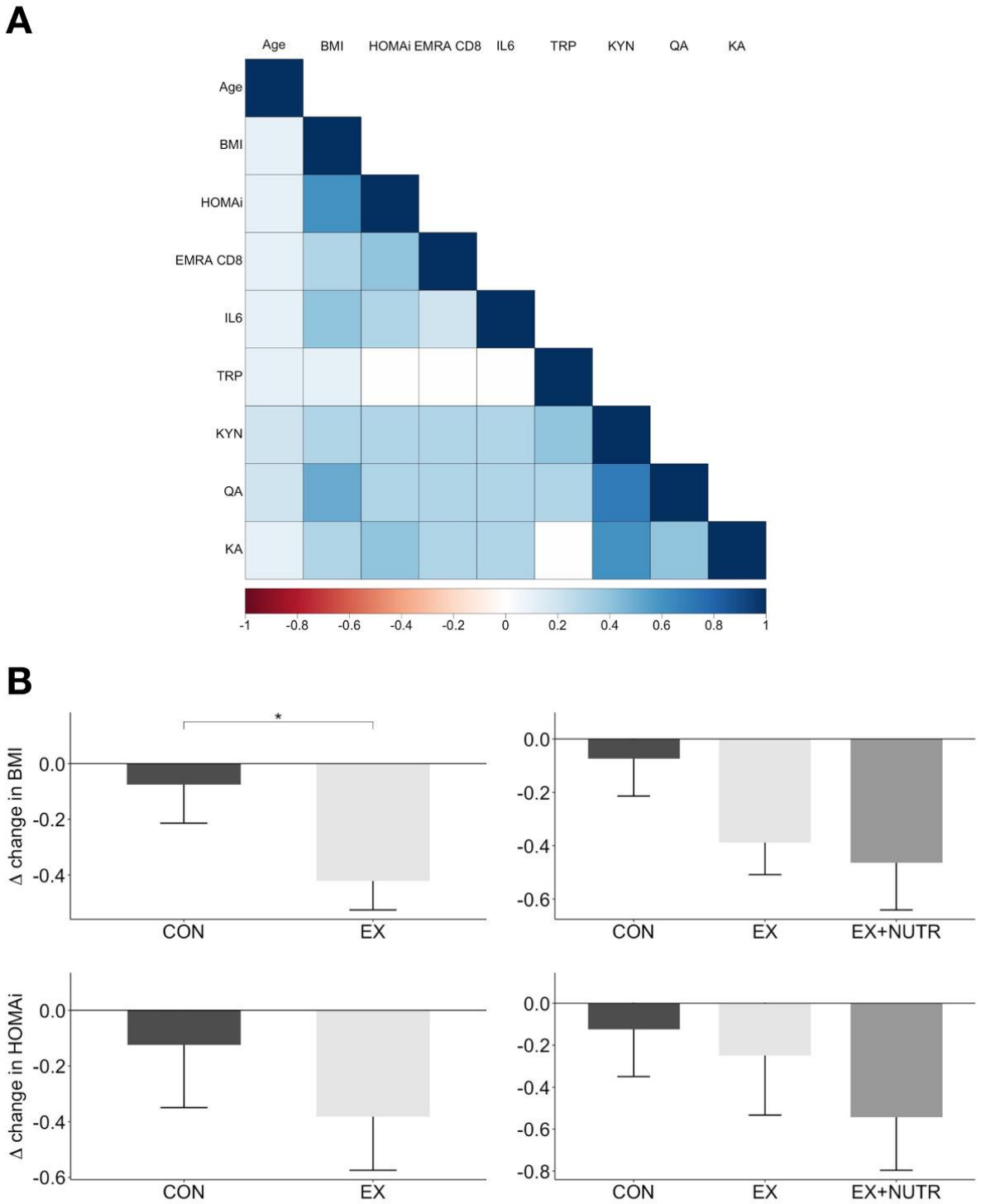


Fig. 2 **A** Heat map illustrating baseline correlations. Pearson's r values are depicted, where the values are given in the coloured scale bar. **B** Absolute change in BMI and HOMA-IR per study group over the twelve-week intervention period ($\Delta = t_{12} - t_0$). *($p < .05$) indicates a significant difference between groups, analysed by rmANCOVA. CON = control group; EX (two-group-comparison) = exercise group (with and without nutrition instructions); EX (three-group-comparison) = exercise group without nutrition instructions; EX + NUTR = exercise group with nutrition instructions; BMI = Body-Mass-Index, HOMAi = HOMA-Index; EMRA CD8 + = Effector Memory re-expressing CD45RA CD8 + cells; TRP = tryptophane; KYN = kynurenine; QA = quinolinic acid; KA = kynurenic acid

Discussion

In this randomized controlled trial, it has been demonstrated that 12 weeks of combined strength/endurance training in the elderly had a positive effect on BMI and HOMA-IR, and attenuated the increase in the proportion of EMRA cells within the peripheral $CD8^+$ T-cell compartment. Concomitant nutritional intervention showed no additional effect. Moreover, training affected the KYN metabolic pathway by increasing KA formation.

Exercise training attenuates T-cell differentiation

The proportion of EMRA cells within the peripheral $CD8^+$ T-cell compartment increased significantly in the CON group, while no changes were found in the EX group. Thus, combined strength/endurance training may attenuate the progressive increase in the $CD8^+$ EMRA T-cell pool with age and seems to be a suitable approach for the delaying of T-cell differentiation. This is consistent with previous data: Vasconcelos et al. (2022) demonstrated in 108 postmenopausal women that both functional and combined strength-endurance training for 16 weeks (three times a week for 45 min each) reduced the proportion of EMRA cells in both the $CD8^+$ and $CD4^+$ T-cell compartments, compared with a control group [23]. In their study, the authors used the same surface markers as were used in this work for cell differentiation (CCR7, CD45RA) and the subjects also completed combined strength-endurance training, highlighting the reproducibility of the results of this work. Philippe et al. (2019) demonstrated an exercise-induced effect on T-cell differentiation in 16 older adults with prediabetes after an intervention period of only three weeks. In the study, subjects completed an aerobic exercise program three times weekly, running uphill (concentric) or downhill (eccentric) depending on the study group. The results showed that both protocols significantly decreased the percentage of $CD4^+$ and $CD8^+$ EMRA T-cells (CCR7-/CD45RO-), while increasing the proportion of central memory as well as naïve T-cells [24]. Dinh et al. (2019) examined the effects of six weeks (2–3 times per week) of exercise training on the number of senescence-prone $CD8^+$ T-cells ($CD57^+$) in 100 women aged ≥ 65 years [25]. They compared different exercise modalities: intensive strength training (80% of maximum repetition, 3 sets of 10 repetitions), strength endurance training (40% of maximum repetition, 2 sets of 30 repetitions), and a control condition (stretching). The authors found a significant decrease in the percentage and absolute count of senescence-prone $CD8^+$ T cells in the strength endurance training group. The magnitude of change was also positively related to the number of training sessions. Interestingly, intense strength training did not affect the senescence-associated cell fraction. These results suggest that both modality and frequency of exercise may determine the training-induced effect on T-cell differentiation. This is consistent with Niemiro et al. (2021), who demonstrated divergent effects of 12-week endurance training (three times a week) on the numbers of $CD4^+$ and $CD8^+$ T-cell subsets in older women at risk of breast cancer depending on training intensity. Consequently, high-intensity training evoked more 'pro-senescent' (decrease in naïve $CD4^+$ T-cells) and moderate-intensity training more 'anti-senescent' (increase in effector memory $CD8^+$ T-cells) effects. However, training showed no significant effects on the $CD8^+$ EMRA T-cell fraction, but the group size of the study was relatively small (n = 8), which should be considered critically [21]. Taken together, the current data suggest that $CD8^+$ T-cell differentiation in the elderly is exercise-sensitive and can be influenced within a few weeks by both endurance and strength endurance training, depending on the training intensity and frequency. Further work comparing different training regimens is needed to make conclusive training recommendations. At present, only speculations can be made about the mechanisms of the training-induced adaptations in the $CD8^+$ T-cell compartment.

It is conceivable that adrenergic stimuli and hemodynamic changes during repetitive exercise lead to a redistribution of highly differentiated T-cells into the blood [20], where they are exposed to pro-apoptotic factors such as reactive oxygen species [26, 27]. Since these cells in particular appear to be sensitive to these factors, they could be eliminated by repeated acute exercise [28] and subsequently shift the ratio of highly differentiated EMRA to naive $CD8^+$ T-cells [29, 30]. This hypothesis is supported by Krüger et al. (2016) who showed that one session of high-intensity training (HIT) induced a higher increase of apoptosis in highly differentiated T-cells ($CD28^-CD57^+$) compared to a continuous exercise regimen [28]. Furthermore, from longitudinal data of Niemiro et al. (2021) it was revealed that changes in $\dot{V}O_2max$ were inversely related to changes in highly differentiated $CD8^+$ EMRA T-cells after physical training. This has been confirmed by cross-sectional data [31],

(See figure on next page.)

Fig. 3 **A** Flow cytometric gating strategy for the analysis of T-cell subsets **B** Absolute changes in proportions of peripheral $CD8^+$ T-cell subsets per study group over the twelve-week intervention period ($\Delta = t_{12} - t_0$). *($p < .05$) indicates a significant difference between groups, analysed using rmANCOVA. **C** Absolute changes of plasma metabolites of the KYN pathway in µmol/L ($\Delta = t_{12} - t_0$). *($p < .05$) indicates a significant difference between groups, analysed using rmANCOVA. CON = control group; EX (two-group-comparison) = exercise group (with and without nutrition instructions); EX (three-group-comparison) = exercise group without nutrition instructions; EX + NUTR = exercise group with nutrition instructions; EMRA CD8 + = Effector Memory re-expressing CD45RA CD8 + cells; TRP = tryptophane, KYN = kynurenine; QA = quinolinic acid; KA = kynurenic acid

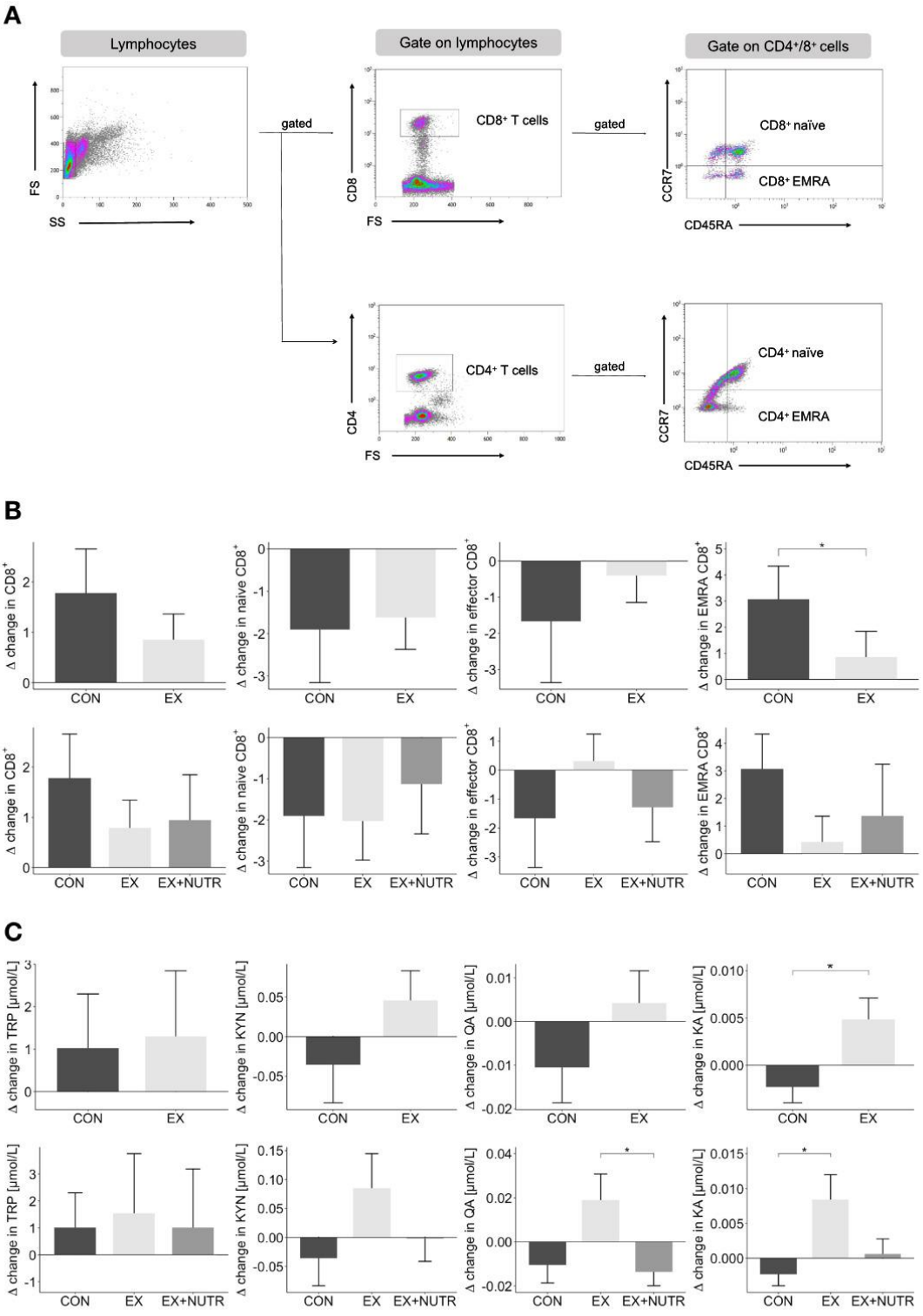


Fig. 3 (See legend on previous page.)

from which it has been shown that VO2max is inversely associated with the number of senescent $CD8^+$ T-cells (KLRG1 + /CD57 + ; KLRG1 + /CD28-). Unfortunately, VO2max was not determined in the work of this study because the focus of the training intervention was on the strength endurance component. Instead, subjects in the EX group performed measurements of maximum strength before and after the intervention phase, but correlation analysis revealed no relationship between changes in maximum strength and changes in the proportion of peripheral $CD8^+$ EMRA T-cells (data not shown). Physical training seems to reduce susceptibility to infection and could suppress reactivation of latent viruses [32, 33]. The increase in EMRA $CD8^+$ T-cells in the control group might be due to the fact that they experienced more infections or a higher burden of latent viral infections than the exercise group. In particular, a persistent CMV infection is a known driver of T-cell differentiation and should be considered as a potential confounder in studies of physical activity and immunity [20]. However, in previous work examining the effect of exercise on $CD8^+$ T-cell differentiation either the CMV status of the subjects at all [21, 23] was not recorded or it was not taken into account in the analyses with regard to an influence on the exercise-induced effects [24, 25]. This must be taken as a caveat and underscores the additional importance of the work of this study. In the work of this study, CMV serostatus was considered as a covariate in the rmANCOVA statistics. In addition, an exploratory subgroup analysis was performed with the CMV serostatus as an additional between-subject factor. This analysis did not reveal any significant 'time x study group x CMV serostatus' interaction effects. Thus, the CMV serostatus does not appear to influence the exercise-induced change on the proportions of peripheral $CD8^+$ T-cell subsets. However, significant between-subjects effects were found for the proportion of $CD8^+$ EMRA T-cells, thereby suggesting a general effect of latent CMV infection on these cells, which is consistent with a recent paper published by the author's group [34].

Regarding dietary modification, no additional effects were observed on the distribution of peripheral $CD8^+$ T-cell subsets. In general, to the best of the authors' knowledge, there is only one study with aged nonhuman primates in which the effects of diet have been investigated on T-cell differentiation. In this it was shown that long-term caloric restriction delayed T-cell differentiation and reduced the production of inflammatory cytokines by memory T-cells [35]. In the EX + NUTR group of the work in this study, mean energy intake decreased by approximately 200 kcal per day after the intervention period, but this was not statistically different from the other groups, as described in a previously published paper of this same study [36]. Therefore, future human studies focusing more specifically on the effect of caloric restriction on T-cell differentiation would be interesting.

Exercise training and dietary measures affect kynurenine pathway

Plasma concentration of KA increased significantly in the EX group compared with the CON group, whereas the concomitant dietary modification attenuated this effect. QA also showed an increase in the EX group, while the concomitant dietary modification had the opposite effect and decreased plasma concentration. Both KA and QA are derived from KYN, which in turn is the main product of tryptophan. KA is synthesized by KYN aminotransferases 1–4 (KAT 1–4) and has neuroprotective properties, whereas QA is closely associated with neuronal excitotoxicity and is produced by kynurenine-3-monooxygenase (KMO) [22]. To the best of the authors' knowledge, the present work is the first in which a significant increase has been demonstrated in the plasma concentration of KA in response to a longitudinal training intervention. In comparable work only an increased gene expression of KAT isoenzymes in skeletal muscle was demonstrated [37, 38]. Moreover, Allison et al. (2019) observed a non-significant increase in KA and a non-significant decrease in QA in peripheral blood [38]. The reason why enhanced expression of KAT did not have a stronger effect on the concentration of KA in peripheral blood is unclear. Epigenetic influences may play a role here. In addition, rapid metabolism or excretion of the synthesized KYN metabolites are also speculated to be causative [39].

Within the work of this study, concomitant balanced dietary modification had a mitigating effect on the exercise-induced changes and resulted in reduced concentrations of KYN pathway metabolites. In particular, peripheral concentrations of QA were shown to have a significant reduction in the EX + NUTR group compared with the EX ONLY group. One possible explanation is that food rich in antioxidant compounds could counteract immune response and tryptophan breakdown by IDO. Jenny et al. (2011) showed that specific compounds like vitamin C and E, and also stilbene resveratrol and coffee flavonoids, were able to delay T-cell activation and IFN-γ production in vitro in mitogen-stimulated human peripheral blood mononuclear cells (PBMC) from healthy donors, followed by reduced activity of the downstream biochemical pathways like tryptophan breakdown by IDO-1 [40]. Since it has already been confirmed in preliminary work that the subjects in the EX + NUTR group consumed significantly more vegetables and fruit [36], this is a conceivable explanatory direction.

In summary, it is noticeable that the QA/KA ratio shifted slightly in favour of KA in the EX ONLY and EX + NUTR group, whereas it shifted in favour of QA in the CON group. In many neurodegenerative diseases, there is an imbalance between KA and QA in the CNS with an accumulation of QA and a decrease in KA [22]. In addition, the development of metabolic diseases has been associated with increased QA concentrations as well as decreased KA synthesis [41, 42]. Based on this, from the decreased QA/KA ratio in the intervention groups of this study it can be cautiously inferred that combined strength endurance training with or without a concomitant nutritional modification may have a moderate neuroprotective effect and could reduce the risk of developing type 2 diabetes mellitus in healthy elderly.

Limitations

A substantially higher dropout rate occurred in the EX + NUTR group (n = 10) compared to the EX ONLY (n = 5) and CON (n = 3) group due to inadherence to the training and nutrition intervention. This should be taken into account when interpreting the data. However, the overall retention of subjects was good (> 80%), suggesting a high day-to-day suitability of the study intervention procedure. In this work, peripheral cell subsets were quantified by flow cytometry as percentages of the $CD8^+$ T-cell fraction. Additional cell enumeration procedures were not performed, thus it was not possible to provide absolute cell concentrations. This is a significant limitation of the results, because it cannot be clearly demonstrated whether an increase or decrease in the proportion of a cell population resulted from an absolute change in that population or was due to a relative change in other cell proportions. Nevertheless, in the context of age-related advancing T-cell differentiation, the relative composition of the different $CD8^+$ subsets is also considered as an important outcome. Moreover, in previous work it has been shown that the relative reduction in peripheral senescence-prone $CD8^+CD57^+$ T-cells (as a percentage of $CD8^+$ T-cells) caused by strength-endurance training is related to an absolute decrease in concentration of these cells in the blood [25]. Thus, highly differentiated $CD8^+$ T-cells such as those in the EMRA subpopulation appear to be exercise sensitive themselves. The parameters of the work in this study were determined only in peripheral blood, and no evidence of changes in other organs or tissue types could be obtained. For example, the quantitative change of the KYN pathway metabolites in cerebrospinal fluid due to physical training would have been interesting. In addition, gene expression analyses of the enzymes involved in the KYN signalling pathway would have been useful. In the case of T-cell subsets, it is not clear at present whether unequal redistribution to extravascular tissues may be responsible for the exercise-induced altered proportions in peripheral blood. Moreover, more sensitive measurement methods of T-cell differentiation exist (e.g., deep T-cell receptor beta sequencing), which should be included in future work.

Conclusions

BMI and HOMA-IR are associated with the proportions of $CD8^+$ EMRA T-cells as well as KYN pathway metabolites. 12 weeks of combined strength and endurance exercise were found to attenuate the age-related increase in the proportion of EMRA subsets within the peripheral $CD8^+$ T-cell pool. In addition, exercise activated the KYN pathway and significantly increased serum level of KA. Thus, combined strength and endurance training seems to be a suitable approach to attenuate $CD8^+$ T-cell differentiation in the elderly and to redirect the KYN pathway towards KA. However, the clinical relevance of these effects needs further investigation.

Methods

Study participants

Ninety six men and women were enrolled from the general population in Hannover, Germany, between August 2018 and March 2019. Recruitment was conducted using a wide distribution via advertisements in local newspapers and public notices. The inclusion and exclusion criteria for each participant were determined using a formalized questionnaire. Inclusion criteria for participation were age ≥ 50 and ≤ 70 years, no regular exercise training aside from the daily activities for at least 2 years, and stable body weight (± 5 kg) for at least 6 months. Exclusion criteria were defined as cardiovascular diseases (angina pectoris, myocardial infarction, stroke, peripheral arterial occlusive disease, heart failure, cardiac arrhythmia), type 1 and 2 diabetes, renal insufficiency and liver diseases, blood coagulation disorders, chronic gastrointestinal disorders (e.g., ulcers, Crohn's disease, pancreatic insufficiency), immunological diseases (e.g. autoimmune diseases), intake of immunosuppressive drugs or laxatives, intake of supplements containing n3-FAs, smoking, alcohol-, drug and/or medicine dependency, pregnancy or lactation, retraction of the consent by the subject, concurrent participation in another clinical study, and participation in a study in the last 30 days. Following the guidelines of the Declaration of Helsinki, written informed consent was obtained from all participants before participation in the study. Ethical approval was provided by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany) (Bo/07/2018, URL: https://www.drks.de/drks_web/setLocale_EN.do). This study is registered in the German Clinical Trial Register (DRKS00014322).

Study design

The present single-centre, randomized, controlled trial in a parallel-group design is based on a cross-sectional study recently published by the authors' group [17]. For this the baseline data (t0) from this publication were used and the subjects completed a 12-week intervention phase in the present study, with a new data collection after the intervention (t12).

After baseline analysis (t0), participants were randomly assigned by an independent researcher to one of two study groups based on covariates (in descending order: sex, BMI, and age): 1) control group (CON), 2) exercise group (EX). Subjects in the EX group were further randomly subdivided according to whether they also received a nutritional intervention (EX + NUTR) or not (EX ONLY). The CON group served as a control group, and participants in this group were asked to maintain their usual level of diet and exercise throughout the 12-week study period. The EX group was instructed to perform exercise training twice per week. Participants in the EX ONLY group were asked to maintain their usual diet. Participants in the EX + NUTR group were asked to balance their diet according to guidelines provided by the managers of the study.

Exercise training

The exercise training was carried out in cooperating fitness centres after thorough instruction by a professional trainer. Each training session consisted of an initial warm-up followed by two rounds of a strength and endurance circuit.

The strength training consisted of six machine-based exercises (Leg Press, Bench Press, Lat Pull-down, Back Extension, Rowing, Abdominal Flexion) covering all major muscle groups. Each of these were performed for one minute. During the first training session, a maximum strength test was performed with three attempts for each exercise. The score of the best of the three trials was used to set the machines to 60% of the participants' maximum strength for the first two weeks of training. For the following six weeks, the load was increased by 10% and again by 5% for the last four weeks. In the last training session, the maximum strength test was repeated analogously to the initial measurement. Endurance training consisted of a four-minute session on cycle ergometers and cross-trainers with a perceived exertion corresponding to a score of 15/20 on the Borg scale [43]. Participants had 30 s of rest between each exercise. Including warm-up and rest periods, the training session could be completed in approximately one hour.

Nutrition intervention

Participants in the EX + NUTR group received individualized nutritional counselling from a professional dietitian at the start. The dietary recommendations were based on the guidelines of the German Nutrition Society (DGE) [44], which are comparable to the recommendations of the World Health Organization (WHO) [45]. They generally included the following advice for the whole time period of the intervention: Daily consumption of 3 servings of vegetables and 2 servings of fruit, consumption of cereal products with an emphasis on whole grains, daily consumption of dairy products such as milk or cheese, limited meat consumption of 300–600 g per week, consumption of fish once or twice per week, and limited consumption of salt and especially sugar.

Monitoring of food intake and physical activity

General compliance with the study instructions was monitored in all groups by fortnightly telephone calls. Compliance to the exercise intervention was additionally assessed using an exercise diary. Furthermore, participants' exercise data were recorded by the cooperating fitness centres and handed over to the study management. The amount of regular physical activity outside the intervention was recorded at baseline, after six weeks, and at the end of the study using the Freiburg Physical Activity Questionnaire [46]. Thus, altered physical activity habits of the participants could be excluded. Participants' dietary intake was monitored at baseline, after six weeks, and at the end of the study using 3-day dietary logs. The records were reviewed by dietitians for completeness, legibility, and plausibility. If necessary, ambiguities were clarified with participants. Energy and nutrient intakes were estimated using the PRODI6.4® software (Nutri-Science GmbH, Freiburg, Germany). In addition, consumption of specific food groups was assessed using the Food Frequency Questionnaire (FFQ) [47]. In the context of a previously published paper of from the same project, a significant increase in vegetables, fruit, and fish intake in the EX + NUTR group could be verified [36]. The subjects' activity levels outside the study intervention, as well as their dietary intake, can be seen in the Supplementary Material (Table S6 and S7).

Anthropometric measurements

Waist and hip circumferences were measured using a measuring tape, when the participant was in a standing position. The Waist-to-hip-ratio (WHR) was calculated. Height was measured with a stadiometer in metres (seca GmbH & Co. KG, Hamburg, Germany) and body weight was measured on a digital scale to the nearest 0.1 kg (seca GmbH & Co. KG, Hamburg, Germany).The BMI was

calculated by the ratio of weight to the squared height. All measurements were carried out by the same study personal.

Blood sampling

Blood was collected in the morning after at least two and a half days of rest, and after overnight fasting (≥10 h) using serum, EDTA, and NaF glucose tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany). Blood was either processed directly (analysis of T-cell subpopulations, blood glucose, insulin resistance) or stored at -80 °C in serum and plasma form for later analyses (analysis of KYN pathway metabolites, IL-6, CMV status).

Analysis of glucose metabolism and insulin resistance

An analysis of markers of glucose metabolism was performed by a certified laboratory (Laborärztliche Arbeitsgemeinschaft für Diagnostik und Rationalisierung e.V., in Hannover, Germany). Fasting glucose was analysed photometrically (Beckman Coulter GmbH, Krefeld, Germany). HbA1c was analysed using high-performance liquid chromatography (HPLC) (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). For the determination of insulin the electrochemiluminescence immunoassay method (ECLIA) using cobas 801e (Roche Diagnostics GmbH, Mannheim, Germany) was applied. Insulin sensitivity was evaluated using the homeostatic model assessment (HOMA): HOMA-IR = fasting insulin (µU/ml) x fasting blood glucose (mg/dl) / 405 [48].

Analysis of IL-6 plasma concentration

The plasma level of IL-6 was determined using a human Magnetic Luminex Assay (Bio-Techne, Abingdon, Oxon, UK) and a Magpix Luminex instrument (Luminex Corp, Austin, Texas, US) according to the manufacturer's instruction.

Analysis of T-cell subpopulations

Peripheral Blood Mononuclear Cells (PBMCs) were isolated from fresh EDTA whole blood by ficoll density gradient centrifugation. After the PBMCs were washed, 1×10^6 cells in 100 µl PBS were stained for 20 min in the dark with 5 µl of different fluorescence-coupled antibodies, respectively (BioLegend Inc., USA & Immuno-Tools GmbH, Germany). The antibody cocktails were composed as follows for the analysis of $CD8^+$ subtypes: anti-CD8-FITC (clone MEM-31), anti-CD197(CCR7)-PE (clone G043H7), and anti-CD45RA-PerCP (clone HI100). The percentages of naïve ($CD45RA^+/CCR7^+$), central memory ($CD45RA^-/CCR7^+$), effector memory ($CD45RA^-/CCR7^-$), and EMRA ($CD45RA^+/CCR7^-$) $CD8^+$ T-cells were quantified by flow cytometer FC 500 using the CXP software (Beckman Coulter, USA). The gating strategy was implemented in accordance with Koch et al. (2008) [4] and is illustrated in Fig. 3A. In addition, the $CD4^+/CD8^+$ T cell ratio was determined.

Analysis of tryptophan metabolites

Tryptophane (Trp) and its metabolites KYN, QA, and KA were measured via high-performance liquid chromatography (HPLC) coupled to a mass spectrometer (MS). The serum was stored in 50 µL aliquots at -80 °C until analysis. The analysis was performed on a Waters ACQUITY UPLC® system equipped with an ACQUITY UPLC® HSS T3 analytical column coupled to a Xevo® TQ-XS triple quadrupole mass spectrometer (Waters, Eschborn, Germany), as described elsewhere [49].

Analysis of Cytomegalovirus (CMV) serostatus

Serum anti-CMV immunoglobulin G (IgG) antibodies were detected using a semi-quantitative sandwich enzyme-linked immunosorbent assay (ELISA-Viditest anti-CMV IgG, VIDIA, Czech Republic). The procedure followed the manufacturer's instructions. End-point optical density was measured by the Emax Plus ELISA reader (Molecular Devices, USA).

Statistical analysis

Distribution of all data was assessed using the Shapiro–Wilk-Test and Gaussian distribution. Outliers in all outcome measures, defined as z-scores > 3, were removed using winsorization. In total, 1.37% of all data points were winsorized with a maximum of three values per parameter (3.8%), as these values were considered to be supraphysiological due to technical errors. Descriptive statistics were generated for all measured variables and the results given as mean ± standard deviation. Baseline characteristics were tested for significant intergroup differences using the independent samples T-Test and the Chi-square test (for nominal data). The Pearson's correlation coefficient was calculated to show baseline associations between age, BMI, HOMA-IR, $CD8^+$ EMRA T-cells, IL-6, and metabolites of KYN pathway among all subjects regardless of their study group. In addition, a Pearson's correlation analysis was performed with the intervention-induced changes (t12-t0) of the same parameters. Subsequently, a manipulation check was performed to determine whether the study intervention had the intended effect on participants. For this purpose, differences in the change in BMI and HOMA-IR between groups were analysed by using a separate baseline-adjusted repeated measures analysis of covariance (rmANCOVA). In addition, paired samples T-tests were performed to test for potential differences in maximum strength values of the intervention group between before and after the study intervention. The effect of the intervention on the proportions of

$CD8^+$ T-cell subsets and the KYN pathway was analysed using rmANCOVA. For this, age, BMI and CMV serostatus, as well-described confounding factors for peripheral T-cell characteristics, and plasma levels of KYN pathway metabolites [17, 34] were included as covariates in the model. An exploratory subgroup analysis was also performed with the CMV serostatus (pos./neg.) as an additional between-subject factor (rmANCOVA with study group x CMV serostatus x time interaction, including age and BMI as covariates). For all outcome measures, at first the EX versus CON group was compared. Additionally, a three-group comparison in which the EX group was subdivided into with/without nutrition intervention was performed. For the case of significant interaction effects within the three-group comparison, a Bonferroni-corrected post-hoc analysis was calculated in order to specify the intergroup differences. The level of significance was set at $p < 0.05$. Statistical analyses were conducted using IBM SPSS statistics version 28.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12979-023-00347-7.

Additional file 1: Table S1. BMI and HOMA-IR and their changes (Δ) between the start (t0) and end (t12) of the intervention. **Table S2.** Proportions of peripheral T cell subpopulations and their changes (Δ) between the start (t0) and end (t12) of the intervention. **Table S3.** Exploratory subgroup analysis regarding the influence of CMV serostatus (on exercise-induced effects) on the proportions of peripheral $CD8^+$ T-cell subsets (rmANCOVA with study group x CMV serostatus x time interaction). **Table S4.** Plasma metabolites and ratios of KYN pathway and their changes (Δ) between the start (t0) and end (t12) of the intervention. **Table S5.** Exploratory subgroup analysis regarding the influence of CMV serostatus (on exercise-induced effects) on peripheral KYN pathway metabolites (rmANCOVA with study group x CMV serostatus x time interaction). **Table S6.** Questionnaire based physical activity levels outside the intervention of the participants at baseline (0), after six weeks (6) and 12 weeks after the intervention (12). **Table S7.** Dietary Intake of food groups at baseline (0), after six weeks (6) and at the end of the intervention (12). **Figure S1.** Trends in outcomes per study group over the 12-week intervention period. **A.** BMI and HOMA-IR. **B.** Proportions of peripheral $CD8^+$ T-cell subsets. **C.** Plasma metabolites of the KYN pathway. # ($p < .05$) indicates a significant difference between groups, analysed using rmANCOVA. CON = control group, EX (two-group-comparison) = exercise group (with and without nutrition instructions), EX (three-group-comparison) = exercise group without nutrition instructions, EX+NUTR = exercise group with nutrition instructions, BMI = Body-Mass-Index, HOMAi = HOMA-Index, EMRA CD8+ = Effector Memory re-expressing CD45RA CD8+ cells, TRP = tryptophane, KYN = kynurenine, QA = quinolinic acid, KA = kynurenic acid.

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Authors' contributions
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Availability of data and materials
The datasets generated and/or analysed during the current study are not publicly available, but are available from the corresponding author on the basis of a reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted at Leibniz University Hanover from August 2018 to March 2019. The research conforms to the principles of the Declaration of Helsinki. The protocol and informed consent were reviewed and approved by the Ethics Commission of the Medical Chamber of Lower Saxony (Hannover, Germany) (Bo/07/2018, URL: https://www.drks.de/drks_web/setLocale_EN.do). This study is registered in the German Clinical Trial Register (DRKS00014322). All subjects provided written informed consent prior to any trial-related procedures.

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The authors declare no competing interests.

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References

1. Cicin-Sain L, Smyk-Pearson S, Currier N, Byrd L, Koudelka C, Robinson T, Swarbrick G, Tackitt S, Legasse A, Fischer M, Nikolich-Zugich D, Park B, Hobbs T, Doane CJ, Mori M, Axthelm MK, Lewinsohn DA, Nikolich-Zugich J. Loss of naive T cells and repertoire constriction predict poor response to vaccination in old primates. J Immunol. 2010;184:6739–45. https://doi.org/10.4049/jimmunol.0904193.
2. Goronzy JJ, Weyand CM. Successful and maladaptive T cell aging. Immunity. 2017;46:364–78. https://doi.org/10.1016/j.immuni.2017.03.010.
3. Wertheimer AM, Bennett MS, Park B, Uhrlaub JL, Martinez C, Pulko V, Currier NL, Nikolich-Žugich D, Kaye J, Nikolich-Žugich J. Aging and cytomegalovirus infection differentially and jointly affect distinct circulating T cell subsets in humans. J Immunol. 2014;192:2143–55. https://doi.org/10.4049/jimmunol.1301721.
4. Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. Immun Ageing. 2008;25:5–6. https://doi.org/10.1186/1742-4933-5-6.
5. Remmerswaal EB, Havenith SH, Idu MM, van Leeuwen EM, van Donselaar KA, Ten Brinke A, van der Bom-Baylon N, Bemelman FJ, van Lier RA, Ten Berge IJ. Human virus-specific effector-type T cells accumulate in blood but not in lymph nodes. Blood. 2012;119(7):1702–12. https://doi.org/10.1182/blood-2011-09-381574.

6. Wills MR, Carmichael AJ, Weekes MP, Mynard K, Okecha G, Hicks R, Sissons JG. Human virus-specific CD8+ CTL clones revert from CD45ROhigh to CD45RAhigh in vivo: CD45RAhighCD8+ T cells comprise both naive and memory cells. J Immunol. 1999;162(12):7080–7.
7. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood. 2003;101(11):4260–6. https://doi.org/10.1182/blood-2002-11-3577.
8. Henson SM, Riddell NE, Akbar AN. Properties of end-stage human T cells defined by CD45RA re-expression. Curr Opin Immunol. 2012;24(4):476–81. https://doi.org/10.1016/j.coi.2012.04.001.
9. Faint JM, Annels NE, Curnow SJ, Shields P, Pilling D, Hislop AD, Wu L, Akbar AN, Buckley CD, Moss PA, Adams DH, Rickinson AB, Salmon M. Memory T cells constitute a subset of the human CD8+CD45RA+ pool with distinct phenotypic and migratory characteristics. J Immunol. 2001;167(1):212–20. https://doi.org/10.4049/jimmunol.167.1.212.
10. Hertoghs KM, Moerland PD, van Stijn A, Remmerswaal EB, Yong SL, van de Berg PJ, van Ham SM, Baas F, ten Berge IJ, van Lier RA. Molecular profiling of cytomegalovirus-induced human CD8+ T cell differentiation. J Clin Invest. 2010;120(11):4077–90. https://doi.org/10.1172/JCI42758.
11. Callender LA, Carroll EC, Beal RW, Chambers ES, Nourshargh S, Akbar AN, Henson SM. Human CD 8+ EMRA T cells display a senescence-associated secretory phenotype regulated by p38 MAPK. Aging cell. 2018;17(1):e12675. https://doi.org/10.1111/acel.12675.
12. Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured? Nutr Rev. 2007;65:173–6. https://doi.org/10.1111/j.1753-4887.2007.tb00358.x.
13. Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nat Rev Drug Discov. 2019. https://doi.org/10.1038/s41573-019-0016-5.
14. Jones SP, Franco NF, Varney B, Sundaram G, Brown DA, De Bie J, Lim CK, Guillemin GJ, Brew BJ. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: Implications for inflammatory and neurodegenerative disease. PLoS ONE. 2015;10:1–18. https://doi.org/10.1371/journal.pone.0131389.
15. Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol. 2010;185:3190–8. https://doi.org/10.4049/jimmunol.0903670.
16. DiNatale BC, Murray IA, Schroeder JC, Flaveny CA, Lahoti TS, Laurenzana EM, Omiecinski CJ, Perdew GH. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. Toxicol Sci. 2010;115(1):89–97. https://doi.org/10.1093/toxsci/kfq024.
17. Boßlau TK, Wasserfurth P, Krüger B, Reichel T, Palmowski J, Nebl J, Weyh C, Schenk A, Joisten N, Stahl F, Thoms S, Gebhardt K, Hahn A, Krüger K. Abdominal obesity-related disturbance of insulin sensitivity is associated with $CD8^+$ EMRA cells in the elderly. Cells. 2021;10(5):998. https://doi.org/10.3390/cells10050998.
18. Yi HS, Kim SY, Kim JT, Lee YS, Moon JS, Kim M, Kang YE, Joung KH, Lee JH, Kim HJ, Chun K, Shong M, Ku BJ. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. Cell Death Dis. 2019;10:249. https://doi.org/10.1038/s41419-019-1494-4.
19. Laurans L, Venteclef N, Haddad Y, Chajadine M, Alzaid F, Metghalchi S, Sovran B, Denis RGP, Dairou J, Cardellini M, Moreno-Navarrete JM, Straub M, Jegou S, McQuitty C, Viel T, Esposito B, Tavitian B, Callebert J, Luquet SH, Federici M, Fernandez-Real JM, Burcelin R, Launay JM, Tedgui A, Mallat Z, Sokol H, Taleb S. Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health. Nat Med. 2018;24(8):1113–20. https://doi.org/10.1038/s41591-018-0060-4.
20. Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity? Nat Rev Immunol. 2019;19(9):563–72. https://doi.org/10.1038/s41577-019-0177-9.
21 Niemiro GM, Coletta AM, Agha NH, Mylabathula PL, Baker FL, Brewster AM, Bevers TB, Fuentes-Mattei E, Basen-Engquist K, Katsanis E, Gilchrist SC, Simpson RJ. Correction: Salutary effects of moderate but not high intensity aerobic exercise training on the frequency of peripheral T-cells associated with immunosenescence in older women at high risk of breast cancer: a randomized controlled trial. Immun Ageing. 2022;19(1):30. https://doi.org/10.1186/s12979-022-00276-x. (**Published 2022 Jun 8**).
22. Joisten N, Kummerhoff F, Koliamitra C, Schenk A, Walzik D, Hardt L, Knoop A, Thevis M, Kiesl D, Metcalfe AJ, Bloch W, Zimmer P. Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training. Exerc Immunol Rev. 2020;26:24–42.
23. Vasconcelos ABS, Aragão-Santos JC, de Resende-Neto AG, Rodrigues LS, Corrêa CB, Schimieguel DM, Camargo EA, de Paula Ramos S, Da Silva-Grigoletto ME. Effects of functional and combined training on subsets of memory T cells and functional fitness of postmenopausal women: A randomized controlled trial. Exp Gerontol. 2022;167:111898. https://doi.org/10.1016/j.exger.2022.111898.
24. Philippe M, Gatterer H, Burtscher M, Weinberger B, Keller M, Grubeck-Loebenstein B, Fleckenstein J, Alack K, Krüger K. Concentric and eccentric endurance exercise reverse hallmarks of T-cell senescence in pre-diabetic subjects. Front Physiol. 2019;10:684. https://doi.org/10.3389/fphys.2019.00684.
25. Cao Dinh H, Njemini R, Onyema OO, Beyer I, Liberman K, De Dobbeleer L, Renmans W, Vander Meeren S, Jochmans K, Delaere A, Knoop V, Bautmans I. Strength endurance training but not intensive strength training reduces senescence-prone T cells in peripheral blood in community-dwelling elderly women. J Gerontol A Biol Sci Med Sci. 2019;74(12):1870–8. https://doi.org/10.1093/gerona/gly229.
26. Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy EC, Pircher H, Simpson RJ. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. Brain Behav Immun. 2014;39:142–51. https://doi.org/10.1016/j.bbi.2013.05.003.
27. Simpson RJ, Bigley AB, Spielmann G, LaVoy EC, Kunz H, Bollard CM. Human cytomegalovirus infection and the immune response to exercise. Exerc Immunol Rev. 2016;22:8–27.
28. Krüger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, Gindler K, Kimmelmann L, Walscheid R, Muders K, Frech T, Eder K, Mooren FC. Apoptosis of T-cell subsets after acute high-intensity interval exercise. Med Sci Sports Exerc. 2016;48(10):2021–9. https://doi.org/10.1249/MSS.0000000000000979.
29. Simpson RJ. Aging, persistent viral infections, and immunosenescence: can exercise "make space"? Exerc Sport Sci Rev. 2011;39(1):23–33. https://doi.org/10.1097/JES.0b013e318201f39d.
30. Mooren FC, Kruger K. Apoptotic lymphocytes induce progenitor cell mobilization after exercise. J Applied Physiol. 2015;119:135–9. https://doi.org/10.1152/japplphysiol.00287.2015.
31. Spielmann G, McFarlin BK, O'Connor DP, Smith PJ, Pircher H, Simpson RJ. Aerobic fitness is associated with lower proportions of senescent blood T-cells in man. Brain Behav Immun. 2011;25(8):1521–9. https://doi.org/10.1016/j.bbi.2011.07.226.
32. Grande AJ, Keogh J, Silva V, Scott AM. Exercise versus no exercise for the occurrence, severity, and duration of acute respiratory infections. Cochrane Database Syst Rev. 2020;4:4. https://doi.org/10.1002/14651858.CD010596.pub3.
33. Simpson RJ, Hussain M, Baker F, Bigley AB, Peek MK, Stowe RP. Cardiorespiratory fitness is associated with better control of latent herpesvirus infections in a large ethnically diverse community sample: evidence from the Texas City stress and health study. Brain Behav Immun. 2017;66:e35. https://doi.org/10.1016/j.bbi.2017.07.128.
34. Joisten N, WencesChirino TY, Boßlau TK, Wasserfurth P, Hahn A, Krüger K, Zimmer P. Older adults with cytomegalovirus reveal increased $CD8^+$ / $CD4^+$ EMRA T cells and elevated systemic levels of kynurenic acid. Immunology. 2022. https://doi.org/10.1111/imm.13609.
35. Messaoudi I, Warner J, Fischer M, et al. Delay of T cell senescence by caloric restriction in aged long-lived nonhuman primates. Proc Natl Acad Sci U S A. 2006;103(51):19448–53. https://doi.org/10.1073/pnas.0606661103.
36. Wasserfurth P, Nebl J, Schuchardt JP, Müller M, Boßlau TK, Krüger K, Hahn A. Effects of exercise combined with a healthy diet or calanus finmarchicus oil supplementation on body composition and metabolic markers-a pilot study. Nutrients. 2020;12(7):2139. https://doi.org/10.3390/nu12072139.
37. Agudelo LZ, Femenía T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, Correia JC, Izadi M, Bhat M, Schuppe-Koistinen I, Pettersson AT, Ferreira DMS, Krook A, Barres R, Zierath JR, Erhardt S, Lindskog M, Ruas JL. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell. 2014;159(1):33–45. https://doi.org/10.1016/j.cell.2014.07.051.

38. Allison DJ, Nederveen JP, Snijders T, Bell KE, Kumbhare D, Phillips SM, Parise G, Heisz JJ. Exercise training impacts skeletal muscle gene expression related to the kynurenine pathway. Am J Physiol Cell Physiol. 2019;316(3):C444–8. https://doi.org/10.1152/ajpcell.00448.2018.
39. Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenic acid: potential in the treatment of psychiatric disorders. CNS Drugs. 2009;23(2):91–101. https://doi.org/10.2165/00023210-200923020-00001.
40. Jenny M, Schroecksnadel S, Fuchs D. Testing for immunomodulatory properties of nanoparticles. J Biomed Nanotechnol. 2011;7(1):11–2. https://doi.org/10.1166/jbn.2011.1175.
41. Yu E, Papandreou C, Ruiz-Canela M, Guasch-Ferre M, Clish CB. Association of tryptophan metabolites with incident type 2 diabetes in the PREDIMED Trial: a case-cohort study. Clin Chem. 2018;64(8):1211–20. https://doi.org/10.1373/clinchem.2018.288720.
42. Mudry JM, Alm PS, Erhardt S, Goiny M, Fritz T. Direct effects of exercise on kynurenine metabolism in people with normal glucose tolerance or type 2 diabetes. Diabetes Metab Res Rev. 2016;32(7):754–61. https://doi.org/10.1002/dmrr.2798.
43. Borg G. Perceived exertion as an indicator of somatic stress. Scand J Rehabil Med. 1970;2:92–8.
44. Deutsche Gesellschaft für Ernährung e.V.: 10 Regeln Der DGE. 2018. https://www.dge.de/ernaehrungspraxis/vollwertige-ernaehrung/10-regeln-der-dge/. Accessed 8 May 2023.
45. World Health Organisation (WHO): Healthy Diet. https://www.who.int/news-room/fact-sheets/detail/healthy-diet (2020). Accessed 27 Dec 2022.
46. Frey I, Berg A, Grathwohl D, Keul J. Freiburger Fragebogen zur körperlichen Aktivität-Entwicklung, Prüfung und Anwendung. Soz- Präventivmedizin Social Prev Med. 1999;44:55–64. https://doi.org/10.1007/BF01667127.
47. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol. 1985;122:51–65. https://doi.org/10.1093/oxfordjournals.aje.a114086.
48. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412–9. https://doi.org/10.1007/BF00280883.
49. Joisten N, Schumann M, Schenk A, Walzik D, Freitag N, Knoop A, Thevis M, Bloch W, Zimmer P. Acute hypertrophic but not maximal strength loading transiently enhances the kynurenine pathway towards kynurenic acid. Eur J Appl Physiol. 2020;120(6):1429–36. https://doi.org/10.1007/s00421-020-04375-9.

Publisher's Note

Gesamtdiskussion

Die Ergebnisse von Boßlau et al. (2021) zeigten eine statistisch signifikante Assoziation zwischen einer reduzierten Insulinsensitivität und einem erhöhten Anteil zirkulierender $CD8^+$ EMRA T-Zellen (als prozentualer Anteil der gesamten $CD8^+$ T-Zell-Population). Außerdem korrelierten höhere Konzentrationen von Kynurenin im peripheren Blut älterer Probanden mit einer verminderten Insulinsensitivität. Darüber hinaus wurden die $CD8^+$ EMRA T-Zellen, als auch die Kynurenin-Konzentration als Mediatorvariablen in der übergewichtsinduzierten Reduktion der Insulinsensitivität ermittelt. Dies wurde durch die Tatsache gestützt, dass insbesondere die Subgruppe der Probanden mit einem als fettleibig eingestuften BMI (> 30 kg/m^2) einen höheren Anteil an $CD8^+$ EMRA T-Zellen sowie erhöhte Kynurenin-Konzentrationen aufwies. In der Arbeit von Boßlau et al. (2023) konnte aufbauend darauf gezeigt werden, dass sich ein 12-wöchiges kombiniertes Kraft-Ausdauertraining positiv auf den BMI sowie die Insulinsensitivität älterer Probanden auswirkt und die progressive Akkumulation von EMRA-Zellen im peripheren $CD8^+$ T-Zell-Kompartiment reduziert. Darüber hinaus beeinflusste das Training den Kynurenin-Stoffwechselweg, indem es die Konzentration der neuroprotektiven Kynurensäure im peripheren Blut erhöhte.

Die Rolle von $CD8^+$ EMRA T-Zellen in der (übergewichtsassoziierten) Reduktion der Insulinsensitivität

In der Arbeit von Boßlau et al. (2021) wurde gezeigt, dass endständig differenzierte $CD8^+$ EMRA T-Zellen im peripheren Blut von adipösen Personen vermehrt akkumulieren. Einerseits beschleunigt eine Adipositas die Thymusinvolution, was zu einer geringeren Produktion naiver T-Zellen und zu einer proportionalen Zunahme hochdifferenzierter T-Zellen führt (Yang et al., 2009). Andererseits verstärkt eine Zunahme des viszeralen Fettgewebes die Differenzierung von T-Zellen aufgrund der Freisetzung von proinflammatorischen Adipokinen und Zytokinen (Shirakawa et al., 2016). Es entsteht ein sich selbst verstärkender Zyklus aus fortschreitender T-Zell-Differenzierung und entzündlicher Zytokinproduktion, da $CD8^+$ EMRA T-Zellen selbst eine Vielzahl von entzündungsfördernden Faktoren sezernieren (Davalos et al., 2010). Bekannt ist, dass Übergewicht und insbesondere viszerale Adipositas die Insulinsensitivität reduzieren und somit die Entwicklung eines Typ 2 Diabetes mellitus begünstigen. Die zugrundeliegenden Mechanismen sind hingegen noch nicht abschließend geklärt. Die Ergebnisse von Boßlau et al. (2021) deuten darauf hin, dass die Akkumulation von $CD8^+$ EMRA T-Zellen in der Entstehung einer übergewichtsinduzierten Insulinresistenz eine pathophysiologische Rolle spielen könnte.

Zwar gewährleistet die große Stichprobe der Arbeit von Boßlau et al. (2021) eine hohe Repräsentativität der Ergebnisse, jedoch können aufgrund des Querschnittsdesigns keine kausalen

Schlüsse gezogen werden. Dennoch basieren die Überlegungen der Arbeit auf dem Wissen um biologische Zusammenhänge und Mechanismen in experimentellen (Tier-)Versuchen, die den statistischen Ansatz legitimieren und die Schlussfolgerungen stützen. Yi et al. (2019) transferierten seneszente $CD8^+$ T-Zellen ($CD44^+CD153^+$) aus Mäusen, die mit einer fettreichen Diät gefüttert wurden, in junge Empfängermäuse, die daraufhin im Vergleich zu Kontrollmäusen eine verschlechterte systemische Glukosetoleranz und eine geringere Insulinsensitivität aufwiesen. Darüber hinaus wiesen die Forscher nach, dass seneszente $CD8^+$ T-Zellen ($CD28^-CD57^+$) bei Menschen mit Prädiabetes zahlreicher in der Leber lokalisiert sind und diese eine Vielzahl von proinflammatorischen Zytokinen und zytotoxischen Substanzen exprimieren. In Zellkulturexperimenten belegten die Autoren außerdem eine direkte Interaktion zwischen seneszenten $CD8^+$ T-Zellen und der hepatogenen Insulinwirkung. Mittels Echtzeit-PCR-Analysen zeigten sie im ersten Schritt, dass die Behandlung mit Insulin die Expression von glukoneogenen Enzymen in menschlichen Leberzellen signifikant reduzierte. Die Unterdrückung der glukoneogenen Gentranskription durch Insulin wurde jedoch im zweiten Schritt durch die Co-Kultur mit seneszenten $CD8^+$ T-Zellen statistisch signifikant gehemmt. Diese Daten legen nahe, dass die hepatische Insulinsensitivität durch seneszente $CD8^+$ T-Zellen beeinträchtigt wird, wobei die genauen molekularen Mechanismen noch unbekannt sind. Wir können nur spekulieren, dass zelluläre Interaktionen oder von $CD8^+$ EMRA T-Zellen sezernierte (proinflammatorische) Moleküle die Insulinsensitivität beeinflussen. Verschiedene entzündungsfördernde Zytokine wie IL-6, TNF-α, Adiponektin und Resistin sind direkt oder indirekt an der Regulierung der Insulinempfindlichkeit beteiligt (Nieto-Vazquez et al., 2009; Kim et al., 2009). In unserer Studie konnten wir jedoch für keines dieser Zytokine eine vermittelnde Rolle feststellen.

12-wöchiges Kraft-Ausdauer-Training vermindert die Progression der $CD8^+$ T-Zell-Differenzierung im Alter

In der Arbeit von Boßlau et al. (2023) wurde gezeigt, dass der Anteil der EMRA-Zellen innerhalb des peripheren $CD8^+$ T-Zell-Kompartiments über die zwölfwöchige Studiendauer in der Kontrollgruppe signifikant zunahm, während er in der Interventionsgruppe unverändert blieb. Somit scheint ein kombiniertes Kraft-Ausdauer-Training die progressive Zunahme des $CD8^+$ EMRA T-Zell-Pools abzuschwächen und könnte ein geeigneter Ansatz zur Verzögerung der fortschreitenden T-Zell-Differenzierung im Alter sein. Dies steht im Einklang mit früheren Daten. Vasconcelos et al. (2022) wiesen bei 108 postmenopausalen Frauen nach, dass sowohl funktionelles als auch kombiniertes Kraft-Ausdauertraining über 16 Wochen (dreimal wöchentlich für jeweils 45 Minuten) den Anteil der EMRA-Zellen sowohl im $CD8^+$ als auch im $CD4^+$ T-Zellkompartiment im Vergleich zu einer Kontrollgruppe reduzierte. In ihrer Studie verwendeten die Autoren die gleichen Oberflächenmarker für die EMRA Zelldifferenzierung (CCR7, CD45RA) und die Probanden absolvierten ebenfalls ein kombiniertes Kraft-

Ausdauertraining, was die Reproduzierbarkeit der Ergebnisse von Boßlau et al (2023) unterstreicht. Philippe et al. (2019) zeigten bei 16 älteren Erwachsenen mit Prädiabetes bereits nach einem Interventionszeitraum von nur drei Wochen eine trainingsinduzierte Wirkung auf die T-Zell-Differenzierung. In der Studie absolvierten die Probanden dreimal wöchentlich ein aerobes Trainingsprogramm, wobei sie je nach Studiengruppe bergauf (konzentrisch) oder bergab (exzentrisch) liefen. Die Ergebnisse zeigten, dass beide Protokolle den Anteil der $CD4^+$ und $CD8^+$ EMRA T-Zellen ($CCR7^-/CD45RO^-$) signifikant verringerten, während der Anteil der zentralen Gedächtniszellen sowie der naiven T-Zellen zunahm. Dinh et al. (2019) untersuchten die Auswirkungen eines sechswöchigen Bewegungstrainings (2-3 Mal pro Woche) auf die Anzahl der seneszenten $CD8^+$ T-Zellen ($CD57^+$) bei 100 Frauen im Alter von ≥ 65 Jahren. Sie verglichen verschiedene Trainingsmodalitäten: Intensives Krafttraining (80 % der maximalen Wiederholungszahl, 3 Sätze mit 10 Wiederholungen), Kraftausdauertraining (40 % der maximalen Wiederholungszahl, 2 Sätze mit 30 Wiederholungen) und eine Kontrollbedingung (Stretching). Die Autoren stellten einen signifikanten Rückgang des Prozentsatzes und der absoluten Anzahl der seneszenten $CD8^+$ T-Zellen in der Kraftausdauertrainingsgruppe fest. Das Ausmaß der Veränderung stand auch in einem positiven Zusammenhang mit der Anzahl der Trainingseinheiten. Interessanterweise hatte intensives Krafttraining keinen Einfluss auf den Anteil der seneszenten Zellen. Diese Ergebnisse deuten darauf hin, dass sowohl die Modalität als auch die Häufigkeit des Trainings die trainingsinduzierte Wirkung auf die T-Zell-Differenzierung bestimmen könnten. Dies steht im Einklang mit der Arbeit von Niemiro et al. (2021). Bei älteren Frauen mit Brustkrebsrisiko wurden unterschiedliche Auswirkungen eines 12-wöchigen Ausdauertrainings (dreimal wöchentlich) auf die Anzahl der $CD4^+$ und $CD8^+$ T-Zell-Untergruppen in Abhängigkeit von der Trainingsintensität nachgewiesen. Dabei hatte ein Training mit hoher Intensität eher "pro-seneszente" (Abnahme der naiven $CD4^+$ T-Zellen) und ein Training mit moderater Intensität eher "anti-seneszente" (Zunahme der $CD8^+$ T-Zellen mit Effektorgedächtnis) Effekte. Das Training zeigte jedoch keine signifikanten Auswirkungen auf die $CD8^+$ EMRA T-Zellfraktion, allerdings war die Gruppengröße der Studie relativ klein (n=8), was kritisch betrachtet werden sollte. Zusammengenommen deuten die aktuellen Daten darauf hin, dass die $CD8^+$ T-Zell-Differenzierung bei älteren Menschen sowohl durch Ausdauer- als auch durch Kraftausdauertraining beeinflusst werden kann und womöglich in einem „verjüngten“ peripheren T-Zell-Profil resultiert. Dabei scheinen Trainingsintensität und -häufigkeit das Ausmaß der Effekte zu determinieren. Um schlüssige Trainingsempfehlungen geben zu können, sind weitere Arbeiten erforderlich, die verschiedene Trainingspläne miteinander vergleichen.

Über die Mechanismen der trainingsinduzierten Anpassungen im Kompartiment der $CD8^+$ T-Zellen kann derzeit nur spekuliert werden. Es ist denkbar, dass adrenerge Reize und hämodynamische Veränderungen bei repetitiver Belastung zu einer Umverteilung hochdifferenzierter EMRA T-Zellen ins

Blut führen (Duggal et al., 2019), wo sie pro-apoptotischen Faktoren wie reaktiven Sauerstoffspezies ausgesetzt sind (Spielmann et al., 2014; Simpson et al., 2016). Da insbesondere terminal differenzierte Zellen empfindlich auf diese Faktoren zu reagieren scheinen (Krüger et al., 2016), könnten sie durch wiederholte akute Belastung eliminiert werden und sich in der Folge das Verhältnis von hochdifferenzierten EMRA zu naiven $CD8^+$ T-Zellen verschieben (Simpson et al., 2011; Mooren et al., 2015). Diese Hypothese wird von Krüger et al. (2016) gestützt, die zeigten, dass eine hochintensive Trainingseinheit (HIT) eine vermehrte Apoptose hochdifferenzierter T-Zellen ($CD28^-CD57^+$) induziert, verglichen mit einer geringeren Trainingsintensität.

In der Arbeit von Boßlau et al. (2023) zeigte eine zusätzliche Ernährungsumstellung nach den Empfehlungen der DGE keine zusätzlichen Effekte auf die Zusammensetzung des peripheren $CD8^+$ T-Zell-Kompartiments. Generell wurde der Einfluss von Ernährung auf die T-Zell-Differenzierung bis dato lediglich in einer Studie mit älteren nicht-menschlichen Primaten untersucht (Messaoudi et al., 2006). Dabei zeigte sich, dass eine langfristige Kalorienrestriktion die T-Zell-Differenzierung verzögerte und die Produktion entzündlicher Zytokine durch Gedächtnis-T-Zellen verringerte. Zukünftige Studien, die sich spezifischer mit den Effekten einer Kalorienrestriktion auf die T-Zell-Differenzierung im Menschen befassen, wären demnach ein interessanter Forschungsansatz.

Die Rolle von Kynurenin in der (übergewichtsassoziierten) Reduktion der Insulinsensitivität

Die Ergebnisse von Boßlau et al. (2021) deuten auf eine potentielle Rolle des Tryptophan-Metaboliten Kynurenin bei der Entwicklung einer gestörten Insulinsensitivität in übergewichtigen älteren Personen hin. Dies deckt sich mit Ergebnissen aus anderen Arbeiten. Munipally et al. (2011) wiesen erhöhte Kynurenin-Serumspiegel in Patienten mit diabetischer Retinopathie nach. Vangipurapu et al. (2020) zeigten an 5181 Probanden, dass die Konzentrationen der Kynurenin-Metaboliten Kynurensäure und Xanthurensäure mit einer verminderten Insulinsekretion und Insulinsensitivität sowie mit einer erhöhten Anfälligkeit für die Entwicklung eines Diabetes mellitus Typ 2 korrelieren. Darüber hinaus wiesen Laurans et al. (2018) in einem Mausmodell nach, dass eine fettreiche Diät die Expression des Schlüsselenzyms IDO in verschiedenen Geweben erhöht, wodurch der Metabolismus von Tryptophan in Richtung Kynurenin gelenkt wird. Gleichzeitig erniedrigte sich die Insulinsensitivität der Versuchstiere. IDO-Knockout-Mäuse zeigten hingegen keine verminderte Insulinsensitivität nach fettreicher Diät. Somit scheinen Kynurenin und/oder seine Metabolite eine kausale Rolle in der Entstehung einer ernährungsbedingt verringerten Insulinsensitivität zu spielen. Die potentiellen Mechanismen wurden in früheren Arbeiten bereits angerissen. Für Xanthurensäure wurde gezeigt, dass sie zur Bildung von Insulinchelat-Komplexen (XA-In) führt, welche antigene Eigenschaften besitzen und eine um 49 % geringere Aktivität als reines Insulin aufweisen (Meyranov et al., 1998). Außerdem

wurde für einzelne Kynurenin-Metabolite eine hemmende Wirkung auf die Insulinsekretion im Pankreas der Ratte (Inubushi et al., 2012) sowie die Auslösung von Apoptose der Betazellen der Bauchspeicheldrüse durch einen Caspase-3-abhängigen Mechanismus nachgewiesen (Malina et al., 2001). Es fehlen jedoch weitere Arbeiten, welche den kausalen Einfluss der Kynurenin-Metabolite in der Entstehung eines Diabetes mellitus Typ 2 verifizieren und die entscheidenden zugrundeliegenden Mechanismen erörtern.

12-wöchiges Kraft-Ausdauer-Training und diätetische Maßnahmen beeinflussen den Kynurenin-Stoffwechsel

Die Arbeit von Boßlau et al. (2023) ist nach Wissen der Autoren die erste überhaupt, die einen signifikanten Anstieg der Plasmakonzentration von Kynurensäure als Reaktion auf eine mehrwöchige Trainingsintervention zeigte (Joisten et al., 2020). In vergleichbaren Arbeiten wurden bis dato lediglich erhöhte Genexpressionen der kynureninsäurebildenden KAT-Isoenzyme in der Skelettmuskulatur nachgewiesen (Agudelo et al., 2014; Allison et al., 2019). Es ist fraglich, wieso die verstärkte Expression von KAT in diesen Arbeiten keinen stärkeren Effekt auf die Kynurensäure-Konzentration im peripheren Blut hatte. Möglicherweise spielen hier epigenetische Einflüsse oder eine schnelle Verstoffwechselung und Ausscheidung der synthetisierten Kynurenin-Metaboliten eine Rolle (Erhardt et al., 2009).

Bei vielen neurodegenerativen Erkrankungen besteht ein Ungleichgewicht zwischen den Downstream-Mataboliten von Kynurenin im ZNS mit einer Anhäufung der neurotoxischen Chinolinsäure und einer Reduktion der neuroprotektiven Kynurensäure (Joisten et al., 2020). Durch das zwölfwöchige Kraft-Ausdauer-Training in der Arbeit von Boßlau et al. (2023) verschob sich das Verhältnis im peripheren Blut zugunsten der Kynurensäure, während es in der Kontrollgruppe zugunsten der Chinolinsäure verschoben wurde. Dabei handelte es sich zwar lediglich um Trends ohne statistische Signifikanz, jedoch stehen die Daten im Einklang mit einer Arbeit von Allison et al. (2019), die einen nicht signifikanten Anstieg von Kynurenäure und einen nicht signifikanten Rückgang von Chinolinsäure im peripheren Blut nach 12-wöchigem Ausdauertraining zeigten. Regelmäßiges Kraft-Ausdauer-Training könnte somit möglicherweise über eine Rebalancierung des Kynurenin-Stoffwechselwegs neuroprotektive Effekte induzieren. Um diese Hypothese zu bestätigen, sind zukünftige Studien über längere Interventionszeiträume erstrebenswert. Außerdem sollten die Konzentrationen der Bluthirnschranken-gängigen Kynurenin-Metabolite zusätzlich im Liquor bestimmt werden, um spezifischere Aussagen über Effekte im zentralen Nervensystem treffen zu können. Die Entwicklung von Diabetes mellitus Typ 2 wurde ebenfalls mit erhöhten Chinolinsäure-Konzentrationen und einer verminderten Kynurensäure-Synthese in Verbindung gebracht (Yu et al., 2018; Mudry et al., 2016). Dies suggeriert, dass ein kombiniertes Kraft-Ausdauer-Training über eine Rebalancierung des

Kynurenin Stoffwechselwegs auch das Diabetes-Risiko älterer Menschen verringern könnte, jedoch bleibt dies zum jetzigen Zeitpunkt spekulativ.

In unserer Studie hatte eine begleitende Ernährungsumstellung nach Leitlinie der DGE eine mildernde Wirkung auf die belastungsinduzierten Veränderungen der Kynurenin-Metabolite. Beispielsweise zeigten sich die peripheren Konzentrationen von Chinolinsäure in der Studiengruppe mit begleitender Ernährungsintervention gegenüber der alleinigen Trainingsintervention (nicht-signifikant) reduziert. Möglicherweise könnten Antioxidantien aus der Nahrung hierbei eine Rolle spielen. Jenny et al. (2011) zeigten, dass spezifische Verbindungen wie Vitamin C und E sowie das Stilben Resveratrol und Kaffeeflavonoide in der Lage sind, die T-Zell-Aktivierung und die IFN-γ-Produktion peripherer mononukleärer Zellen in vitro zu mildern, was in einer verringerten Aktivität nachgeschalteter Stoffwechselwege, wie dem Tryptophan-Abbau durch IDO-1, resultiert. Da in einer zuvor veröffentlichten Pilotstudie aus dem gemeinsamen Projekt von Boßlau et al (2021 & 2023) bereits bestätigt wurde, dass die Probanden mit Ernährungsintervention, verglichen mit jenen ohne Ernährungsumstellung, signifikant mehr Gemüse und Obst konsumierten (Wasserfurth et al., 2020), erscheint dies ein denkbarer Erklärungsansatz.

12-wöchiges Kraft-Ausdauer-Training und diätetische Maßnahmen senken den BMI und beeinflussen die Insulinsensitivität

12-wöchiges Kraft-Ausdauer-Training reduzierte den BMI signifikant im Vergleich zur Kontrollgruppe und eine begleitende Ernährungsintervention verstärkte diesen Effekt (Boßlau et al., 2023). Die Insulinsensitivität wurde in der Interventionsgruppe gesteigert, wobei dies nicht in statistisch signifikanten Unterschieden zur Kontrollgruppe resultierte. Die Gründe hierfür können vielfältig sein. Ein Vergleich mit anderen Arbeiten lässt jedoch vermuten, dass die Interventionsdauer, die Trainingshäufigkeit und -intensität sowie der Anteil der Ausdauerübungen im Rahmen der Studie von Boßlau et al. (2023) zu gering war, um stärkere Anpassungen zu erzielen. O'Donovan et al. (2005) konnten eine signifikante Zunahme der Insulinsensitivität nach 24-wöchigem Ausdauertraining verzeichnen. Dabei trainierten die Probanden dreimal wöchentlich bei 60-80 % ihrer VO_{2max} und einem Gesamtenergieverbrauch von 400kcal pro Einheit. Short et al. (2003) erzielten eine gesteigerte Insulinsensitivität mit einem 16-wöchigen Ausdauertrainingsregime (3x wöchentlich à 40 Minuten bei 80% der maximalen Herzfrequenz). Im Gegensatz dazu trainierten die Probanden von Boßlau et al. (2023) nur zweimal wöchentlich über zwölf Wochen und die Ausdauerkomponente pro Trainingseinheit umfasste lediglich 16 Minuten. Möglicherweise verhinderten diese moderaten Trainingsvorgaben größere Anpassungen hinsichtlich der Insulinsensitivität und generell der verschiedenen Studienparameter. Nichtsdestotrotz wurde das Trainingsregime bewusst ausgewählt, um eine realistische Umsetzbarkeit des Trainings im Alltag und somit eine hohe extrinsische Reliabilität

unserer Ergebnisse zu gewährleisten. Dass dies gelungen ist, wird durch eine über 80-prozentige Studienadhärenz der Probanden bestätigt (Boßlau et al., 2023).

Ausblick

Übergewicht begünstigt das Auftreten vieler internistischer Krankheiten, von denen insbesondere Personen am Ende des BMI-Spektrums betroffen sind. Stoffwechselkrankheiten entwickeln sich oft langsam und allmählich, jedoch können beginnende pathologische Veränderungen bereits bei als klinisch gesund eingestuften Personen wie in der Arbeit von Boßlau et al. (2021) festgestellt werden. Die Erkenntnis, dass $CD8^+$ EMRA T-Zellen sowie Metabolite des Kynurenin-Stoffwechselwegs einen Beitrag in der Pathophysiologie einer reduzierten Insulinsensitivität spielen könnten, ist aus zweierlei Gründen wichtig. Zum einen könnten Analysen dieser Parameter einen prädiktiven Marker für eine beginnende Insulinresistenz darstellen. Zum anderen wäre es wichtig, potentielle molekulare Wechselwirkungen zu identifizieren, wie $CD8^+$ EMRA T-Zellen oder Kynurenin-Metabolite mit insulinsensitiven Organen interagieren. Somit könnten möglicherweise neue Targets in der Behandlung und Prävention des Diabetes mellitus Typ 2 aufgedeckt werden. In diesem Zusammenhang ist weitere Grundlagenforschung erforderlich. Insbesondere gilt es in zukünftigen Arbeiten, die heterogene $CD8^+$ EMRA T-Zell-Fraktion anhand weiterer Oberflächenmarker besser zu charakterisieren. Außerdem gibt es empfindlichere Messmethoden für die T-Zell-Differenzierung, beispielsweise Deep T-Cell Receptor Beta Sequencing, die in zukünftige Arbeiten einbezogen werden sollten. So könnte es gelingen, zielgerichtetere Aussagen hinsichtlich potentieller Pathomechanismen und Therapiemöglichkeiten zu treffen. Ein kombiniertes Kraft-Ausdauer-Training scheint den peripheren $CD8^+$ EMRA T-Zell-Pool zu beeinflussen, jedoch sind die zugrundeliegenden Mechanismen unklar. Sowohl eine vermehrte Apoptose dieser Zellen unter sportlicher Belastung als auch eine Umverteilung in extravaskuläre Gewebe ist denkbar. Hierzu bedarf es weiterer Arbeiten. Insbesondere wäre es interessant zu untersuchen, wie sich körperliches Training auf $CD8^+$ EMRA T-Zellen im Fettgewebe auswirkt, da ihre dortige Präsenz zur lokalen Entzündung beiträgt und die Insulinsensitivität der Fettzellen reduziert (Wanjalla et al., 2019). Darüber hinaus sollten auch funktionelle Aspekte der $CD8^+$ EMRA Zellpopulation in zukünftigen Studien berücksichtigen werden. Möglicherweise könnte Kraft-Ausdauer-Training die proinflammatorische Zytokinproduktion der $CD8^+$ EMRA T-Zellen in verschiedenen Geweben vermindern und auf diese Weise die Progression einer Insulinresistenz verhindern. Die klinische Relevanz der Metabolite des Kynurenin-Stoffwechselwegs wurde bislang primär im Rahmen neurodegenerativer Erkrankungen diskutiert und verifiziert. Die Daten von Boßlau et al. (2021) sowie weiterer zitierter Arbeiten deuten jedoch zusätzlich auf eine pathophysiologische Rolle im Rahmen des Diabetes mellitus Typ 2 hin. Diese gilt es durch neue Studienansätze zu belegen und hinsichtlich der zugrundeliegenden Mechanismen zu analysieren.

Literaturverzeichnis

Agudelo LZ, Femenía T, Orhan F, Porsmyr-Palmertz M, Goiny M, Martinez-Redondo V, Correia JC, Izadi M, Bhat M, Schuppe-Koistinen I, Pettersson AT, Ferreira DMS, Krook A, Barres R, Zierath JR, Erhardt S, Lindskog M, Ruas JL. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell. 2014;159(1):33-45. https://doi.org/10.1016/j.cell.2014.07.051.

Ahmed B, Sultana R, Greene MW. Adipose tissue and insulin resistance in obese. Biomedecine & pharmacotherapie, 2021; 137, 111315. https://doi.org/10.1016/j.biopha.2021.111315.

Allison DJ, Nederveen JP, Snijders T, Bell KE, Kumbhare D, Phillips SM, Parise G, Heisz JJ. Exercise training impacts skeletal muscle gene expression related to the kynurenine pathway. Am J Physiol Cell Physiol. 2019;316(3):C444–C448. https://doi.org/10.1152/ajpcell.00448.2018.

Callender LA, Carroll EC, Beal RW, Chambers ES, Nourshargh S, Akbar AN, Henson SM. Human CD 8+ EMRA T cells display a senescence-associated secretory phenotype regulated by p38 MAPK. Aging cell. 2018;17(1). https://doi.org/10.1111/acel.12675.

Cao Dinh H, Njemini R, Onyema OO, Beyer I, Liberman K, De Dobbeleer L, Renmans W, Vander Meeren S, Jochmans K, Delaere A, Knoop V, Bautmans I. Strength Endurance Training but Not Intensive Strength Training Reduces Senescence-Prone T Cells in Peripheral Blood in Community-Dwelling Elderly Women. J Gerontol A Biol Sci Med Sci. 2019;74(12):1870-1878. https://doi.org/10.1093/gerona/gly229.

Davalos, A.R.; Coppe, J.P.; Campisi, J.; Desprez, P.Y. Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev. 2010, 29, 273-83. https://doi.org/10.1007/s10555-010-9220-9.

DiNatale BC, Murray IA, Schroeder JC, Flaveny CA, Lahoti TS, Laurenzana EM, Omiecinski CJ, Perdew GH. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. Toxicol Sci. 2010;115(1):89-97. https://doi.org/10.1093/toxsci/kfq024.

Duggal NA, Niemiro G, Harridge SDR, Simpson RJ, Lord JM. Can physical activity ameliorate immunosenescence and thereby reduce age-related multi-morbidity?. Nature Reviews Immunology. 2019;19(9):563-72. https://doi.org/10.1038/s41577-019-0177-9.

Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenic acid: potential in the treatment of psychiatric disorders. CNS drugs. 2009;23(2):91–101. https://doi.org/10.2165/00023210-200923020-00001.

Faint JM, Annels NE, Curnow SJ, Shields P, Pilling D, Hislop AD, Wu L, Akbar AN, Buckley CD, Moss PA, Adams DH, Rickinson AB, Salmon M. Memory T cells constitute a subset of the human CD8+CD45RA+ pool with distinct phenotypic and migratory characteristics. J Immunol. 2001;167(1):212-20. https://doi.org/10.4049/jimmunol.167.1.212.

Franceschi, C. Inflammaging as a major characteristic of old people: can it be prevented or cured?. Nutr Rev. 2007;65:173-76. https://doi.org/10.1111/j.1753-4887.2007.tb00358.x.

Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood. 2003;101(11):4260-6. https://doi.org/10.1182/blood-2002-11-3577.

Goronzy JJ, Fang F, Cavanagh MM, Qi Q, Weyand CM. Naive T cell maintenance and function in human aging. J Immunol. 2015;194(9):4073-4080. https://doi.org/10.4049/jimmunol.1500046.

Goronzy JJ, Weyand CM. Successful and Maladaptive T Cell Aging. Immunity. 2017;46(3):364-378. https://doi.org/10.1016/j.immuni.2017.03.010.

Henson SM, Riddell NE, Akbar AN. Properties of end-stage human T cells defined by CD45RA re-expression. Curr Opin Immunol. 2012;24(4):476-81. https://10.1016/j.coi.2012.04.001.

Hertoghs KM, Moerland PD, van Stijn A, Remmerswaal EB, Yong SL, van de Berg PJ, van Ham SM, Baas F, ten Berge IJ, van Lier RA. Molecular profiling of cytomegalovirus-induced human CD8+ T cell differentiation. J Clin Invest. 2010;120(11):4077-90. https://doi.org/10.1172/JCI42758.

Inubushi T, Kamemura N, Oda M, Sakurai J, Nakaya Y, Harada N, Suenaga M, Matsunaga Y, Ishidoh K, Katunuma N. L-Tryptophan Suppresses Rise in Blood Glucose and Preserves Insulin Secretion in Type-2 Diabetes Mellitus Rats. J. Nutr. Sci. Vitaminol. 2012;58:415–422. https://doi.org/10.3177/jnsv.58.415.

Jacomet F, Cayssials E, Basbous S, et al. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. Eur J Immunol. 2015;45(7):1926-1933. https://doi.org/10.1002/eji.201545539.

Jenny M, Schroecksnadel S, Fuchs D. Testing for immunomodulatory properties of nanoparticles. J Biomed Nanotechnol. 2011;7(1):11–2. https://doi.org/10.1166/jbn.2011.1175.

Joisten N, Kummerhoff F, Koliamitra C, Schenk A, Walzik D, Hardt L, Knoop A, Thevis M, Kiesl D, Metcalfe AJ, Bloch W, Zimmer P. Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training. Exerc Immunol Rev. 2020;26:24-42.

Jones SP, Franco NF, Varney B, Sundaram G, Brown DA, De Bie J, Lim CK, Guillemin GJ, Brew BJ. Expression of the kynurenine pathway in human peripheral blood mononuclear cells: Implications for inflammatory and neurodegenerative disease. PLoS One. 2015;10:1–18. https://doi.org/10.1371/journal.pone.0131389.

Kim, J.H.; Bachmann, R.A.; Chen, J. Interleukin-6 and insulin resistance. Vitam Horm. 2009, 80, 613-633. https://doi.org/10.1016/S0083-6729(08)00621-3.

Koch S, Larbi A, Derhovanessian E, Ozcelik D, Naumova E, Pawelec G. Multiparameter flow cytometric analysis of CD4 and CD8 T cell subsets in young and old people. Immun Ageing. 2008;25:5-6. https://doi.org/10.1186/1742-4933-5-6.

Krüger K, Alack K, Ringseis R, Mink L, Pfeifer E, Schinle M, Gindler K, Kimmelmann L, Walscheid R, Muders K, Frech T, Eder K, Mooren FC. Apoptosis of T-Cell Subsets after Acute High-Intensity Interval Exercise. Med Sci Sports Exerc. 2016;48(10):2021-29. https://doi.org/10.1249/MSS.0000000000000979.

Laurans L, Venteclef N, Haddad Y, Chajadine M, Alzaid F, Metghalchi S, Sovran B, Denis RGP, Dairou J, Cardellini M, Moreno-Navarrete JM, Straub M, Jegou S, McQuitty C, Viel T, Esposito B, Tavitian B, Callebert J, Luquet SH, Federici M, Fernandez-Real JM, Burcelin R, Launay JM, Tedgui A, Mallat Z, Sokol H, Taleb S. Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health. Nature medicine. 2018;24(8):1113–1120. https://doi.org/10.1038/s41591-018-0060-4.

Makinodan T. Studies on the influence of age on immune response to understand the biology of immunosenescence. Exp Gerontol. 1998;33(1-2):27-38. https://doi.org/10.1016/s0531-5565(97)00058-2.

Malina, HZ, Richter C, Mehl M, Hess OM. Pathological apoptosis by xanthurenic acid, a tryptophan metabolite: Activation of cell caspases but not cytoskeleton breakdown. BMC Physiol. 2001; 1, 7. https://doi.org/10.1186/1472-6793-1-7.

Messaoudi I, Warner J, Fischer M, et al. Delay of T cell senescence by caloric restriction in aged long-lived nonhuman primates. Proc Natl Acad Sci U S A. 2006;103(51):19448-19453. https://doi.org/10.1073/pnas.0606661103.

Meyramov G, Korchin V, Kocheryzkina N. Diabetogenic activity of xanturenic acid determined by its chelating properties? Transplant. Proc. 1998;30:2682–2684. https://doi.org/10.1016/s0041-1345(98)00788-x.

Mezrich JD, Fechner JH, Zhang X, Johnson BP, Burlingham WJ, Bradfield CA. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. J Immunol. 2010;185:3190–8. https://doi.org/10.4049/jimmunol.0903670.

Mooren FC, Kruger K. Apoptotic lymphocytes induce progenitor cell mobilization after exercise. J. Applied Physiol. 2015;119:135-139. https://doi.org/10.1152/japplphysiol.00287.2015.

Mudry JM, Alm PS, Erhardt S, Goiny M, Fritz T. Direct effects of exercise on kynurenine metabolism in people with normal glucose tolerance or type 2 diabetes. Diabetes/metabolism research and reviews. 2016;32(7):754–761. https://doi.org/10.1002/dmrr.2798.

Munipally PK, Agraharm Satish G, Valavala VK, Gundae S, Turlapati NR. Evaluation of indoleamine 2,3-dioxygenase expression and kynurenine pathway metabolites levels in serum samples of diabetic retinopathy patients. Archives of Physiology and Biochemistry. 2011;117(5):254–258. https://doi.org/10.3109/13813455.2011.623705.

Nakajima T, Schulte S, Warrington KJ, et al. T-cell-mediated lysis of endothelial cells in acute coronary syndromes. Circulation. 2002;105(5):570-575. https://doi.org/10.1161/hc0502.103348.

Niemiro GM, Coletta AM, Agha NH, Mylabathula PL, Baker FL, Brewster AM, Bevers TB, Fuentes-Mattei E, Basen-Engquist K, Katsanis E, Gilchrist SC, Simpson RJ. Correction: Salutary effects of moderate but not high intensity aerobic exercise training on the frequency of peripheral T-cells associated with immunosenescence in older women at high risk of breast cancer: a randomized controlled trial. Immun Ageing. 2022;19(1):30. https://doi.org/10.1186/s12979-022-00276-x.

Nieto-Vazquez, I.; Fernández-Veledo, S.; Krämer, D.K.; Vila-Bedmar, R.; Garcia-Guerra, L.; Lorenzo, M. Insulin resistance associated to obesity: the link TNF-alpha [published correction appears in Arch Physiol Biochem. 2009;115(2):117]. Arch Physiol Biochem. 2008, 114, 183-194. https://doi.org/10.1080/13813450802181047.

O'Donovan G, Kearney EM, Nevill AM, Woolf-May K, Bird SR. The effects of 24 weeks of moderate- or high-intensity exercise on insulin resistance. Eur J Appl Physiol. 2005;95(5-6):522-8. https://doi.org/10.1007/s00421-005-0040-5.

Philippe M, Gatterer H, Burtscher M, Weinberger B, Keller M, Grubeck-Loebenstein B, Fleckenstein J, Alack K, Krüger K. Concentric and Eccentric Endurance Exercise Reverse Hallmarks of T-Cell Senescence in Pre-diabetic Subjects. Front Physiol. 2019;10:684. https://doi.org/10.3389/fphys.2019.00684.

Platten M, Nollen EAA, Röhrig UF, Fallarino F, Opitz CA. Tryptophan metabolism as a common therapeutic target in cancer, neurodegeneration and beyond. Nat. Rev. Drug Discov. 2019. https://doi.org/10.1038/s41573-019-0016-5.

Shirakawa, K.; Yan, X.; Shinmura, K.; Endo, J.; Kataoka, M.; Katsumata, Y.; Yamamoto, T.; Anzai, A.; Isobe, S.; Yoshida, N.; Itoh, H.; Manabe, I.; Sekai, M.; Hamazaki, Y.; Fukuda, K.; Minato, N.; Sano, M. Obesity accelerates T cell senescence in murine visceral adipose tissue. J Clin Invest. 2016, 126, 4626-4639. https://doi.org/10.1172/JCI88606.

Short KR, Vittone JL, Bigelow ML, Proctor DN, Rizza RA, Coenen-Schimke JM, Nair KS. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. Diabetes. 2003;52(8):1888-96. https://doi.org/10.2337/diabetes.52.8.1888.

Simpson RJ, Bigley AB, Spielmann G, LaVoy EC, Kunz H, Bollard CM. Human cytomegalovirus infection and the immune response to exercise. Exerc Immunol Rev. 2016;22:8-27.

Simpson RJ. Aging, persistent viral infections, and immunosenescence: can exercise "make space"? Exerc Sport Sci Rev. 2011;39(1):23-33. https://doi.org/10.1097/JES.0b013e318201f39d.

Spielmann G, Bollard CM, Bigley AB, Hanley PJ, Blaney JW, LaVoy EC, Pircher H, Simpson RJ. The effects of age and latent cytomegalovirus infection on the redeployment of CD8+ T cell subsets in response to acute exercise in humans. Brain, behavior, and immunity 2014;39:142–51. https://doi.org/10.1016/j.bbi.2013.05.003.

Spyridopoulos I, Martin-Ruiz C, Hilkens C, et al. CMV seropositivity and T-cell senescence predict increased cardiovascular mortality in octogenarians: results from the Newcastle 85+ study. Aging Cell. 2016;15(2):389-392. https://doi.org/10.1111/acel.12430.

Vangipurapu J, Silva LF, Kuulasmaa T, Smith U, Laakso M. Microbiota-Related Metabolites and the Risk of Type 2 Diabetes. Diabetes Care. 2020;43:1319–1325. https://doi.org/10.2337/dc19-2533.

Vasconcelos ABS, Aragão-Santos JC, de Resende-Neto AG, Rodrigues LS, Corrêa CB, Schimieguel DM, Camargo EA, de Paula Ramos S, Da Silva-Grigoletto ME. Effects of functional and combined training on subsets of memory T cells and functional fitness of postmenopausal women: A randomized controlled trial. Exp Gerontol. 2022;167:111898. https://doi.org/10.1016/j.exger.2022.111898.

Wanjalla CN, McDonnell WJ, Barnett L, Simmons JD, Furch BD, Lima MC, Woodward BO, Fan R, Fei Y, Baker PG, Ram R, Pilkinton MA, Mashayekhi M, Brown NJ, Mallal SA, Kalams SA, Koethe JR. Adipose Tissue in Persons With HIV Is Enriched for CD4+ T Effector Memory and T Effector Memory RA+ Cells, Which Show Higher CD69 Expression and CD57, CX3CR1, GPR56 Co-expression With Increasing Glucose Intolerance. Front Immunol. 2019;10:408. https://doi.org/10.3389/fimmu.2019.00408.

Wasserfurth P, Nebl J, Schuchardt JP, Müller M, Boßlau TK, Krüger K, Hahn A. Effects of Exercise Combined with a Healthy Diet or Calanus finmarchicus Oil Supplementation on Body Composition and Metabolic Markers-A Pilot Study. Nutrients. 2020;12(7):2139. https://doi.org/10.3390/nu12072139.

Yang, H.; Youm, Y.H.; Vandanmagsar, B.; Rood, J.; Kumar, K.G.; Butler, A.A.; Dixit, V.D. Obesity accelerates thymic aging. Blood. 2009, 114, 3803-12. https://doi.org/10.1182/blood-2009-03-213595.

Yi, H.S.; Kim, S.Y.; Kim, J.T.; Lee, Y.S.; Moon, J.S.; Kim, M.; Kang, Y.E.; Joung, K.H.; Lee, J.H.; Kim, H.J.; Chun, K.; Shong, M.; Ku, B.J. T-cell senescence contributes to abnormal glucose homeostasis in humans and mice. Cell Death Dis. 2019, 10, 249. https://doi.org/10.1038/s41419-019-1494-4.

Yu E, Papandreou C, Ruiz-Canela M, Guasch-Ferre M, Clish CB. Association of Tryptophan Metabolites with Incident Type 2 Diabetes in the PREDIMED Trial: A Case-Cohort Study. Clinical chemistry. 2018;64(8):1211–20. https://doi.org/10.1373/clinchem.2018.288720.

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Gießen, den 10. Juli 2023

Liste aller Publikationen

An folgenden Publikationen habe ich während meiner Zeit als Doktorand außerhalb meines Promotionsprojektes mitgewirkt:

2023

Joisten, N., Wences Chirino, T. Y., Boßlau, T. K., Wasserfurth, P., Hahn, A., Krüger, K., & Zimmer, P. (2023). Older adults with cytomegalovirus reveal increased $CD8^+$ /$CD4^+$ EMRA T cells and elevated systemic levels of kynurenic acid. *Immunology*, *169*(1), 113–116. https://doi.org/10.1111/imm.13609

2022

Reichel, T., Hacker, S., Palmowski, J., Boßlau, T. K., Frech, T., Tirekoglou, P., Weyh, C., Bothur, E., Samel, S., Walscheid, R., & Krüger, K. (2022). Neurophysiological Markers for Monitoring Exercise and Recovery Cycles in Endurance Sports. *Journal of sports science & medicine*, *21*(3), 446–457. https://doi.org/10.52082/jssm.2022.446

2021

Wasserfurth, P., Nebl, J., Rühling, M. R., Shammas, H., Bednarczyk, J., Koehler, K., Boßlau, T. K., Krüger, K., Hahn, A., & Das, A. M. (2021). Impact of Dietary Modifications on Plasma Sirtuins 1, 3 and 5 in Older Overweight Individuals Undergoing 12-Weeks of Circuit Training. *Nutrients*, *13*(11), 3824. https://doi.org/10.3390/nu13113824

Simpson, R. J., Boßlau, T. K., Weyh, C., Niemiro, G. M., Batatinha, H., Smith, K. A., & Krüger, K. (2021). Exercise and adrenergic regulation of immunity. *Brain, behavior, and immunity*, *97*, 303–318. https://doi.org/10.1016/j.bbi.2021.07.010

2020

Reichel, T., Boßlau, T. K., Palmowski, J., Eder, K., Ringseis, R., Mooren, F. C., Walscheid, R., Bothur, E., Samel, S., Frech, T., Philippe, M., & Krüger, K. (2020). Reliability and suitability of physiological exercise response and recovery markers. *Scientific reports*, *10*(1), 11924. https://doi.org/10.1038/s41598-020-69280-9

Palmowski, J., Reichel, T., Boßlau, T. K., & Krüger, K. (2020). The effect of acute running and cycling exercise on T cell apoptosis in humans: A systematic review. *Scandinavian journal of immunology*, *91*(2), e12834. https://doi.org/10.1111/sji.12834

Wasserfurth, P., Nebl, J., Schuchardt, J. P., Müller, M., Boßlau, T. K., Krüger, K., & Hahn, A. (2020). Effects of Exercise Combined with a Healthy Diet or *Calanus finmarchicus* Oil Supplementation on Body Composition and Metabolic Markers-A Pilot Study. *Nutrients*, *12*(7), 2139. https://doi.org/10.3390/nu12072139

Wasserfurth, P., Nebl, J., Boßlau, T. K., Krüger, K., Hahn, A., & Schuchardt, J. P. (2021). Intake of *Calanus finmarchicus* oil for 12 weeks improves omega-3 index in healthy older subjects engaging in an exercise programme. *The British journal of nutrition*, *125*(4), 432–439. https://doi.org/10.1017/S0007114520002809

2019

Palmowski, J., Boßlau, T. K., Ryl, L., Krüger, K., & Reichel, T. (2019). Managing immune health in sports–A practical guide for athletes and coaches [Infektprävention im Leistungssport–Ein praktischer Leitfaden für Trainer und Sportler]. *Deutsche Zeitschrift fur Sportmedizin 70 (2019), Nr. 10*, *70*(10), 219-226.