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Thema: Biomarker bei Adipositas unter besonderer Berücksichtigung der Neuronen-spezifischen Enolase

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Abkürzungsverzeichnis

AD	Alzheimer’s disease
BDNF	Brain-derived neurotrophic factor
BMI	Body-mass-index
GM	Gray matter
GMV	Gray matter volume
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
MCI	Mild cognitive impairment
NNE	Non-neuronal enolase
NSE	Neuron-specific enolase
SHIP	Study of Health in Pomerania
SPARE-AD	Spatial Patterns of Abnormality for Recognition of early Alzheimer’s disease
SPARE-BA	Spatial Pattern of Atrophy for Recognition of brain aging
WC	Waist circumference
WHO	World Health Organisation
WHR	Waist-to-hip-ratio

1. Einleitung

1.1 Adipositas

Die Prävalenz von Adipositas hat sich seit 1975 weltweit beinahe verdreifacht. Mehr als 1,9 Milliarden Erwachsene waren 2016 übergewichtig, davon 650 Millionen adipös. Die World Health Organisation (WHO) definiert Übergewicht als einen Body-mass-index (BMI) ≥25 kg/m² und Adipositas als BMI ≥30 kg/m² [1]. Ein pathologisch erhöhter BMI stellt einen der Hauptrisikofaktoren für zahlreiche chronische Erkrankungen dar. Zu den wichtigsten und bekanntesten schädlichen Folgen des übermäßigen Körpergewichts zählen kardiovaskuläre Erkrankungen, Hypertonie, Diabetes mellitus Typ 2 und maligne Tumorerkrankungen [2]. Da eine dramatische Zunahme von Übergewicht im Kindesalter beobachtet wird, entwickeln sich diese Erkrankungen immer früher [1]. Die schädigende Wirkung des Körperfetts setzt früher ein und wirkt zudem durch die alternde Bevölkerung immer länger auf den Organismus. Daher hat Adipositas zunehmend gravierende Auswirkungen auf die Lebenserwartung [2]. Die Weltpopulation altert und nimmt an Gewicht zu – folglich werden wir in der Zukunft mit dem Problem vieler übergewichtiger älterer Menschen konfrontiert werden. Allerdings kann vom BMI nicht auf die Verteilung des Körperfetts geschlossen werden [3]. Muskulöse, schlanke Menschen weisen einen höheren BMI auf, der in den höheren Kategorien als adipös klassifiziert wird. Dahingegen sind Menschen mit einem niedrigen BMI nicht unbedingt schlank, da der BMI nicht auf die fettfreie Körpermasse schließen lässt [3].

Insbesondere abdominelle Adipositas ist eng mit den schädlichen Folgen von Adipositas verknüpft [4]. Der Taillenumfang (waist circumference, WC) scheint dabei neben dem Verhältnis von Taillen- zu Hüftumfang (waist-to-hip-ratio, WHR) und dem Verhältnis von Taillenumfang zu Körpergröße einer der besten Indikatoren für multiple kardiovaskuläre Risikofaktoren zu sein [3, 5]. Der WC wird in der Mitte zwischen der letzten palpablen Rippe und dem Beckenkamm in Zentimetern gemessen [2]. Er ist abhängig von Herkunft, Alter und Geschlecht. In der europäischen Bevölkerung wird ein Wert von ≥80 cm bei Frauen und ≥94 cm bei Männern als angemessenes Maß für die Vorhersage multipler Risikofaktoren angesehen [2].

1.2 Zerebrale Auswirkungen von Adipositas

Es gibt zunehmend wissenschaftliche Hinweise, dass sich die schädlichen Folgen von Übergewicht und Adipositas auf das zentrale Nervensystem erstrecken [8, 9].

Tier- [9], und Humanstudien [11, 12] zeigen die Auswirkungen von Adipositas auf neuronale Struktur und Funktion. In diesem Zusammenhang wurde Adipositas mit einer vermehrten Schädigung der grauen Substanz in Verbindung gebracht [3, 8, 12–15]. Auch hier war der WC im Vergleich zum BMI das bessere Maß, um auf Veränderungen der grauen Substanz zu schließen [12], insbesondere bei Frauen [3]. Aktuelle Studien bringen insbesondere die Kombination eines erhöhten BMIs mit einer erhöhten WHR mit einem gesteigerten Risiko für Atrophien der grauen Substanz in Verbindung [14]. Die Mechanismen, die Adipositas mit einer verringerten grauen Substanz und kognitiven Veränderungen verbinden, verbleiben Gegenstand aktueller Diskussionen. In diesem Zusammenhang offenbarten wissenschaftliche Studien die Assoziation zwischen Adipositas und erhöhtem oxidativen Stress [16] und chronischer Entzündung [17]. Vor diesem Hintergrund nahmen Janowitz et al. [12] fortschreitende Veränderungen des Gehirns an, die durch das vermehrte Fettgewebe induziert und aufrecht erhalten werden. Pro-inflammatorische Zytokine wie Tumornekrosefaktor α sind im Fettgewebe hochreguliert und begünstigen den neuronalen Zelluntergang im Gehirn [18]. Die mit Adipositas assoziierte Entzündung begünstigt und beschleunigt das Voranschreiten der Insulinresistenz, deren Ausmaß mit einer Abnahme kognitiver Funktionen in Verbindung gebracht wurde [19]. Die systemische pro-inflammatorische Antwort könnte dabei aus einer vermehrten Produktion pro-inflammatorischer und einer Abnahme anti-inflammatorischer Faktoren resultieren, die dadurch metabolische Erkrankungen vorantreibt [20]. Darüber hinaus beschleunigt Adipositas neurodegenerative Prozesse, die bekannterweise im alternden Gehirn auftreten [21]. Adipositas stößt strukturelle Veränderungen des Gehirns an, die sich im Alter in einer gestörten Gedächtnisfunktion zeigen [13]. Adipositas im mittleren Lebensalter wird mit einer beschleunigten Hirnalterung und einer Verschlechterung der kognitiven Leistungsfähigkeit im höheren Lebensalter in Verbindung gebracht [11] und gilt als Risikofaktor für neurodegenerative Erkrankungen wie Morbus Alzheimer (Alzheimer's disease, AD) [21, 22]. Es wird sogar diskutiert, dass AD eine Folgeerkrankung eines lebenslangen erhöhten BMI ist und nicht nur eine Erkrankung des höheren Lebensalters [23].

Die beeinträchtigte Gedächtnisleistung im Alter und bei Adipositas könnte möglicherweise aus einer Abnahme der präfrontalen und thalamischen grauen Substanz (gray matter, GM) resultieren [13].

Aktuelle Studien etablierten Indices, die Muster der Gehirnatrophie in kernspintomographischen Aufnahmen erfassen [24]. Sie ermöglichen die Unterscheidung zwischen altersbedingter Atrophie (Spatial Pattern of Atrophy for Recognition of brain aging, SPARE-BA) und einer Atrophie, die insbesondere bei klinisch diagnostiziertem AD vorkommt (Spatial Patterns of Abnormality for Recognition of early Alzheimer's disease, SPARE-AD). Dabei zeigt der SPARE-AD Index den Übergang von normaler Gedächtnisleistung zu leichten kognitiven Einschränkungen (mild cognitive impairment, MCI) und den Progress zu AD an. In diesem Kontext stellten Habes et al. [24] in einer männlichen Subkohorte der Study of Health in Pomerania (SHIP), deren Daten auch der vorliegenden Arbeit zugrunde liegen, die Assoziation zwischen WC und fortgeschrittenen Mustern der Gehirnatrophie dar.

1.3 Biomarker

Um Aussagen bezüglich des Vorhandenseins und des Ausmaßes zerebraler Veränderungen und Schäden machen zu können, werden gehirnspezifische Biomarker benötigt. Biomarker sind objektiv messbare Größen. Sie erfassen als Indikatoren normale und pathologische biologische Prozesse sowie Veränderungen durch therapeutische Interventionen. Biomarker liefern wertvolle Informationen in der Diagnostik, Prognose, Verlaufs-, und Therapiekontrolle verschiedener Erkrankungen [25]. Dabei können Biomarker als zusätzliches diagnostisches Mittel bei der Identifikation von Patienten mit einer bestimmten Erkrankung oder Fehlfunktion eingesetzt werden. Verwendung finden sie auch beim Staging und der Erfassung des Krankheitssausmaßes.

In diesem Zusammenhang zeichnet sich zunehmend ab, dass die Neuronen-spezifische Enolase (neuron-specific enolase, NSE) und der brain-derived neurotrophic factor (BDNF) geeignete Biomarker für Adipositas-assoziierte Veränderungen des Gehirns sein könnten.

1.3.1 NSE

NSE überführt als Enzym der Glycolyse 2-Phosphoglycerat in Phosphoenolpyruvat [26]. NSE besteht aus zwei γ-Untereinheiten und ist eng mit dem Differenzierungsstatus reifer Nervenzellen verknüpft [28, 29]. In Neuronen kommt NSE vor allem im Zytoplasma vor. Da NSE nicht sezerniert werden kann, zeigt ein Anstieg von NSE in Liquor oder Serum neuronale Schäden an [29]. Mit dem Allan Brain Atlas konnten die Gehirnregionen mit der höchsten NSE-Expression identifiziert werden. In Frontal- und Parietallappen, insbesondere in Claustrum und Cerebellum, fand sich die höchsten Expression des NSE-Gens [30]. Vor allem bei Frauen zeigten die Konzentrationen von NSE im Serum eine negative Korrelation mit der Dichte der GM in den Amygdalae, Hippocampi [29] und Cerebellum [31].

Konzentrationsschwankungen von NSE resultieren aus zerebrovaskulären Erkrankungen, Schlaganfall [32] und traumatischer Hirnschädigung [33]. Die Auswirkung des Alters auf NSE ist unklar: Manche Studien berichteten über eine Erhöhung der Konzentration von NSE im Liquor mit voranschreitendem Lebensalter [34]. Dem gegenüber stehen die Ergebnissen von Streitbürger et al. [29], die keine Veränderungen im Serum feststellen konnten.

Aktuelle Studien zeigten Unterschiede zwischen normalen Alterungsprozessen und AD bezüglich der Veränderungen der grauen Substanz. Der normale Alterungsprozess ist insbesondere mit einer Reduktion der grauen Substanz im Frontal- und Parietallappen assoziiert [35]. MCI und AD sind dagegen durch einen ausgeprägter Verlust von GM im limbischen System einschließlich des Temporallappens, Hippocampus und parahippocampalen Gyrus gekennzeichnet [36]. Zerebrale Veränderungen durch Alter oder AD können durch SPARE-BA und SPARE-AD [24] unterschieden werden. In der Literatur fanden sich bislang keine Studien, die Assoziationen zwischen Mustern der Gehirnalterung und NSE-Konzentrationen untersuchten. Daher zielte unsere Studie darauf ab, hier einen möglichen Zusammenhang aufzudecken [7].

Zusätzlich zu den Auswirkungen des voranschreitenden Lebensalters, beeinflusst ein erhöhtes Körpergewicht das Volumen der grauen Substanz (gray matter volume, GMV) [12]. Die Studie von Mueller et al. [30] lieferte erste Erkenntnisse über eine Verbindung zwischen Adipositas-assoziierten Veränderungen von GMV und NSE Konzentrationen. Die Autoren beschrieben eine inverse Korrelation zwischen NSE und der Dichte der GM in Hippocampus und Cerebellum bei übergewichtigen jungen

Erwachsenen. Eine Verknüpfung zwischen Adipositas und einer Degeneration von GMV wurde in der SHIP-Population bereits beschrieben [12]. Daher untersuchten wir eine mögliche Verbindung zwischen diesen Veränderungen der GMV und NSE-Konzentrationen bei Adipositas [7].

1.3.2 BDNF

BDNF ist essenziell für die Funktionsfähigkeit von Synapsen und ist an neuronalen Reparaturprozessen beteiligt [37]. Er reguliert den Energiehaushalt, indem er mit mehreren Neuropeptiden wie Leptin interagiert [38]. Als wichtiger Wachstumsfaktor im zentralen und peripheren Nervensystem spielt BDNF eine Schlüsselrolle bei Neurogenese und Gedächtnisleistungen [39]. Tierstudien zeigten eine Adipositas-assoziierte gestörte Neurogenese bei Mäusen, die fettreich ernährt wurden [41, 42]. Park et al. [41] beschrieben verminderte Konzentrationen von BDNF und ein reduziertes Wachstum neuronaler Vorläuferzellen, die mit der beeinträchtigten Neurogenese im Hippocampus einhergingen. Humanstudien dagegen erbrachten heterogene Ergebnisse bezüglich einer Verbindung zwischen Adipositas und BDNF-Spiegeln: Es fanden sich erniedrigte [42], erhöhte [43] und nicht beeinflusste [44] BDNF-Werte bei Adipositas. Bei dieser unklaren Datenlage analysierten wir in einer großen epidemiologischen Kohorte die Assoziation zwischen BDNF und Adipositas.

2 Material und Methoden

Die Daten wurden im Rahmen der Study of Health in Pomerania (SHIP) erhoben. Das Projekt besteht aus zwei Studien: Der initialen Kohorte (SHIP-0) mit den Folgeuntersuchungen (SHIP-1, SHIP-2), sowie einer parallel zu SHIP-2 verlaufenden Erhebung (SHIP-TREND). Die Werte von NSE, BDNF und Vitamin D wurden in einer Teilprobe von SHIP-TREND bei 1.000 Teilnehmern ohne klinischen Diabetes mellitus Typ 2 erfasst. NSE, BDNF und Vitamin D wurden im Serum gemessen. Detaillierte Informationen über klinische Untersuchungsmethoden, Laboranalysen, Bildgebung und statistische Verfahren können den anhängenden Publikationen entnommen werden: Studie 1 [7], Studie 2 [6].

2.1 Analysen in Studie 1 [7] :NSE

Um geschlechtsabhängige Unterschiede zu erheben, wurde die Stichprobe in Männer und Frauen aufgeteilt. Zur Erfassung BMI-abhängiger Unterschiede wurde die Stichprobe in Übergewichtige (BMI ≥25 kg/m²) und Normal-, und Untergewichtige (BMI <25 kg/m²) eingeteilt. Um Unterschiede zwischen den Gruppen festzustellen, wurde bei kategorischen Variablen (Geschlecht, Medikation) der X^2-Test und bei kontinuierliche Daten (Alter, NSE-Werte) der unabhängige T-Test durchgeführt. Die weiteren Berechnungen erfolgten mit linearen Regressionsanalysen. Analysen bezüglich Assoziationen zwischen NSE-Spiegeln und Alter, Geschlecht, BMI, vaskulären Risikofaktoren (WC, Blutdruck, Bluthochdruck, Einnahme von Antihypertensiva, HDL-, LDL-, Cholesterol-, Triglyzeridwerte, Einnahme von Lipidsenkern, Rauchstatus) sowie GM wurden durchgeführt. Des Weiteren wurde die Assoziation zwischen NSE-Werten und SPARE-BA oder SPARE-AD untersucht. Darüber hinaus wurden Analysen mit NSE und GM als abhängige Variable durchgeführt.

2.2 Analysen in Studie 2 [6]: BDNF, Vitamin D, Adipositas, Depression

Die Studienpopulation wurde in drei Gruppen geteilt. Gruppe 1 enthielt weder depressive noch adipöse Studienteilnehmer, Gruppe 2 Depressive oder Adipöse und Gruppe 3 Depressive und Adipöse.

Die Assoziation zwischen BDNF und Adipositas, sowie Vitamin D und Depression wurde untersucht. Hierbei wurde zunächst der Zusammenhang zwischen BDNF und Vitamin D in einer partiellen Korrelationsanalyse berechnet. Anschließend wurde die Assoziation zwischen BDNF und Vitamin D mit Adipositas und Depression in multivariablen binären logistischen Regressionsmodellen analysiert. Mit einer multinominalen logistischen Regression wurde der Zusammenhang zwischen BDNF oder Vitamin D und der Depressionsschwere untersucht. In multivariablen linearen Regressionsmodellen wurde die Assoziation zwischen BDNF oder Vitamin D und WHR berechnet.

3 Ergebnisse

3.1 Studie 1 [7]

3.1.1 Stichprobencharakteristika

Die Hauptanalyse umfasste 901 Versuchspersonen (55,4% weiblich). Der Vergleich zwischen männlichen und weiblichen Versuchspersonen zeigte bei Frauen niedrigere Werte für GM, BMI, WC, Blutdruck und Triglyzeride und höhere high-density lipoprotein- (HDL) und Cholesterinwerte. Bezüglich Alter, NSE, low-density lipoprotein (LDL), Antihypertensiva und Lipidsenkern fanden sich keine Unterschiede. Der BMI stieg mit zunehmendem Alter linear an. Die Analyse BMI-abhängiger Differenzen zeigte Unterschiede zwischen Übergewichtigen und Normalgewichtigen. In der Gruppe mit einem BMI ≥25 kg/m² war der Anteil an Männern größer und das Alter höher. Es zeigten sich hier höhere Blutdruck-, und Lipidwerte mit der Ausnahme von HDL, das erniedrigt war. Ebenso war die Einnahme von Antihypertensiva und Lipidsenkern in der Gruppe der Übergewichtigen höher. GMV war in der Gruppe der Übergewichtigen erniedrigt. Übergewichtige hatten im Vergleich zu Normalgewichtigen niedrigere NSE-Werte, aber der Unterschied war hier nicht statistisch signifikant.

3.1.2 Assoziation zwischen NSE und Alter

Es fand sich keine lineare Beziehung zwischen Alter und NSE-Werten. In geschlechtsdifferenzierten Auswertungen war die Assoziation zwischen NSE und Alter nur bei Frauen signifikant. Es zeigte sich bei Männern ein U-förmiger, bei Frauen ein J-förmiger Zusammenhang zwischen Alter und NSE: Junge Frauen wiesen niedrigere NSE-Werte auf, die mit zunehmendem Alter anstiegen. Männer zeigten höhere NSE-Werte in der Jugend mit einem Abfall im Alter. In einem Alter von ca. 60 Jahren ähnelten sich die NSE-Werte der Geschlechter.

3.1.3 Assoziationen zwischen NSE und BMI und kardiovaskulären Risikofaktoren

Die NSE-Werte nahmen mit zunehmendem BMI und WC im linearen Modell ab. Im nicht-linearen Modell zeigte sich eine negative, U-förmige Assoziation: Bis zu einem BMI von 25 kg/m² stiegen die NSE-Werte an und sanken bei höheren BMI-Werten.

Um altersabhängige Unterschiede bei Übergewicht-assoziierten zerebralen Schäden aufzudecken, stratifizierten wir die Stichprobe nach Alter. Hier zeigte sich nur bei der ältesten Stichprobe (>60 Jahre) ein signifikanter Zusammenhang zwischen NSE und BMI. Bei der einzelnen Betrachtung von Übergewichtigen und Normalgewichtigen zeigte sich nur bei übergewichtigen Älteren eine signifikante Assoziation zwischen NSE-Werten und BMI.
Bezüglich anderer kardiovaskulärer Risikofaktoren wie Hypertonie, Blutdruck, Blutfetten und Raucherstatus zeigten nur HDL und aktuelles Rauchen eine signifikante negative Assoziation mit NSE.

3.1.4 Assoziationen zwischen NSE und GMV, SPARE-BA und SPARE-AD

NSE-Werte und GMV waren nicht miteinander assoziiert. Die Stratifizierung der Stichprobe in Übergewichtige und Normalgewichtige erbrachte keine signifikanten Unterschiede. Übergewichtige mit höherem Alter zeigten eine negative Assoziation mit GMV, aber der Zusammenhang war nicht statistisch signifikant. Bei Normalgewichtigen konnte dieser Zusammenhang nicht festgestellt werden. Es fanden sich keine signifikanten Assoziationen zwischen NSE und SPARE-BA oder SPARE-AD, weder in der gesamten Stichprobe, noch bei über 65-jährigen. SPARE-BA und SPARE-AD waren jedoch mit GMV assoziiert.

3.2 Studie 2 [6]

3.2.1 Stichprobencharakteristika

Die Hauptanalyse umfasste 3.926 Versuchspersonen (46,9% weiblich). 2.539 waren weder depressiv noch adipös (Gruppe 1), 1.285 entweder depressiv oder adipös (Gruppe 2) und 102 depressiv und adipös (Gruppe 3). Frauen waren sowohl häufiger depressiv oder adipös, als auch depressiv und adipös. Nicht depressive und adipöse Personen waren jünger, hatten eine geringere WHR und höhere Vitamin D-Spiegel als Personen in den anderen zwei Gruppen. Die Einnahme von Antidepressiva war bei Depressiven und Adipösen am höchsten. Bezüglich BDNF-Spiegeln und Thrombozytenzahlen zeigten sich keine signifikanten Unterschiede zwischen den Gruppen.

3.2.2 Assoziationen zwischen BDNF, Adipositas, Vitamin D und Depression

Im logistischen Regressionsmodell zeigte sich keine Assoziation zwischen BDNF und Depression sowie BDNF und Adipositas. Vitamin D hingegen war sowohl mit Depression als auch mit Adipositas negativ assoziiert. Zwischen der Depressionsschwere und BDNF gab es keinen Zusammenhang, während sich zwischen der Depressionsschwere und Vitamin D eine negative Assoziation zeigte.
Es zeigte sich eine schwache Korrelation zwischen BDNF und Vitamin D, wobei die Interaktion zwischen BDNF und Vitamin D nicht signifikant war. Die Interaktionen zwischen BDNF bzw. Vitamin D und Adipositas waren nicht signifikant.

3.3.3 Assoziation zwischen BDNF und Vitamin D mit WHR

Zwischen BDNF und WHR sowie zwischen Vitamin D und WHR fand sich ein signifikanter, nicht-linearer Zusammenhang. Hierbei zeigte das Modell mit BDNF eine U-förmige Assoziation mit einem Minimum des BDNF-Werts bei 23.000 pg/ml. Im Modell mit Vitamin D fand sich eine lineare Abnahme der WHR ausgehend von Vitamin D-Werten bei 25 ng/ml und eine konstante WHR unterhalb dieses Wertes.

4 Diskussion

4.1 Assoziation zwischen NSE und Alter

4.1.1 NSE in Liquor und Serum

Über den Einfluss des Lebensalters auf NSE-Konzentrationen finden sich in der Literatur unterschiedliche Ergebnisse. Dies ist unter anderem dadurch bedingt, dass NSE entweder im Liquor oder im Serum gemessen wurde [7]. Bei der Interpretation von NSE-Konzentrationen ist es wichtig, die unterschiedlichen Lokalisationen und Substrate zu berücksichtigen, in denen NSE erhoben wurde. Gemäß aktueller Forschungslage wird das Ergebnis davon beeinflusst, ob NSE im Serum, im lumbalen oder ventrikulären Liquor gemessen wurde. Das Verhältnis zwischen NSE in ventrikulärem Liquor und NSE im Serum beläuft sich auf 1:1. Die vom Gehirn abgeleitete, intrathekale NSE Fraktion im Liquor wird mit >99% angegeben. Allerdings nimmt die NSE-Konzentration zwischen ventrikulärem und lumbalem Liquor ab. Die durchschnittlichen NSE-Konzentrationen im lumbalen Liquor ändern

sich im Vergleich zu denen im ventrikulären Liquor nur wenig. Das Blut dahingegen spiegelt die Dynamik der ventrikulären NSE-Konzentrationen aufgrund eines lokal erhöhten Blut-Gehirn-Gradienten recht gut wieder [45]. Darüber hinaus ist die Bestimmung von NSE im Blut weniger invasiv und kann in größeren Kohorten einfacher durchgeführt werden.

Bislang findet sich nur im Liquor eine positive Assoziation zwischen NSE-Werten und Alter. Im Serum dagegen sind die NSE-Konzentrationen nicht mit dem Alter assoziiert [7]. Die unterschiedlichen Ergebnisse könnten folglich aus einer anderen Dynamik von NSE in Liquor oder Serum resultieren. Darüber hinaus lassen Studien auf einen nur geringen neuronalen Verlust im Rahmen der normalen Gehirnalterung schließen, was sich folglich kaum auf NSE Werte auswirken würde [46].

4.1.2 NSE bei AD

Kernspintomographische Muster der normalen Gehirnalterung (SPARE-BA) zeigten keine Assoziation mit NSE-Konzentrationen. Allerdings zeigte sich auch kein Zusammenhang zwischen SPARE-AD und NSE-Werten [7].

In Anbetracht des ausgedehnten neuronalen Zelltods bei AD haben Studien einen möglichen Zusammenhang zwischen Änderungen der NSE-Konzentrationen im Verlauf von AD untersucht. Allerdings kamen die Studien zu unterschiedlichen Ergebnissen [48, 49]. Obwohl sich zwischen Patienten mit AD und der Kontrollgruppe meist kein signifikanter Unterschied der NSE-Werten findet, zeigte sich eine inverse Assoziation zwischen kognitiven Tests wie dem Mini-mental-state Test und der NSE-Konzentration [47]. Während Probanden mit niedrigen Ergebnissen im Mini-mental-state Test höhere NSE-Spiegel aufwiesen, waren bei Patienten mit AD die NSE-Spiegel invers mit der Schwere der morphologischen Gehirnveränderungen assoziiert. Dabei nahmen die NSE-Konzentrationen mit der Schwere der durch das MRT erfassten Gehirnatrophie ab. Durch die Ergebnisse aktueller Studien zeichnet sich allerdings ab, dass der Zusammenhang zwischen AD und NSE-Konzentrationen nur schwach und klinisch irrelevant ist [49–51].

4.1.3 Geschlechtsspezifische Unterschiede der Hirnalterung

Zunehmend stellen sich geschlechtsspezifische Unterschiede bei der Gehirnalterung [46] heraus, die sich auch auf NSE-Konzentrationen auswirken [7]: Während bei Frauen die NSE-Werte mit zunehmendem Alter ansteigen, nehmen sie bei Männern

im Alter ab. Als Erklärungsansatz für dieses Ergebnis können geschlechtsspezifische Unterschiede im Hormonsystem herangezogen werden. Die Spiegel von Geschlechtshormonen wie Estradiol und Testosteron nehmen im Alter bei beiden Geschlechtern ab. Während die Estradiolwerte nach der Menopause bei Frauen recht steil abfallen, nehmen die Testosteronwerte bei Männern langsamer ab [51]. Ältere Frauen weisen sowohl niedrigere Estradiol-, als auch Testosteronwerte auf als Männer [52]. Niedrige Estradiolwerte sind mit kognitiver Leistungsabnahme und AD in Verbindung gebracht worden [53]. Im Tiermodell konnte das Voranschreiten von AD durch die gezielte Estradiolgabe sogar positiv beeinflusst werden [54]. Insbesondere postmenopausale Frauen mit einem schlechten metabolischen Gesundheitsstatus und Hypertonie profitierten von einer Hormontherapie sowohl durch metabolische als auch durch kognitive Verbesserung [55]. Aktuelle Studien zeigen die neuroprotektive Wirkung von Estradiol insbesondere im Hippocampus [56]. NSE wird, unter anderem, insbesondere im Hippocampus exprimiert. Daher könnte die Abnahme von Estradiol-vermittelten neuroprotektiven Effekten einen beschleunigten neuronalen Zelluntergang bedingen. Dies würde sich in steigenden NSE-Werten niederschlagen, wie in unserer Studie beobachtet wurde [7].

4.2 Assoziationen zwischen NSE, BMI und GMV

4.2.1 NSE bei Hirnschädigung

Als Biomarker für neuronale Schäden kennzeichnen erhöhte NSE-Werte eine akute Hirnschädigung, insbesondere die Zerstörung von GMV. So finden sich erhöhte NSE-Werte infolge zerebraler Minderperfusion bei Herzstillstand, Schlaganfall [32] sowie nach traumatischer Rückenmarks- [26], und Hirnschädigung [57]. Hier zeigte sich ein Zusammenhang zwischen der durch das Glasgow Coma Scale erfassten Schwere der Hirnschädigung und der Höhe der NSE-Werte. Höhere NSE-Werte waren mit einer schlechteren Prognose und einer erhöhten Sterblichkeit assoziiert, woraus sich ein Zusammenhang zwischen dem Ausmaß der geschädigten Hirnmasse und der Konzentration von NSE ableiten lässt. Darüber hinaus diskutieren aktuelle Studien, dass NSE infolge traumatischer Rückenmarksschädigung an die Plasmamembran migriert und dort neurodegenerative Prozesse anstoßen kann [26].

4.2.2 NSE und Adipositas-assoziierte Komorbiditäten

Studien zeigen eine Assoziation zwischen neuronalen Schäden und Adipositas sowie Adipositas-assoziierten Komorbiditäten wie Hypertonie und Hyperlipidämie [59, 60]. Auch eine inverse Korrelation mit GMV wird beschrieben [30]. Als Marker für Neurodestruktion wären bei adipösen Patienten erhöhte NSE-Werte zu erwarten. Dementsprechend zeigte sich eine negative Korrelation zwischen der Dichte der GM in Cerebellum und Hippocampus und NSE-Konzentrationen bei übergewichtigen jungen Erwachsenen [30].

Des Weiteren scheinen altersabhängige Mechanismen die Assoziation zwischen BMI und NSE zu beeinflussen. Bei Übergewichtigen fand sich eine negative Assoziation zwischen NSE und BMI nur im höheren, nicht aber im jüngeren Lebensalter [7]. Auch der Zusammenhang zwischen NSE und Hypertonie weist altersabhängige Unterschiede auf. Studien weisen darauf hin, dass erhöhte NSE-Werte bei Hypertonikern subklinische Schäden anzeigen können [58]. Demgegenüber fand sich keine Assoziation zwischen NSE und Hypertonie in einer Stichprobe, in der die Übergewichtigen im Durchschnitt älter als die Kontrollgruppe der Normalgewichtigen sind [7].

4.2.3 NSE und BMI

Es stellte sich heraus, dass NSE-Werte bei Übergewicht und Adipositas unter anderem von der Höhe des BMIs abhängen. Es zeigte sich eine parabolische Assoziation zwischen NSE-Werten und BMI, mit abfallenden NSE-Konzentrationen ab einem BMI >25 kg/m² [7]. Diese Beobachtung steht den Ergebnissen von Mueller et al. [30] gegenüber, die eine positive Assoziation zwischen NSE und BMI konstatieren.

Im Folgenden werden mehrere Theorien diskutiert, um die abnehmende NSE-Werte ab einem BMI von 25 kg/m² erklären.

4.2.3.1 Degeneration von GMV

Bei schwerer Adipositas ist anzunehmen, dass die negativen Effekte des übermäßigen Körperfetts schon seit längerer Zeit auf das Gehirn wirken. Folglich ist hier eine reduzierte GMV zu erwarten [12]. Die verminderte GMV könnte sich in einem Abfall der NSE-Konzentrationen zeigen, da immer weniger NSE enthaltende Hirnmasse vorhanden wäre. Sinkende NSE-Spiegel wurden bei einem erhöhten

Grad der Hirnatrophie bei AD festgestellt [47]. Entsprechend kann ein ähnlicher Verlauf der NSE-Spiegel bei einer Adipositas-bedingten Degeneration von GMV erwartet werden. In diesem Kontext muss das Alter der Studienteilnehmer mit erhöhtem BMI berücksichtigt werden, was die unterschiedlichen Studienergebnisse erklären könnte [9, 31]. In jüngeren Jahren hat das schädliche Körperfett noch nicht so lange auf das Gehirn eingewirkt, wie bei älteren adipösen Menschen. Daher wird die GMV bei Jüngeren noch nicht so weit reduziert sein, dass sich diese Degeneration in niedrigeren NSE-Konzentrationen niederschlägt. Im Gegenteil: Die akute Neurodestruktion könnte durch erhöhte NSE-Werten sichtbar werden [30]. Außerdem fanden sich parallele Veränderungen von BMI und Verlust der Dichte der GM: Je höher der BMI, desto größer zeigte sich die Abnahme der Dichte der GM. Ähnliche Beobachtungen der hirnatrophischen Prozesse wurden auch bei älteren Personen mit MCI gemacht. Mit zunehmendem Lebensalter würde dementsprechend die Abnahme von GMV bei pathologischem BMI weiter voranschreiten, bis sich dies schließlich in einem Abfall der NSE-Werte äußern würde [7].

4.2.3.2 Neuroinflammation

Es kann angenommen werden, dass Adipositas fortschreitende Veränderungen des Gehirns anstößt. Durch Adipositas induzierter oxidativer Stress [16] und chronische Entzündungsprozesse [60, 61] werden in diesem Zusammenhang diskutiert. Der erhöhte oxidative Stress wirkt sich in diesem Rahmen auch über die Beeinflussung neurotrophischer Faktoren wie BDNF auf die neuronale Lebensdauer und kognitive Funktionen aus [61]. Die genauen biologischen Mechanismen, die zu einer durch Adipositas bedingten Inflammation führen, sind sehr komplex [62]. Zahlreiche Studien belegen eine Adipositas-assoziierte systemische Entzündung, die sich bis auf das Gehirn erstreckt und die kognitive Leistungsfähigkeit beeinträchtigt [61–63]. Gemäß aktueller Forschungslage erhöht Adipositas im mittleren Lebensalter, insbesondere ein erhöhter WC, sogar das Risiko an AD zu erkranken. Eine der Hauptursachen von Übergewicht und Adipositas ist eine unangemessen fettreiche Ernährung. Insbesondere der Hippocampus ist vulnerabel gegenüber den schädigenden Einflüssen einer fettreichen Ernährung [60, 64]. Dabei spielen andauernd erhöhte Spiegel von freien Fettsäuren eine wichtige Rolle im Rahmen einer stetigen niedrig-gradigen Entzündung [59]. Tierstudien zeigen hier die Induktion von inflammatorischen Zytokinen sowie eine geschädigte neuronale Plastizität. Diese

Veränderungen führen zu Einbußen im Hippocampus-abhängigen Gedächtnis, sind aber bei einer Ernährungsumstellung größtenteils reversibel [64]. Auch regelmäßiges aerobes Training kann durch die Reduktion von oxidativem Stress und der Erhöhung neurotrophischer Faktoren Adipositas-assoziierte Schäden positiv beeinflussen [61].
Bei Adipositas kommt es zu einer übermäßigen Produktion von Adipokinen durch das weiße Fettgewebe [66, 67]. Pro-inflammatorische Zytokine wie Interleukin 6 und Tumornekrosefaktor α sowie vom Fettgewebe produzierte Hormone wie Leptin stoßen mehrere pathologische Prozesse an und rufen eine Entzündungsantwort in der Mikroglia hervor. Dies wiederum löst eine positive Rückkoppelungsschleife aus, bei der noch mehr Zytokine und Entzündungsfaktoren produziert werden, was die Inflammation letztendlich zunehmend steigert. Die Auswirkungen der Adipokine auf das Gehirn bedingen eine erhöhte Vulnerabilität gegenüber schädigenden und pathologischen Prozessen.

Vorausgehende Studien weisen auf eine Beteiligung von NSE bei der Zellaktivierung, Produktion von Zytokinen und Chemokinen, sowie der Induktion von neuronalem Zelluntergang hin [26]. Diese sind im Rahmen akuter neuronaler Schädigung zu beobachten, womit die steigenden NSE-Werte bis zu einem BMI von 25 kg/m² erklärt werden können. Interessanterweise bringen neueste Studienergebnisse eine Erhöhung von NSE-Werten mit neuroprotektiver Aktivität und neuronaler Lebensdauer, Differenzierung und Migration nach Rückenmarksverletzungen in Verbindung. Die Mechanismen, die entweder für die neuroprotektive oder destruktive Wirkung von NSE verantwortlich sind, sind Gegenstand aktueller Forschung [26, 68]. Unter anderem ist die Lokalisation von NSE für die angestoßenen neurodestruktiven oder protektiven Prozesse bedeutsam. Normalerweise wird NSE im Zytoplasma exprimiert. Im Rahmen von Neuroinflammation kann NSE an die Plasmamembran migrieren. Über eine erhöhte Antigenexpression kommt es zu einer verstärkten Zellinvasion mit nachfolgenden Schäden der Extrazellularmatrix. Die vermehrte Produktion pro-inflammatorischer Zytokine und Sauerstoffradikale durch an der Zelloberfläche befindliche NSE führt ebenfalls zu einer verstärkten Neurodegeneration [26]. Daher scheint die Regulation von NSE und dem NSE-abbauenden Enzym, Cathepsin X, ein therapeutischer Zielpunkt zu sein [68]. Als Enzym der Glycolyse ist NSE aber auch an der Zellproliferation und -regeneration beteiligt.

Die optimale Kontrolle und Balance der NSE-Expression und Aktivität scheint daher ein therapeutischer Ansatzpunkt zu sein. In diesem Rahmen kann die Höhe der NSE-Werte über die Rolle als Biomarker hinaus als therapeutisches Ziel angesehen werden.

4.2.3.3 Veränderungen des Glukosemetabolismus

Niedrigere NSE-Werte bei schwerer Adipositas könnten des Weiteren auf Veränderungen des Glukosemetabolismus zurückzuführen sein. Als Enzym der Glykolyse ist NSE direkt am Glukosemetabolismus beteiligt. Aktuelle Studien berichten über einen verminderten Glukosestoffwechsel in einigen Gehirnregionen von Patienten mit MCI, der neben einer zunehmenden Atrophie des Hippocampus den Symptomen von AD vorausgeht [67, 68]. In diesem Kontext zeigte sich ein Zusammenhang zwischen BMI und einem reduzierten zerebralem Glukosestoffwechsel präfrontal und im anterioren Gyrus cinguli [71]. Veränderungen des präfrontalen Metabolismus gingen dabei mit geringerer Lernfähigkeit und einer Störung von Exekutivfunktionen einher. Die Gehirnaktivierung bei kognitiver Stimulation zeigte allerdings keine Assoziation mit dem BMI oder neuropsychologischen Tests. Als zugrunde liegende Mechanismen, die den gestörten Glukosestoffwechsel mit bedingen, werden unter anderem eine durch Adipositas verursachter zerebrale Vasokonstriktion, endotheliale Dysfunktion, oxidativer Stress, sowie ein reduzierter zerebraler Blutfluss diskutiert [69].

4.2.3.4 Störungen der neuronale Differenzierung

Ein weiterer Erklärungsansatz für niedrigere NSE-Werte bei Adipositas betrifft die neuronale Differenzierung. NSE ist spezifisch für reife Nervenzellen und ist eng mit dem neuronalen Differenzierungsstatus verknüpft [27].

NSE besteht aus zwei Untereinheiten. Abhängig von Gewebe und Entwicklungsstatus werden unterschiedliche Kombinationen der Untereinheiten exprimiert. Im menschlichen Gehirn finden sich Kombinationen aus α und γ Untereinheiten. NSE besteht aus zwei γγ- Untereinheiten und ist spezifisch für adulte Neuronen mit voll ausgebildeten synaptischen Verbindungen. In frühen Stadien der Hirnentwicklung finden sich hohe Konzentrationen von nicht-neuronen spezifischer Enolase (non-neuronal enolase, NNE), die aus zwei α- Einheiten zusammengesetzt ist. Während der neuronalen Migration und des Reifungsprozesses des Gehirns

bilden sich Hybridenolasen (αγ), die den Wechsel von NNE zu NSE anzeigen. Nachdem die Neuronen ihre volle synaptische Vernetzung in ihrer endgültigen Lage erreicht haben, findet der finale Übergang zu NSE statt. Allerdings differenzieren sogar im adulten Gehirn nicht alle NNE zu NSE. Dies könnte sich in erniedrigten NSE-Werten zeigen und auf eine gestörte neuronale Differenzierung hinweisen.
Wie bereits erwähnt, sind ernährungsabhängige Veränderungen spezifisch für den Hippocampus [40] und NSE ist insbesondere mit Strukturen des Hippocampus assoziiert. Daher kann vermutet werden, dass die Adipositas-bedingten Veränderungen in neuronalen Reifungsprozessen und Neurogenese sich bis auf die Entwicklung von NSE erstrecken.

Im Rahmen der Exprimierung unterschiedlicher Enolasen (γγ oder αγ) mit niedrigeren NSE-Werten infolge gestörter neuronaler Differenzierung wird aktuell noch eine weitere Theorie diskutiert [68]. Während NSE mit zwei γγ-Einheiten in Neuronen exprimiert wird, findet sich NNE in Mikroglia, Astrozyten und Oligodendrozyten. Folglich wären bei einer inflammatorischen Reaktion auch erhöhte NNE-Werte zu erwarten. Die unterschiedliche Lokalisation in Neuronen und Gliazellen deutet möglicherweise darauf hin, dass NSE sowohl an inflammatorischen, als auch an neurotrophischen Prozessen beteiligt ist und eine regulierende Funktion bei neuronaler Differenzierung, Wachstum, Untergang und Überleben einnimmt .

4.2.3.5 BDNF und Vitamin D

Der gestörten neuronalen Differenzierung und Neurogenese könnten Veränderungen in BDNF- und Vitamin D-Stoffwechsel zugrunde liegen.
Eine gestörte neuronale Differenzierung im Hippocampus geht mit einer Abnahme sowohl von BDNF als auch einem verringertem Wachstum neuronaler Progenitorzellen einher [41]. Von einem Zusammenhang zwischen Änderungen der BDNF-Konzentrationen und Adipositas wurde berichtet [6]. Die Richtung der Assoziation sowie die genauen Mechanismen sind Gegenstand aktueller Diskussion. Es finden sich Berichte über keine [44], eine negative [42] als auch eine positive [43] Assoziation zwischen BDNF und dem BMI. Hier muss berücksichtigt werden, dass nicht alle Studien für Thrombozyten adjustiert waren und daher nicht direkt vergleichbar sind. Eine mögliche Erklärung für einen U-förmigen Zusammenhang

zwischen BDNF und WHR könnte auf die neuroprotektiven Eigenschaften von BDNF bei Adipositas-assoziierter Neuroinflammation zurückzuführen sein [6]. Da BDNF für die synaptische Funktion essenziell ist, führen verminderte Konzentrationen zu Störungen in BDNF-abhängigen Mechanismen, die unter anderem in die synaptische Plastizität im Hippocampus involviert sind. Dabei sind die Ausschüttung von Neurotransmittern, Neuritenwachstum, Gedächtnis und Lernen betroffen.

Vitamin D-Mangel ist mit Adipositas assoziiert [8, 71, 72]. Resultieren könnten die niedrigeren Vitamin D-Spiegel aus einer mangelnden Aufnahme und einem veränderten Stoffwechsel bei Adipositas [71, 72]. Darüber hinaus scheinen niedrige Vitamin D-Spiegel prädisponierend für eine übermäßige Gewichtszunahme zu sein, während hohe Vitamin D-Spiegel mit einer geringeren Gewichtszunahme assoziiert sind [74].
Wissenschaftliche Studien lassen einen Zusammenhang zwischen Vitamin D-Mangel und einem erhöhten Risiko für Erkrankungen des zentralen Nervensystems vermuten [75]. Unter anderem spielt Vitamin D im Rahmen der neuronalen Entwicklung, dem Neuritenwachstum und der neuronalen Differenzierung eine wichtige Rolle. Dabei ist Vitamin D in Produktion und Ausschüttung neurotrophischer Faktoren, die essenziell für die neuronale Differenzierung sind, involviert.
Darüber hinaus scheint Vitamin D neuroprotektive Effekte zu besitzen. Vitamin D beeinflusst dabei den zerebralen Calcium- und Glutathionstoffwechsel und schützt das Nervensystem so vor oxidativem Stress. So lassen klinische Studien einen Zusammenhang zwischen neurodegenerativen Erkrankungen wie AD und Morbus Parkinson und Vitamin D-Mangel und vermuten.
Die genauen Pathomechanismen, die durch veränderte BDNF- und Vitamin D-Spiegel bei Adipositas zum Tragen kommen, sind noch nicht abschließend geklärt. Abschließend kann angenommen werden, dass es durch diese Veränderungen zu Abweichungen in multiplen zerebralen Stoffwechselprozessen kommt, die letztendlich zu Beeinträchtigungen in neuronaler Integrität und Funktionsfähigkeit führen.

4.3 Ausblick

Zusammenfassend kann im Einklang mit der aktuellen Studienlage eine unmittelbare neuronale Schädigung in den Anfangsstadien von Adipositas angenommen werden. Mit zunehmender Dauer und dem Fortschreiten der Adipositas scheinen die Auswirkungen des übermäßigen Körperfetts komplexe und tiefgreifende neuronale Folgen nach sich zu ziehen.

Übermäßiges Körpergewicht und die damit verbundenen Auswirkungen auf Gesundheit, zerebrale Struktur und Funktion werden insbesondere durch die alternde Bevölkerung ein zunehmendes globales Problem darstellen. Daher ist es essenziell, pathologische Folgen frühzeitig zu erfassen, um therapeutisch intervenieren zu können. Bislang werden Biomarker bei Adipositas nur im Rahmen von Studien und nicht routinemäßig erhoben. Es zeigte sich, dass Änderungen der Werte von Markern wie NSE und BDNF mit den schädlichen Auswirkungen des Körperfetts auf das Gehirn assoziiert sind. Um sie im Rahmen von Prävention und Therapiemonitoring einsetzen zu können, bedarf es noch weiterer Forschung und Evaluation, da von den jeweiligen Serumspiegeln nicht direkt auf den Grad der Hirnschädigung bei Adipositas geschlossen werden kann.

Die zukünftige Erfassung von Biomarkern bei Adipositas im klinischen Alltag könnte so die Therapieadhärenz von Patienten verbessern und durch gezielte Interventionen bei Risikopatienten ein Fortschreiten neuronaler Schäden verhindern.

5 Zusammenfassung

Adipositas stellt weltweit ein zunehmendes Problem dar. Es besteht kein Zweifel an den systemischen schädlichen Auswirkungen des übermäßigen Körperfetts. Auch das Nervensystem ist von den pathologischen Prozessen betroffen, die durch Adipositas angestoßen werden. Die genauen Mechanismen, die diesen Prozessen zugrunde liegen, sind noch unklar. Auch gibt es bislang keine klinisch etablierten Biomarker, die eine gezielte Diagnostik und ein Therapiemonitoring der neuronalen Schäden ermöglichen. NSE ist ein Marker für Neurodestruktion. Bei Adipositas und Demenz weisen Studien auf das Potenzial von NSE als Marker für die zerebralen Auswirkungen dieser Erkrankungen hin. Daher behandelt diese Dissertation die Zusammenhänge zwischen NSE, BMI, GMV und Alter. Darüber hinaus wurde die Assoziation zwischen dem weiteren Biomarker BDNF sowie Vitamin D und Adipositas untersucht. Die Daten wurden im Rahmen der SHIP-Studie in einer Teilstichprobe (SHIP-TREND) erhoben.

Es zeigten sich altersabhängig geschlechtsspezifische Unterschiede der NSE-Spiegel. Während bei Frauen die NSE-Werte im Alter anstiegen, sanken sie bei Männern. Zwischen NSE-Werten und BMI fand sich eine parabolische Assoziation mit fallenden NSE-Werten ab einem BMI ≥25 kg/m². Kein Zusammenhang fand sich zwischen NSE und GMV, Alter und magnetresonanz-tomographischen Mustern der Gehirnalterung. Zwischen Vitamin D und Adipositas fand sich eine inverse Assoziation, zwischen BDNF und der WHR ein U-förmiger Zusammenhang. Als zugrunde liegende Pathomechanismen werden geschlechtsspezifische Unterschiede der Hirnalterung, neuronale Degeneration, Veränderungen des neuronalen Glukosemetabolismus und der neuronalen Differenzierung sowie Neuroinflammation diskutiert.

Im Einklang mit der aktuellen Studienlage kann im Frühstadium von Adipositas eine akute neuronale Schädigung angenommen werden. Jedoch scheint das Fortschreiten und Andauern von Adipositas tiefgreifende Veränderungen durch das überschüssige Körperfett anzustoßen, die sich auf neuronaler Ebene manifestieren. Weitere Studien zur Evaluierung von Biomarkern bei Adipositas sind nötig, um klinisch wirksame Handlungsstrategien entwickeln zu können.

Die zukünftige Erfassung von Biomarkern bei Adipositas im klinischen Alltag könnte so die Therapieadhärenz von Patienten verbessern und durch gezielte Interventionen bei Risikopatienten ein Fortschreiten neuronaler Schäden verhindern.

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7 Anhang

7.1 Verwendete Zeitschriftenartikel

Die dieser kumulativer Arbeit zugrundeliegenden wissenschaftlichen Artikel sind nachfolgend aufgelistet. Die Artikel sind auf den nachfolgenden Seiten reproduziert.

1 **Hoffmann, J**., Janowitz, D., Auwera, S.V. der, Wittfeld, K., Nauck, M., Friedrich, N., Habes, M., Davatzikos, C., Terock, J., Bahls, M., Goltz, A., Kuhla, A., Völzke, H., Grabe, H.J.: Association between serum neuron-specific enolase, age, overweight, and structural MRI patterns in 901 subjects. Transl. Psychiatry. 7, 1272 (2017). doi:10.1038/s41398-017-0035-0, (IF: 4,730).

2 Goltz, A., Janowitz, D., Hannemann, A., Nauck, M., **Hoffmann, J**., Seyfart, T., Völzke, H., Terock, J., Grabe, H.J.: Association of Brain-Derived Neurotrophic Factor and Vitamin D with Depression and Obesity: A Population-Based Study. Neuropsychobiology. 1–11 (2018). doi:10.1159/000489864, (IF 1,421).

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ARTICLE Open Access

Association between serum neuron-specific enolase, age, overweight, and structural MRI patterns in 901 subjects

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Abstract

Serum neuron-specific enolase (sNSE) is considered a marker for neuronal damage, related to gray matter structures. Previous studies indicated its potential as marker for structural and functional damage in conditions with adverse effects to the brain like obesity and dementia. In the present study, we investigated the putative association between sNSE levels, body mass index (BMI), total gray matter volume (GMV), and magnetic resonance imaging-based indices of aging as well as Alzheimer's disease (AD)-like patterns. Subjects/Methods: sNSE was determined in 901 subjects (499 women, 22–81 years, BMI 18–48 kg/m^2), participating in a population-based study (SHIP-TREND). We report age-specific patterns of sNSE levels between males and females. Females showed augmenting, males decreasing sNSE levels associated with age (males: $p = 0.1052$, females: $p = 0.0363$). sNSE levels and BMI were non-linearly associated, showing a parabolic association and decreasing sNSE levels at BMI values >25 ($p = 0.0056$). In contrast to our hypotheses, sNSE levels were not associated with total GMV, aging, or AD-like patterns. Pathomechanisms discussed are: sex-specific hormonal differences, neuronal damage/differentiation, or impaired cerebral glucose metabolism. We assume a sex-dependence of age-related effects to the brain. Further, we propose in accordance to previous studies an actual neuronal damage in the early stages of obesity. However, with progression of overweight, we assume more profound effects of excess body fat to the brain.

Introduction

The global prevalence of obesity has more than doubled since 1980. In 2014, >1.9 billion adults were overweight (body mass index (BMI) ≥25 kg/m²), from which over 600 million were obese (BMI ≥30 kg/m²)[1]. The global world population is aging and gaining weight; therefore, we will face the problem of many overweight elderly people in the future. There is growing evidence that the consequences of excess body weight extend to the brain. Specifically, human[2] and animal studies[3] reported effects of obesity on neuronal structure and function.

For example, obesity has been associated with increased gray matter (GM) damage[4–6]. However, the mechanism of how obesity, GM reductions, and cognitive alterations are related remains subject of ongoing discussion. Many studies reported midlife obesity to increase the risk for cognitive impairment[2], and neurodegenerative diseases like Alzheimer's disease (AD)[7]. Obesity accelerates neurodegenerative processes known in the aging brain[8], and initiates structural cerebral alterations that result in impairments of memory performance in aging[5].

Recent studies established indices that acquire cerebral atrophy patterns. They allow to distinguish between age-dependent atrophy (spatial pattern of atrophy for

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recognition of brain aging, SPARE-BA), and atrophy specifically found in clinically diagnosed AD cases (spatial patterns of abnormality for recognition of early AD, SPARE-AD)[9] from magnetic resonance (MR) images. The SPARE-AD index is predictive for transition from normal cognition to mild cognitive impairment[10], and further on to AD[11]. Habes et al.[9] demonstrated that waist circumference (WC) was associated with more advanced brain aging patterns in a male sub-cohort in our study population.

In order to give evidence for the presence and extent of cerebral damage and alterations, we need brain-specific markers. In this context, there is growing evidence, that the neuron-specific enolase (NSE) could be a good candidate. NSE is an enzyme of the glycolytic pathway and is closely related to the differentiated state of mature nerve cells[12]. In neurons, NSE is primarily localized in the cytoplasm. Since NSE cannot be secreted by cells, an increase of NSE in cerebrospinal fluid (CSF) or serum is a marker for neuronal damage[13]. Using the Allen Brain Atlas, it was shown that NSE gene expression is increased in frontal and parietal lobes, claustrum, and cerebellum, with claustrum and cerebellum showing the highest gene expression[14].

Previous studies demonstrated that different regions are affected by GM reduction in normal aging or AD. Cerebral alterations in aging or AD can be distinguished by SPARE-BA or SPARE-AD[9], but no study investigated the association between aging patterns and serum neuron-specific enolase (sNSE) levels so far.

In addition of the effects of aging on GM, an increased body weight influences gray matter volume (GMV)[4]. First evidence of a putative link between obesity-associated alterations in GMV and sNSE levels was established by the study of Mueller et al.[14]. The authors described an inverse correlation between sNSE and GM density in hippocampal and cerebellar regions in overweight young adults. Therefore, we sought to investigate if sNSE levels would be indicative of these alterations in GMV associated with obesity in our population-based sample.

Additionally, medication may interfere with measurements of cerebral volume and function. Therefore, we controlled calculations also for antihypertensive and lipid-lowering drugs.

Based on findings in literature, we hypothesized, that

1. sNSE levels are positively associated with age,
2. sNSE levels are positively associated with increasing BMI or WC,
3. sNSE levels are negatively associated with GMV and structural aging patterns (SPARE-BA and SPARE-AD).

Materials and methods

Participants

We collected data in course of the population-based Study of Health in Pomerania (SHIP)[15–17]. The study comprises adult residents living in three cities and 29 communities, with a total population of 212,157. SHIP-TREND contains a stratified random sample of 8,016 adult Caucasians aged 20–79 years (baseline). Effectively, a total number of 4,420 subjects participated in the study. Baseline information was collected between 2008 and 2011. The only exclusion criterion for SHIP-TREND was participation in the parallel running study SHIP-0. The data of sNSE were collected within a subsample of SHIP-TREND, consisting of 1,000 participants without clinical diabetes mellitus.

The study was approved by the ethics committee of the University of Greifswald adhering to the Declaration of Helsinki. All subjects provided written informed consent.

Clinical examination and medication

Clinical examination procedures have been described previously[4].

Smoking was defined as current smoking (occasional; 1–14 cigarette(s) per day; ≥15 cigarettes per day), former smoking (occasional; 1–14 cigarette(s) per day; ≥15 cigarettes per day), and never smoking.

Current medication was recorded using Anatomical Therapeutic Chemical classification codes[18]. For our analysis, we used information on antihypertensive medication (C02*, C03*, C07*, C08*, and C09*) and lipid-lowering drugs (C10*).

Laboratory analyses

Fasting blood samples (fasting ≥8 h) were drawn between before noon from the cubital vein of subjects in the supine position and analyzed immediately or stored at −80 °C. Serum concentrations of sNSE were determined using an immunoassay (cobas e 411 analyzer, Roche Diagnostics GmbH, Mannheim, Germany) with a functional sensitivity of 0.05 µg/L. The interassay variation was 4.4%.

Photometry was used to quantify high-density lipoprotein cholesterol (HDL-C) concentrations (Hitachi 704, Roche Diagnostics). Comparability in the longitudinal HDL-C analyses was ensured by using baseline HDL-C concentrations as the reference, and calculating corrected follow-up HDL-C concentrations based on a previous published conversion formula ($HDL\_fu\_corr = -80 + (1.158 \times HDL\_fu)$)[19]. We quantified serum low-density lipoprotein (LDL-C) by applying a precipitation procedure using dextran sulphate (Immuno, Heidelberg, Germany) on an Epos 5060 (Eppendorf, Hamburg, Germany). LDL-C, HDL-C, and total cholesterol were measured in mmol/L as dimensional scores.

The laboratories analysing the samples of SHIP, participate in the official German external quality proficiency testing programmes. As often as available, all assays were calibrated against the international reference preparations.

Magnetic resonance imaging

We asked participants to undergo whole-body magnetic resonance imaging (MRI). The image acquisition parameters of the whole-body MRI scans in SHIP have been described previously[20]. The images were acquired with 1.5 Tesla scanner (Magnetom Avanto: Siemens Medical Solutions, Erlangen, Germany). MRI images were available for all 901 subjects with information on sNSE. Subjects who fulfilled exclusion criteria against MRI (e.g., pregnancy, cardiac pacemaker), who refused participation and subjects with a stroke, Parkinson's disease, epilepsy, hydrocephalus, enlarged ventricles, pathological lesions, history of cerebral tumor, and multiple sclerosis were excluded from the present analyses. Moreover, we excluded images with severe inhomogeneities of the magnetic field, strong movement artefacts, and images, who failed for quality control. Within the voxel based morphometry (VBM), 8 toolbox homogeneity check was conducted. After exclusion, 832 subjects remained in the sample (Supplementary Fig. 4). For detailed information for exclusion criteria of MRI of the brain in SHIP-TREND, see Supplementary Fig. 5.

For structural examination, the three-dimensional T1-weighted axial MRI sequence with the following parameters was used: 1.900 ms repetition time, 3.4 ms echo time, flip angle = 15, and a voxel size of 1.0 × 1.0 × 1.0 mm. GM, white matter, and the volume of the CSF were determined using SPM 8 with the VBM toolbox for spatially normalization by the means of high-dimensional DARTEL, bias correction, and segmentation into the different tissue classes of the T1 images. Intracranial volume (ICV) was calculated as the sum of GM, white matter, and CSF.

MRI pattern classification

The SPARE-AD is an index previously developed using a support vector machine classifier that allows distinguishing between atrophy patterns in regions typically affected by clinical AD. It predicts the transformation from normal cognition to mild cognitive impairment and further on to clinical AD. Furthermore, we included in this study the SPARE-BA index to capture brain aging patterns of atrophy. The method of SPARE-BA has been described in more details earlier[9].

Statistical analysis

In 99 subjects, sNSE was not measurable, thus data about sNSE levels were available for $N = 901$ participants. To detect sex-dependent differences on a descriptive level, we divided the sample in females and males. To detect BMI-dependent differences on a descriptive level, we divided the sample in two groups. The overweight group contained participants with a BMI ≥25 kg/m², the normal-weighted group subjects with a BMI <25 kg/m². The χ^2 test was used to evaluate differences between groups for categorical variables (e.g., sex or medication) and the independent samples t test to compare means of continuous data (e.g., age or sNSE concentrations). Associations between sNSE levels and age, sex, BMI, vascular risk factors, or GMV were performed using linear regression analyses with robust estimates with STATA/MP version 13.1 (StataCorp, TX 77845, USA). Bootstrap analyses with 1,000 replicates were used to evaluate the robustness of the models. This did not change the results of calculations of the association between sNSE and age, specifically the sex-separated analyses ($p = 0.008$), nor the results of calculations of a non-linear association between sNSE, BMI, and WC (BMI $p = 0.0056$, WC $p = 0.0049$). In calculations concerning an association between sNSE and BMI, the non-linear gave a better model fit (R^2 increase from 2.2 to 2.9%). Thus, we included cubic splines for BMI. Statistical significance was defined as $p < 0.05$. Hypertension was defined by a systolic blood pressure (BP) ≥140 Hg and/or a diastolic BP ≥90 and/or antihypertensive intake. We further tested for associations between sNSE levels and SPARE-BA or SPARE-AD. Linear regression analyses with robust estimates on the dependent variable sNSE or GMV were performed. The analyses were adjusted for age and sex as basic confounders. As we observed an interaction term between age and sex, we included this interaction term in all further regression models. Analyses for GM were additionally adjusted for ICV and analyses for blood lipids and blood pressure were additionally adjusted for medication. Tests for non-linearity were performed graphically using lowess-smoothing plots for the full sample as well as sex-separated to assess possible interaction effects with sex. Analyses for blood pressure, hypertension, and blood lipids were controlled for medication. Specifically, all calculations were adjusted for age, sex, and age × sex interaction. Calculations on triglycerides, LDL-C, HDL-C, total cholesterol were additionally adjusted for lipid-lowering drugs. Calculations on systolic and diastolic blood pressure were additionally adjusted for antihypertensives.

Results

Sample characteristics

In total, we included 901 subjects (499 (55.4%) female) for the main analysis (Supplementary Table 1).

Table 1 shows the descriptive statistics of sex-dependent differences in the sample. Compared with males, females had lower GMV, BMI, WC, systolic and diastolic BP, hypertension, and triglycerides, but higher HDL-C and total cholesterol on a descriptive level. The difference between males and females regarding smoking nicotine was also significant: Females were more often current smokers, whereas males were more often former

Table 1 Descriptive sample characteristics: group comparison of females and males

	Females *N*/mean	±SD	Males *N*/mean	±SD	*p* value
Participants	499		402		
Age (y)	50.45	±13.09	50.16	±14.16	0.7449
NSE (µg/L)	8.73	±3.81	8.95	±3.57	0.3739
GMV (cm^3)*	557.77	±55.66	606.1	±65.68	**<0.001**
BMI (kg/m^2)	26.83	±4.86	27.7	±3.68	**0.0029**
WC (cm)	82.85	±11.71	94.09	±10.95	**<0.001**
WC ≥88 (cm), women/≥102 (cm) men	379		315		**0.027**
Systolic BP in mmHg	118.83	±15.59	131.2	±15.25	**<0.001**
Diastolic BP in mmHg	74.35	±15.59	79.21	±9.89	**<0.001**
Hypertension	178		179		**<0.001**
Smoking nicotine					**<0.001**
Current	254		130		
Former	141		184		
Never					
Triglycerides in mmol/L	1.28	±0.67	1.58	±1.03	**<0.001**
LDL-C in mmol/L	3.4	±0.9	3.44	±0.88	0.5386
HDL-C in mmol/L	1.61	±0.36	1.32	±0.3	**<0.001**
Total cholesterol in mmol/L	5.59	±1.04	5.37	±1.03	**0.0022**
Antihypertensives	153		109		0.137
Lipid-lowering drugs	34		36		0.143

BMI body mass index, *BP* blood pressure, *GMV* gray matter volume, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *NSE* neuron-specific enolase, *WC* waist circumference, *y* years
*Data available for 832 subjects; bold values represent statistical significant *p*-values defined as $p < 0.05$

Table 2 Descriptive sample characteristics: group comparisons in terms of the BMI

	BMI <25 *N*/mean	±SD or (%)	BMI ≥25 *N*/mean	±SD or (%)	*p* value
Participants	302	(33.5)	599	(66.5)	
Sex					**<0.001**
Women	203	(40.7)	296	(59.3)	
Men	99	(24.6)	303	(75.4)	
Age (y)	44.95	±13.4	53.0	±12.8	**<0.001**
NSE (µg/L)	9.0	±3.6	8.8	±3.7	0.407
GMV (cm^3)*	590.4	±66.5	576.8	±63.2	**0.004**
WC (cm)	76.2	±7.2	93.7	±10.6	**<0.001**
WC ≥88 (cm), women	1	(0.7)	147	(99.3)	**<0.001**
WC ≥102 (cm), men	0	(0.0)	104	(100.0)	**<0.001**
Systolic BP in mmHg	116.4	±13.9	128.4	±16.4	**<0.001**
Diastolic BP in mmHg	72.4	±8.2	78.6	±9.6	**<0.001**
Hypertension	45	(12.6)	312	(87.4)	**<0.001**
Smoking nicotine					**0.013**
Current	72	(23.8)	118	(19.7)	
Former	89	(29.5)	236	(39.4)	
Never	140	(46.4)	244	(40.7)	
Triglycerides in mmol/L	1.0	±0.4	1.6	±1.0	**<0.001**
LDL-C in mmol/L	3.1	±0.9	3.5	±0.9	**<0.001**
HDL-C in mmol/L	1.6	±0.4	1.4	±0.3	**<0.001**
Total cholesterol in mmol/L	5.3	±1.0	5.6	±1.0	**<0.001**
Antihypertensives	33	(12.6)	229	(87.4)	**<0.001**
Lipid-lowering drugs	6	(8.6)	64	(91.4)	**<0.001**

BMI body mass index, *BP* blood pressure, *GMV* gray matter volume, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *NSE* neuron-specific enolase, *WC* waist circumference, *y* years
*Data available for 832 subjects; bold values represent statistical significant *p*-values defined as $p < 0.05$

smokers. No differences were seen for age, NSE, LDL-C, antihypertensive and lipid-lowering drugs in the group comparisons. Table 2 shows the descriptive analysis of BMI-dependent differences in the sample. Men were more often overweight than women were, and overweight subjects were older compared to individuals with normal weight ($p < 0.001$). Figure 1 shows a scatter plot with the linear increase of BMI during aging. GMV was lower in the overweight group ($p = 0.004$) in a subcohort of 832 subjects with MRI assessment. Obesity-associated comorbidities as hypertension and increased blood lipids were more often in the overweight group ($p < 0.001$), as well as antihypertensive intake ($p < 0.001$). Apart from HDL-C, all blood lipids (triglycerides, LDL-C, and total cholesterol) and lipid-lowering drug intake were higher in the overweight group ($p < 0.001$). Former smokers were more often in the obese group. Overweight showed somewhat lower sNSE levels than normal weighted, but the difference was not significant.

Association between sNSE with age

We did not find linear association between age and sNSE levels ($p = 0.205$, beta = 0.0122) (Table 3). As we observed different age effects in males and females, we

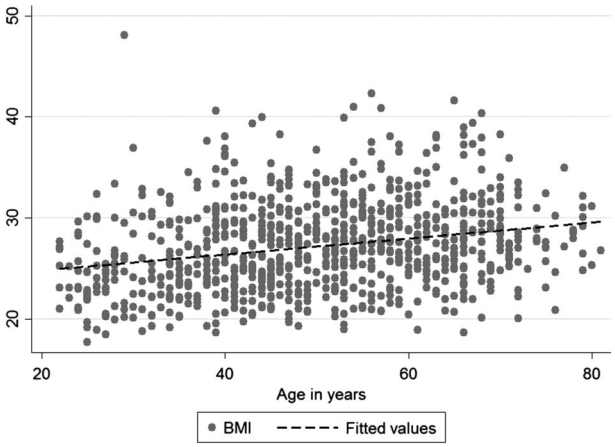

Fig. 1 Increasing patterns of BMI during aging. Subjects show an increasing BMI with advancing age. Linear trend line, no adjustment. BMI in kg/m²

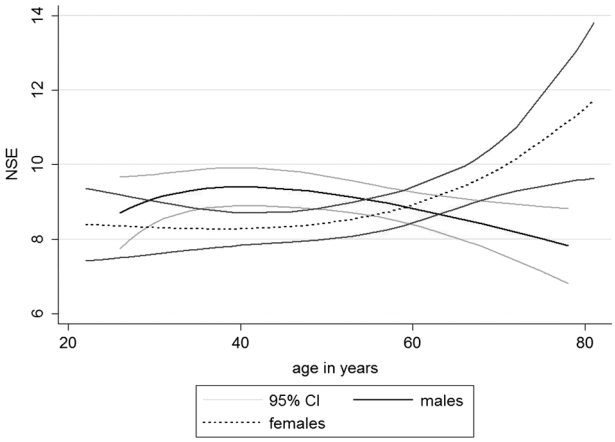

Fig. 2 Non-linear age-dependent sNSE levels in men and women. Women show constant low sNSE levels in younger years and increasing levels in higher ages, whereas men show increasing sNSE levels in young adulthood and a decrease in older ages. About an age of 60 years, sNSE values are similar in men and women. Sex-specific differences in the hormonal balance may serve as explanatory approach. After menopause, women have both lower levels of estradiol and testosterone. The missing estradiol-mediated neuroprotective effects may result in increased neural death, showing in increased sNSE levels. sNSE in µg/L

Table 3 Association of sNSE levels with age (non-linear), sex, and sex × age interaction

	Beta	SE	*T*	*p* value
Age (y)*#			$F = 1.05$	0.37
Sex*	−0.221	0.2467	−0.9	0.371
Age × sex interaction	0.05	0.019	2.64	0.009

sNSE serum neuron-specific enolase
*Unadjusted
#Variables were treated non-linear as splines; no betas, SE possible

included sex-stratified cubic splines for age (four knots equally placed; males: 26, 44, 57, 72 and females: 28, 45, 56, 71) and included an age × sex interaction term. We observed a significant age–sex interaction ($p = 0.009$). The levels of sNSE in aging are depicted as scatter plots separately for males and females in Supplementary Fig. 1. In sex-specific analyses, the association between age and sNSE was not significant ($p = 0.1052$) for males, whereas females showed a significant association between age and sNSE levels ($p = 0.0363$). For males, a U-shaped and for females a J-shaped relation between age and sNSE was observed (Fig. 2).

Association between sNSE and BMI

sNSE levels decreased with increasing BMI in the linear model (Supplementary Fig. 2). In the non-linear model, sNSE values and BMI showed a U-shaped negative association (Supplementary Fig. 3). These effects were significant for both, BMI ($p = 0.0056$) and WC ($p = 0.0042$), apart from an influence of age and sex. Specifically, sNSE levels increased with higher BMI values up to a BMI of 25 kg/m² and decreased thereafter (Fig. 3). *p* values for the association between sNSE levels and BMI or WC were similar; therefore, we performed the following analyses with BMI only. To investigate presumed age-dependent differences in overweight-associated cerebral impairment, we stratified the sample for age. Regarding a possible interaction between BMI and age, the interaction term was not significant ($p = 0.244$), but when stratifying the sample into different age groups (age <40, age 40–60, age >60), only the oldest sample revealed a significant association between sNSE and BMI ($p = 0.02$). When regarding the overweight and normal-weighted separately, a significant association between sNSE levels and BMI was specific for overweight elderly ($p = 0.0365$). Concerning hypertension, blood pressure, blood lipids and smoking status, only HDL ($p = 0.016$), and current smoking ($p = 0.019$) exhibited a significant inverse association with sNSE in the full sample (Table 4).

Association between sNSE and GMV, SPARE-BA and SPARE-AD

sNSE levels were not associated with GMV ($p = 0.963$) after adjustment for ICV (Table 5). Stratifying the sample into subjects with and without overweight parallel to Mueller et al., results were also not significant (BMI <25: $p = 0.854$; BMI ≥25: $p = 0.416$). Overweight elderly aged >60 years, showed a negative association with GMV, yet the association was not significant. In normal-weighted elderly, this tendency was not seen (BMI <25: $p = 0.65$; BMI ≥25: $p = 0.068$). Also no significant associations with

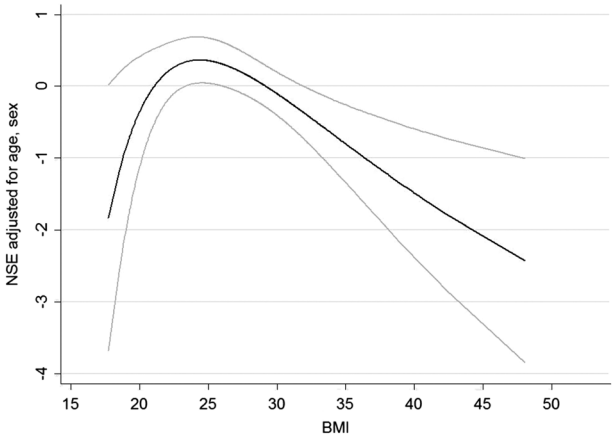

Fig. 3 Non-linear association between sNSE levels and BMI. sNSE levels increase up to a BMI of >25, and decline with higher BMI values. Obesity-associated neuronal damage may reflect the increasing sNSE levels up to a BMI <25. In subjects with a very high BMI (>25), the excess body fat affected cerebral structures over a long time, what could result in decreased GMV. This reduced GMV could show in a drop of sNSE levels, as there would be less and less GMV containing NSE. Other explanatory approaches for decreasing sNSE levels at BMI levels >25 are impairments in glucose metabolism and neuronal differentiation. NSE in µg/L

sNSE and SPARE-BA ($p = 0.928$) or SPARE-AD ($p = 0.643$) were observed in the total sample or in elderly aged >65 years.

However, SPARE-BA and SPARE-AD were associated with GMV ($p < 0.001$).

Discussion

We showed sex-dependent differences in the patterns of sNSE levels with increasing age, with an increase of sNSE levels in elderly women.

sNSE levels and BMI were non-linearly associated, showing a parabolic association with decreasing sNSE levels at BMI values >25 kg/m².

We could not substantiate the hypothesis of an inverse association between sNSE levels and GMV, yet we saw the tendency in elderly subjects with overweight. Atrophy patterns in brain aging (SPARE-BA) or for AD-like patterns of atrophy (SPARE-AD) were not associated with sNSE levels.

Association between sNSE and age

The effect of age on NSE concentrations is differently discussed in the literature, partly because NSE levels may be assessed in CFS or in serum[13, 21, 22]. Until now, only NSE levels in CSF and age were positively associated[22]. In contrast, serum NSE levels showed no age-dependent alterations in two studies with $N = 108$ and $N = 41$ probands[13, 21]. Corresponding to previous studies based on sNSE levels[13, 21], we report no significant linear association between NSE and age. Considering the extensive neural loss in the course of AD, studies have aimed to show disease-associated alterations in sNSE levels. However, studies produced inconsistent results concerning sNSE levels in AD[23, 24]. Chaves et al.[23] found no differences in sNSE levels between AD patients and healthy elderly controls.

However, we did find significant non-linear sex-specific associations with aging. Specifically, females showing augmented, males decreasing sNSE levels associated with aging. We saw no association between sex and sNSE levels, which is in line with previous studies[13], but an interaction between sex and age on sNSE levels. It seems contradictive that there was no association between sex and sNSE levels, but an association between sNSE levels

Table 4 Associations of sNSE levels with BMI, WC, and lipids

	Beta	SE	*T*	*p* value
BMI (kg/m²)[#]			$F = 4.22$	**0.0056**
WC (cm)[#]			$F = 4.44$	**0.0042**
Triglycerides in mmol/L*	−0.19	0.1379	−1.38	0.168
LDL-C in mmol/L*	0.1076	0.152	0.71	0.479
HDL-C in mmol/L*	0.8991	0.3734	2.41	**0.016**
Total cholesterol in mmol/L*	0.1611	0.1369	1.18	0.24
Lipid-lowering drugs[1]	−0.0684	0.4902	−0.14	0.889
Smoking nicotine				
Current (reference)				
Former	−0.2799	0.2903	−0.96	0.335
Never	−0.7749	0.3309	−2.34	**0.019**

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *sNSE* serum neuron-specific enolase, *WC* waist circumference
Associations adjusted for age (non-linear), sex, and age × sex interaction
[#]Variables were treated non-linear as splines; no betas, SE possible
*Additionally adjusted for lipid-lowering drugs; bold values represent statistical significant p-values defined as $p < 0.05$

Table 5 Association of sNSE levels with GMV and SPARE-BA and SPARE-AD

	Beta	SE	*T*	*p* value
GMV*	−0.0116	0.2462	−0.05	0.963
SPARE-BA	−0.0001	0.0106	−0.09	0.928
SPARE-AD	0.0045	0.0097	0.46	0.643

GMV gray matter volume, *sNSE* serum neuron-specific enolase, *SPARE-AD* spatial patterns of abnormality for recognition of early Alzheimer's disease, *SPARE-BA* spatial pattern of atrophy for recognition of brain aging, *WC* waist circumference
Associations adjusted for age (non-linear), sex, and age × sex interaction
GMV, SPARE-AD/BA treated as outcome
*Additionally adjusted for ICV, data available for 832 subjects

and sex depending on age. Specific differences between the sNSE levels of men and women may only show with advancing age. Therefore, no association would be found when regarding participants of all ages, for the differences between males and females would be leveled.

To our knowledge, our study is the first to describe a divergence of age-dependent sNSE levels in women and men in a large sample from the general population ($N = 901$). One may speculate sex-specific differences in the hormonal balance may serve as an explanatory approach. However, currently no research has further explored this association. Levels of sex steroids as estradiol and testosterone decline with age in both sexes. After menopause, estradiol levels show sharp descends in women, whereas testosterone levels decline more gradually in men. Elderly women have both lower levels of estradiol and testosterone[25], accompanied by higher levels of cholesterol. This may be explained by increasing NSE levels, as enolases mobilize cholesterol[26].

There is growing evidence for estradiol mediating neuroprotective effects in the hippocampus[27]. Since NSE is related to hippocampal structures, a possible explanation for our results is that the decline of estradiol-mediated neuroprotective effects is accompanied by accelerated neuronal death eventually resulting in increased sNSE levels.

Association between sNSE and BMI

Our hypotheses of a positive association between sNSE levels and BMI was based on the study of Mueller et al[14]. They described an inverse correlation between GM density in cerebellum and hippocampus and sNSE levels in $N = 27$ overweight subjects. They hypothesized that increased sNSE levels are a result of obesity-associated structural damage of GM. Accordingly, obesity-associated neuronal damage could reflect our findings of increasing sNSE levels up to a BMI <25. Moreover, the trend towards higher sNSE levels in overweight subjects in our study confirms the findings of Mueller et al. They described sNSE levels to be in the reference range in all subjects, but the overweight showed levels near the upper limit (18.3 µg/L)[14].

We report a parabolic association between sNSE levels and BMI, with decreasing sNSE levels at BMI values >25. Moreover, in higher ages only overweight subjects showed a negative association with sNSE levels. Yet, we could not see the tendency in young adults, as depicted in the sample of Mueller et al. This leads to the assumption, that there may be age-dependent differences concerning an association between sNSE levels in overweight subjects.

Association between sNSE and GMV, SPARE-BA and SPARE-AD

We did not see a linear association between GMV or SPARE-BA or SPARE-AD sNSE levels in our sample. As a marker for neuronal injury, elevated sNSE levels result from an actual cerebral destruction, especially from GM damage. sNSE levels have shown to correlate positively with the severity level of the head injury, and therefore with the extent of brain cell damage[28]. It could be assumed, in subjects with a very high BMI, the excess body fat affected cerebral structures over a long time, resulting in decreased GMV. This reduced GMV could show in a drop of sNSE levels, as there would be less and less GMV containing NSE. Decreased sNSE levels have been reported in higher extent of brain atrophy in AD[23], accordingly similar dynamics of sNSE levels can be expected in an obesity-related negative association with GMV.

The sample of Mueller et al. contained young adults (average age in the overweight group 26.4 ± 5.4 years), whereas in our sample the average age was in middle adulthood (average age in the overweight group 53.0 ± 12.8). Thus, the detrimental effects of body fat could not have affected the brain for a long time. Further, we have seen a non-significant association between sNSE levels and GMV in obese elderly, but not in non-obese subjects, which is also in agreement with the findings of Mueller et al[14]. The study of Mueller et al.[14] stated, that the higher the BMI, the greater the loss of GM density, comparable with mild cognitive impairment in the elderly. An induction of progressive brain alterations in obese subjects might be discussed, as studies revealed obesity-associated increased oxidative stress[29], and chronic inflammation[30].

Furthermore, decreased sNSE levels could result from alterations in the glucose metabolism: As glycolytic enzyme, NSE is directly involved in the metabolism of glucose and obesity-related impairment in glucose metabolism in the frontal cortex have been reported[31].

Another explanatory approach is neuronal differentiation. NSE is a specific marker for mature nerve cells, and is closely correlated to the differentiated state[12]. Depending on tissue and development state, different combinations of the subunits are expressed. γγ is specific for mature neurons with full synaptic connections, whereas during neuronal migration, hybrid enolases (αγ) are found (non-neuronal enolase)[32]. Obesity-related impairments on hippocampal neurogenesis have been demonstrated in animal studies with mice receiving a high-fat diet[33, 34]. As NSE is associated with hippocampal structures, it can be speculated that obesity-related alterations of neuronal maturation and neurogenesis extend to the development of NSE.

In conclusion, we propose in accordance to previous studies an actual neuronal damage in the early stages of obesity. However, with progression of overweight, we assume more profound effects of excess body fat to the brain.

Due to the cross-sectional design, we cannot investigate if the alterations in sNSE levels precede the onset of obesity or are resulting from the adverse effects of an enhanced bodyweight. As one study showed that a high BMI in mid-life was associated with temporal atrophy 24 years later[35] and NSE is an indicator for neuronal injury, the current data support the second hypothesis.

Strength and limitations

Strength of this study is the cross-sectional population-based sample. Thus, we can assume a sample, which is representative for the population. Stratification by age adds valuable information specific for particular age groups. With consideration of pattern classifiers in brain atrophy, we are able to make statements concerning the impact of average or increased aging processes on sNSE levels.

We measured height and weight by research assistants, therefore, we can rule out false statements concerning the BMI. As we performed analyses with BMI and WC, we can preclude effects that are unique for one measurement of obesity. We did not include data for deficits in glucose metabolism, thus this consideration should be examined in further research. To prevent confounding, we controlled for medication. However, we cannot rule out other confounding factors that may have affected the calculations.

Conclusions

Our study presents an analysis of a well-described cohort with a large number of participants, providing valuable and novel insights into alterations of sNSE levels in aging and obesity. We were able to show that sNSE levels and obesity are nonlinearly associated, even after adjusting for important confounders. Moreover, only in women, age was associated with increasing sNSE levels, thus we assume sex-specific pathomechanisms. With the determination of NSE levels in serum, we provide a valuable, easy determinable marker to get insights into brain-specific cellular alterations. Future studies, focusing on cerebral manifestations of obesity and the effect of their therapy would help to get to better and more individualized therapy strategies and to prevent or even undo cognitive impairment.

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Authors' contributions

All authors were involved in review and final approval of the manuscript. Specifically, M.N., N.F., M.H., C.D., J.H., D.J. and H.V. were involved in data collection, study design, and development of methods. S.V.d.A., J.H., D.J. and K.W. were involved in data analysis. M.B., H.J.G., A.G., A.K., and J.T. critically revised the manuscript for important intellectual content. J.H. and D.J. were responsible for writing the manuscript.

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Competing interests

The authors declare that they have no competing financial interests.

Supplementary information

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Association of Brain-Derived Neurotrophic Factor and Vitamin D with Depression and Obesity: A Population-Based Study

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Keywords
Brain-derived neurotrophic factor · Vitamin D · Depression · Obesity

Abstract
Background: Depression and obesity are widespread and closely linked. Brain-derived neurotrophic factor (BDNF) and vitamin D are both assumed to be associated with depression and obesity. Little is known about the interplay between vitamin D and BDNF. We explored the putative associations and interactions between serum BDNF and vitamin D levels with depressive symptoms and abdominal obesity in a large population-based cohort. ***Methods:*** Data were obtained from the population-based Study of Health in Pomerania (SHIP)-Trend ($n = 3,926$). The associations of serum BDNF and vitamin D levels with depressive symptoms (measured using the Patient Health Questionnaire) were assessed with binary and multinomial logistic regression models. The associations of serum BDNF and vitamin D levels with obesity (measured by the waist-to-hip ratio [WHR]) were assessed with binary logistic and linear regression models with restricted cubic splines. ***Results:*** Logistic regression models revealed inverse associations of vitamin D with depression (OR = 0.966; 95% CI 0.951–0.981) and obesity (OR = 0.976; 95% CI 0.967–0.985). No linear association of serum BDNF with depression or obesity was found. However, linear regression models revealed a *U*-shaped association of BDNF with WHR ($p < 0.001$). ***Conclusion:*** Vitamin D was inversely associated with depression and obesity. BDNF was associated with abdominal obesity, but not with depression. At the population level, our results support the relevant roles of vitamin D and BDNF in mental and physical health-related outcomes.

A. Goltz and D. Janowitz contributed equally to this work.

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Introduction

Depression and obesity are 2 widespread medical conditions with major public health implications [1, 2]. Both conditions are closely linked; recently, a bidirectional association was detected: obesity increases the risk of developing depression by 55%, whereas depressed patients have a 58% increased risk of developing obesity [3]. Little is known about the interplay between BDNF and vitamin D. In a recently published study, obesity was related to a reduced BDNF concentration in the hippocampus and vitamin D treatment increased this concentration [4]. However, that study was conducted in rats only, and therefore results may not apply to humans.

BDNF is, among others, an important growth factor in the central and peripheral nervous system, especially in hippocampal and cortical areas [5]. It plays a key role in adult neurogenesis as well as in the processes of learning and memorizing [5]. Reduced serum BDNF levels have been found to be associated with depressive symptoms or impaired memory performance. Given that BDNF passes the blood-brain barrier and circulating peripheral BDNF levels reflect central BDNF levels [6], BDNF has been suggested as a potential biomarker for different mental health conditions and particularly mood disorders [7, 8]. BDNF is expressed not only in neuronal but also in non-neuronal peripheral tissues [9, 10]. Most of the peripheral BDNF is stored in platelets [11, 12]. Recent evidence suggests that low BDNF levels in patients with depressive disorders mainly result from changes in platelet and megakaryocyte counts [12, 13]. Moreover, previous studies have revealed moderating effects of genetic epistasis between the BDNF Val66Met polymorphism (rs6265) with serotonin transporter 1 functional SNP (rs25531) and childhood adversity on depression susceptibility [14, 15].

The association between BDNF and obesity is only insufficiently understood. Proinflammatory cytokines like tumor necrosis factor are upregulated in adipose tissue of obese individuals and promote neuronal death in the brain [16, 17]. Results from Nakahashi et al. [9] showed that BDNF is involved in neuronal repair processes. Furthermore, BDNF modulates energy homeostasis by interacting with several neuropeptides, including leptin [18]. Leptin is mostly expressed in adipose tissue and regulates, among others, glucose and lipid metabolism [19]. A study from 2006 reported lower serum BDNF levels in 328 obese children and adolescents after adjusting for platelet counts [20]. In contrast, other studies with small sample sizes ($n < 100$) described higher BDNF levels in obese women compared to healthy controls [21–23]. Those previous studies mainly defined obesity based on the body mass index (BMI). Unlike the BMI, which does not provide information on regional body fat distribution [24], the waist-to-hip ratio (WHR) is an index for subcutaneous as well as intra-abdominal adipose tissue. It is more strongly related to cardiovascular diseases and overall mortality than BMI [25, 26].

Vitamin D deficiency is a considerable global health problem [27, 28]. The latitude and season determine the amount of vitamin D produced by the skin [29], the major source of vitamin D, followed by diet and dietary supplements. Thus, in Europe, for example, but also in North America, circulating 25-hydroxy vitamin D levels have a large seasonal variation [30] with lower levels of vitamin D and higher proportions of vitamin D deficiency observed in winter compared to summer [29]. Several studies including a meta-analysis of 14 studies have suggested that vitamin D deficiency is associated with depressive symptoms [31–33]. A cross-sectional study from Finland reported that vitamin D was inversely associated with depression even after adjusting for lifestyle, sociodemographic, and metabolic factors [34]. Previous studies have also detected an association of low vitamin D levels with depressive disorders and assigned a key role to vitamin D in various physiological processes [35, 36]. Vitamin D receptors are widespread throughout the central nervous system as well as other tissues and influence early childhood brain development and adult brain function [36]. Vitamin D initiates the synthesis of serotonin, a hormone strongly linked to depression [37]. By activating transcription of the serotonin-synthesizing gene [35] vitamin D modifies the production and release of neurotrophic factors via membrane-associated and nuclear vitamin D receptors in neuronal and non-neuronal cells of the central nervous system [36]. In fact, low vitamin D levels promote depressive disorders [35, 38, 39]. Although previous studies have revealed an inverse association between vitamin D and depressive disorders, recent studies report inconsistent results. For instance, a retrospective study with more than 500 participants found no association between low vitamin D levels and depression [40].

Vitamin D deficiency is also associated with obesity [41–43]. For example, inverse associations of vitamin D with total body fat and the presence of the metabolic syndrome in middle aged and elderly subjects have been described [44]. These observations might be related to the reduced bioavailability of vitamin D in obesity, caused by an increased uptake in adipose tissue [45].

In summary, conflicting results regarding the association of BDNF and vitamin D with depression and obesity

have been reported. Additionally, previous studies have been limited by small sample sizes [5, 21–23] or lacking adjustment for confounders like platelets and fibrinogen for associations with BDNF [21–23] and like smoking for associations with vitamin D [31]. We addressed these limitations by investigating a large population-based study of men and women: the Study of Health in Pomerania (SHIP)-Trend. Based on data of this cohort we elaborated the associations of BDNF and vitamin D with depressive symptoms or obesity. We hypothesized that both BDNF and vitamin D are inversely associated with depression and obesity and that BDNF and vitamin D show interactions with depression and obesity. The aim of our study was to investigate these associations in a large population-based sample to extend previously reported findings and to establish a basis for future research. Further knowledge about BDNF and vitamin D in association with depression and obesity may allow methods to prevent disease or support therapeutic options, i.e. vitamin D supplementation.

Materials and Methods

SHIP is a population-based project in northeast Germany that consists of 2 independent cohorts: SHIP and SHIP-Trend. Its objective is to determine the prevalence and incidence of risk factors and diseases and to investigate associations among risk factors and diseases [46, 47]. Both cohorts were selected from the general population in West Pomerania, a region in the northeast of Germany (latitude: 54° north). In both cohorts, only individuals with German citizenship were included. Moreover, participation in the SHIP cohort was an exclusion criterion for participation in the SHIP-Trend cohort.

The present cross-sectional study includes data from the SHIP-Trend baseline examination [46–48], in which vitamin D and BDNF levels were determined. In detail, a stratified random sample of 10,000 adults (net sample size: n = 8,826) aged 20–79 years was drawn from the local population registries. Stratification variables were age, sex, and city/county of residence. Among the invited individuals, 4,420 men and women chose to participate (50.1% response) in the baseline examinations between 2008 and 2012. Further details on the study design, protocols, and sampling methods have been reported elsewhere [46, 47]. All investigations were carried out in accordance with the Declaration of Helsinki, including written informed consent from all participants. The survey and study methods were approved by an institutional review board (SHIP-Trend [BB 39/09]: Ethics Committee of the University of Greifswald).

Interview and Physical Examination

The SHIP-Trend examinations were performed throughout the year. The season of examination was defined as winter (December to May) or summer (June to November). Trained and certified interviewers collected information on medical history, sociodemographic, and health-related factors via a computer-assisted interview. Physical activity and smoking status were assessed by self-report. Participants were defined as physically inactive if they reported less than 1 h of physical activity per week during summer and winter. Women were classified as pre- or postmenopausal based on age and self-reported menstrual cycling. All women younger than 40 years of age as well as women between 40 and 60 years of age, who reported menstrual cycling, were defined as premenopausal, and all other women were defined as postmenopausal. Data on current depressive symptoms were collected using the Patient Health Questionnaire (PHQ-9) [49]. Depression was defined as a PHQ-9 score ≥10 out of 27 achievable points. The PHQ-9 is a self-report measure of depressive disorders. It consists of 9 items that are rated according to how much a symptom has bothered during the last 2 weeks, each on a scale of 0–3. The items match the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria of major depression [50]. We used an item total score which summarizes the scores of the single items, ranging from 0 to 27. A cut-off point of 10 or above represents a good diagnostic method of screening for depressive disorders [49]. Moreover, the PHQ-9 can be used to define depression severity [50]. For this, PHQ-9 scores are categorized into the following 5 groups: none (<5), mild (5–9), moderate (10–14), moderately severe (15–19), and severe (≥19) [50]. In our study the number of subjects with PHQ-9 scores ≥15 was low (n = 63; 1.6%), and therefore we collapsed the 2 upper categories.

All study participants were asked to bring all medications taken in the last 7 days prior to the examination. The drugs were classified according to the anatomical therapeutic chemical classification system (ATC) [51]. Antidepressant drugs were defined as ATC N06A, antidiabetic drugs as ATC A10, lipid-lowering drugs as ATC C10, and antihypertensive drugs as ATC C02, C03, C07, C08, and C09. The physical examination included measurement of anthropometric parameters with calibrated scales. Waist circumference was measured midway between the lower rib margin and the iliac crest on the horizontal plane. Hip circumference was measured at the greatest circumference between the highest point of the iliac crest and the crotch. Both waist and hip circumferences were measured in centimeters. The WHR was calculated from the respective measures (waist circumference divided by hip circumference). We defined obesity according to the German Society for Sports Medicine and Prevention was a WHR >0.85 in females and >1.0 in males [52].

Blood Sampling and Laboratory Measurements

In SHIP-Trend single-occasion blood samples were drawn from the cubital vein of participants in the supine position following standardized procedures. The sampling was performed between 7:30 a.m. and 1:00 p.m. The majority (61.2%) of the study participants provided fasting (>8 h) blood samples, and the remaining samples (38.8%) were obtained from nonfasting subjects. A maximum of 65.5 mL of blood was collected in 13 tubes, including EDTA, citrate, serum, and PAXgene tubes. Directly after sampling, EDTA and serum tubes were cooled down to 4 °C, while citrate tubes were stored at room temperature. Hourly transport to the central laboratory (Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald) was arranged. After arrival at the laboratory, the samples were immediately processed. When necessary, samples were centrifuged at 2,550 g for 15 min at 8 °C. The samples were then analyzed or stored at –80 °C in the Integrated Research Biobank (LiCONiC, Lichtenstein). BDNF levels were measured in serum with a quantitative sandwich en-

Table 1. Characteristics of the study population

Characteristics	No depression and no obesity (n = 2,539)	Depression or obesity (n = 1,285)	Depression and obesity (n = 102)	p
Sex				<0.010
Male	53.0	42.6	24.5	
Premenopausal women	24.8	19.7	20.6	
Postmenopausal women	22.1	37.7	54.9	
Age, years	48.0 (36.0–61.0)	57.0 (46.0–67.0)	54.0 (47.0–61.0)	<0.001
Smoking				0.038
Nonsmokers	36.3	36.3	30.4	
Former smokers	35.6	39.6	39.2	
Current smokers	28.1	24.1	30.4	
Physically inactive	51.0	57.2	63.7	<0.001
Antidiabetic drugs	4.6	12.9	14.7	<0.001
Lipid-lowering drugs	10.2	18.5	17.7	<0.001
Antihypertensive drugs	29.2	51.0	53.9	<0.001
Antidepressant drugs	3.5	7.0	22.6	<0.001
Sleep disorders[1]			85.7	
WHR	0.84 (0.80–0.93)	0.92 (0.87–1.01)	0.90 (0.87–1.00)	<0.001
PHQ-9	3.0 (1.0–5.0)	3.0 (1.0–7.0)	12.0 (10.0–15.0)	<0.001
BDNF, pg/mL	21,363 (17,775–25,331)	21,363 (17,250–25,721)	22,487 (16,003–26,763)	0.772
PLT, GPT/L	222 (191–259)	227 (190–264)	231 (187–280)	0.201
Fibrinogen, g/L	2.90 (2.40–3.40)	3.20 (2.70–3.60)	3.30 (2.70–3.80)	<0.001
25(OH)D, ng/mL	23.3 (17.3–29.9)	21.4 (16.4–27.3)	18.7 (14.4–24.9)	<0.001
Season				0.415
Winter	51.8	51.8	45.1	
Summer	48.3	48.3	54.9	

The total number of patients was 3,926. Values are presented as medians (first to third quartiles) or percent. Group differences were tested with Kruskal-Wallis (continuous data) or χ^2 tests (nominal data). BDNF (serum), brain-derived neurotrophic factor; PHQ-9, Patient Health Questionnaire; PLT, platelet count; WHR, waist-to-hip ratio; 25(OH)D, 25-hydroxy vitamin D; no depression, PHQ-9 score <10; depression, PHQ-9 score ≥10; not obese, WHR <0.85 (females) or <1.00 (males); obese, WHR ≥0.85 (females) or ≥1.00 (males). [1] Proportion of participants with sleep disorders, including insomnia or hypersomnia or inability to fall and stay asleep, for at least 2 weeks among 102 subjects with depression and obesity.

zyme immunoassay technique (Quantikine Human Free BDNF Immunoassay, R&D Systems, Inc., Minneapolis, MN, USA). Two concentrations of control material were measured. The coefficients of variation for BDNF were 14.95% at low levels (129 pg/mL) and 5.81% at high levels (667 pg/mL) of control material. Serum 25-hydroxy vitamin D levels were measured on an IDS-iSYS Multi-Discipline Automated Analyzer (Immunodiagnostic Systems Limited, Frankfurt am Main, Germany). Three concentrations of control material were measured. The coefficients of variation for vitamin D were 11.6 at low, 9.1 at medium, and 10.6% at high levels of control material. The participants' vitamin D status was defined as deficient (vitamin D <20 ng/mL) or sufficient (vitamin D ≥20 ng/mL) according to the recommendation of the German Nutrition Society [53]. Serum creatinine was measured with a modified kinetic Jaffé method (Siemens Dimension Vista; Siemens Healthcare Diagnostics, Eschborn, Germany). The estimated glomerular filtration rate was estimated according to the 4-variable Modification of Diet in Renal Disease formula [54]. Fibrinogen levels were determined in citrate plasma according to Clauss with a BCS-XP analyzer (Siemens Healthcare Diagnostics). Platelets were counted in EDTA whole-blood samples using Sysmex XT2000, XE5000, or SE9000 analyzers (Sysmex, Kobe, Japan) or Advia (Siemens Healthcare Diagnostics).

Selection of the Study Population

We excluded from the SHIP-Trend participants (n = 4,420) all men and women with missing data on exposure, outcome, or confounders (n = 441) for the statistical analyses. From the remaining subjects we excluded all of those (overlap exists) with renal disease, defined as an estimated glomerular filtration rate <30 mL/min/1.73 m^2, or missing information on renal disease (n = 7), suspected hyperparathyroidism, defined as parathyroid hormone levels >120 pg/mL (n = 7), intake of parathyroid hormone or vitamin D supplements (ATC H05AA and A11CC, n = 32), and all pregnant women. The resulting study population consisted of 3,926 participants aged between 20 and 84 years.

Statistical Analyses

Statistical analyses were performed using SPSS version 23 (SPSS Inc., Chicago, IL, USA) and SAS 9.4 (SAS Institute Inc., Cary, NC, USA). The general characteristics of our study participants grouped into (1) not depressed and not obese, (2) depressed or obese, and (3) depressed and obese individuals are given in Table 1. Categorical data are given as proportions; continuous data are given as medians (1st to 3rd quartiles). For group comparisons Kruskal-Wallis or χ^2 tests were used. $p < 0.05$ was considered statistically significant.

Table 2. Associations of BDNF or vitamin D serum levels with depression or obesity

Outcome	Model	BDNF				Vitamin D				BDNF × vitamin D interaction	
		OR (95% CI)	*p*	χ^2 (*p*)	R^2	OR (95% CI)	*p*	χ^2 (*p*)	R^2	OR (95% CI)	*p*
Depression	1	0.997 (0.977–1.017)	0.754	0.1 (0.753)	<0.001	0.973 (0.959–0.987)	<0.001	15.5 (<0.001)	0.010	1.002 (1.000–1.004)	0.105
	2	0.992 (0.972–1.013)	0.451	46.8 (<0.001)	0.029	0.976 (0.962–0.990)	<0.001	58.1 (<0.001)	0.036	1.002 (1.000–1.005)	0.057
	3	0.995 (0.974–1.017)	0.681	50.1 (<0.001)	0.031	0.966 (0.951–0.981)	<0.001	70.0 (<0.001)	0.044	1.002 (1.000–1.005)	0.065
Obesity	1	1.000 (0.989–1.011)	0.954	<0.1 (0.950)	<0.001	0.974 (0.966–0 981)	<0.001	47.9 (<0.001)	0.017	1.000 (0.998–1.001)	0.467
	2	0.997 (0.986–1.009)	0.656	353.1 (<0.001)	0.121	0.980 (0.972–0.988)	<0.001	377.1 (<0.001)	0.129	0.999 (0.998–1.000)	0.165
	3	0.995 (0.982–1.007)	0.393	411.8 (<0.001)	0.141	0.976 (0 967–0.985)	<0.001	383.3 (<0.001)	0.131	0.999 (0.998–1.000)	0.117

Results from binary logistic regression models (n = 3,926). Depression was defined as a Patient Health Questionnaire score ≥10. Obesity was defined as a waist-to-hip ratio ≥0.85 (females) or ≥1.00 (males). In the logistic regression models, an increase of 1,000 pg/mL in BDNF and of 1 ng/mL in vitamin D was modeled. Model 1 = unadjusted; model 2 = adjusted for sex, age, physical activity, and smoking and for WHR in depression models; model 3 = model 2 + platelets and fibrinogen in BDNF models or + season in vitamin D models. The overall model fit is given by the χ^2 *(p)* values as well as Nagelkerke's R^2. BDNF, brain-derived neurotrophic factor.

We firstly assessed whether BDNF and vitamin D serum levels were related by performing a partial correlation analysis adjusting for sex, age, platelets, and season. Afterwards, we examined the associations of BDNF and vitamin D (continuous exposure variables) with depression or obesity (dichotomous outcome variables) in multivariable binary logistic regression models. In these models we also examined whether the participants' vitamin D status was a potential effect modifier by testing the interaction of BDNF and vitamin D. We additionally used multinomial logistic regression to assess whether increasing BDNF or vitamin D serum levels (continuous exposure variables) are related to higher odds of being not depressed and not obese compared to being depressed and obese (categorized outcome variable). We further assessed the associations between BDNF or vitamin D (continuous exposure variables) and depression severity (categorized outcome variable) in multinomial logistic regression and used multivariable linear regression models to assess the associations between BDNF or vitamin D (continuous exposure variables) and the WHR (continuous outcome variable). To account for nonlinearity, we added restricted cubic splines with 3 knots to the linear regression models. The three knots were prespecified, located at the 5th, 50th, and 95th percentiles, resulting in one component of the spline function, which was named BDNF' or vitamin D', respectively. Whether the overall effect of the exposure was different from zero was tested with a Wald χ^2 test. We report β-coefficients with standard errors SE and p values from the linear regression models and OR with 95% CI and p values from the logistic regression models. To describe the overall model fit we reported F and p values as well as the adjusted R^2 for the linear regression models and χ^2 and p values as well as Nagelkerke's R^2 for the binary and multinomial logistic regression models. To take confounding into account, we calculated 3 sets for each model with different adjustments. Model 1 was unadjusted; model 2 was adjusted for sex, age, physical inactivity, and smoking and additionally for WHR in models with depression as the outcome; and model 3 was additionally adjusted for platelets and fibrinogen in models with BDNF as exposure or for season in models with vitamin D as exposure. To account for multiple testing, we used the Bonferroni correction to assess statistical significance in the regression models. In total we tested the associations between 2 exposure variables (BDNF and vitamin D) and 5 outcome variables (depression, obesity, depression/obesity group, depression severity, and WHR), yielding a Bonferroni-corrected statistical significance at $p < 0.005$ (i.e., 0.05/10).

Results

Table 1 shows the baseline characteristics of the study sample stratified into not depressed or not obese (n = 2,539), depressed or obese (n = 1,285), and depressed and obese (n = 102) individuals. Women were overrepresented in the depressed or obese group and also in the depressed and obese group. Not depressed and not obese subjects were younger and less often physically inactive and had a lower WHR and higher vitamin D concentrations than subjects in the other 2 groups. Moreover, intake of antidiabetic, lipid-lowering, or antihypertensive medication was substantially less frequent in subjects without depression or obesity than in the other groups. Antidepressant drug intake was highest among depressed and obese individuals, with more than 22% of subjects in

Table 3. Associations of BDNF or vitamin D serum levels with depression and obesity

Model	Outcome	BDNF				Vitamin D			
		OR (95% CI)	p	χ^2 (p)	R^2	OR (95% CI)	p	χ^2 (p)	R^2
1	No depression and no obesity	0.994 (0.963–1.027)	0.731			1.059 (1.032–1.086)	<0.001		
	Depression or obesity	0.993 (0.961–1.026)	0.653	0.26 (–0.8783)	<0.001	1.032 (1.005–1.059)	0.018	62.1 (<0.001)	0.02
	Depression and obesity	Reference				Reference			
		Joint effect	0.878			Joint effect	<0.001		
2	No depression and no obesity	1.008 (0.975–1.041)	0.656			1.046 (1.016–1.076)	0.002		
	Depression or obesity	1.003 (0.970–1.037)	0.866	309.4 (<0.001)	0.098	1.031 (1.003–1.060)	0.028	2,757.2 (<0.001)	0.652
	Depression and obesity	Reference				Reference			
		Joint effect	0.690			Joint effect	0.004		
3	No depression and no obesity	1.004 (0.970–1.040)	0.814			1.061 (1.029–1.095)	<0.001		
	Depression or obesity	0.997 (0.962–1.033)	0.859	368.6 (<0.001)	0.116	1.043 (1.013–1.074)	0.005	2,764.3 (<0.001)	0.654
	Depression and obesity	Reference				Reference			
		Joint effect	0.503			Joint effect	<0.001		

Results from multinomial logistic regression models (n = 3,926). In the multinomial logistic regression models, an increase of 1,000 pg/mL in BDNF and of 1 ng/mL in vitamin D was modeled. The joint effect of the exposure was tested with a Wald χ^2 test. Model 1 = unadjusted; model 2 = adjusted for sex, age, physical activity, and smoking; model 3 = model 2 + platelets and fibrinogen in BDNF models or + season in vitamin D models. The overall model fit is given by the χ^2 (p) values as well as Nagelkerke's R^2. BDNF, brain-derived neurotrophic factor.

reporting a respective medication intake. BDNF concentrations and platelet counts were similar between the 3 groups.

The logistic regression models revealed no association between BDNF and depression or obesity, while vitamin D was inversely associated with both outcomes in all models (Table 2). Thus, increasing vitamin D levels was associated with decreased odds of depression or obesity. The fully adjusted multinomial logistic regression model (Table 3) also revealed no associations with BDNF but associations with vitamin D. We found that a 1 ng/mL increase in vitamin D levels was related to a 6.1% higher odds of being not depressed and not obese versus being depressed and obese ($p < 0.001$). We further observed 4.3% higher odds of being either depressed or obese versus being depressed and obese with a 1 ng/mL increase in vitamin D levels ($p = 0.005$). However, this association was on the threshold of statistical significance after correction for multiple testing. The results from the previous 2 analyses were supported by the observations regarding depression severity. There was no association between BDNF and depression severity but there was one between vitamin D and depression severity (Table 4). In the fully adjusted model 3, an increase in vitamin D of 1 ng/ml was associated with an OR of 1.076 (95% CI 1.037–1.116, $p < 0.001$) for having a PHQ-9 score <5 compared to a PHQ-9 score ≥15. Overall, the associations of vitamin D with depression or depression severity remained significant after correction for multiple testing, and only the odds for moderate versus moderately severe or severe depression missed the Bonferroni-corrected statistical significance threshold.

We found a very weak partial correlation between BDNF and vitamin D (partial correlation coefficient 0.006, $p < 0.001$) but the interaction of BDNF and vitamin D missed statistical significance in the model for depression (Table 2; model 1: $p = 0.105$, model 2: $p = 0.057$, and model 3: $p = 0.065$). Interactions of BDNF and vitamin D on obesity were also not statistically significant (Table 2; model 1: $p = 0.467$, model 2: $p = 0.165$, and model 3: $p = 0.117$).

Finally, we assessed the associations of BDNF and vitamin D with WHR (Table 5; Fig. 1). We found significant nonlinear associations of BDNF or vitamin D with WHR. Regarding BDNF, the fully adjusted model showed a *U*-shaped association with a nadir at 23,000 pg/mL of BDNF. Regarding vitamin D, the fully adjusted model demonstrated a linear decrease in WHR starting at vitamin D concentrations of about 25 ng/mL and a constant WHR when the vitamin D level was below this threshold. These associations were also robust to the correction for multiple testing.

Table 4. Associations of BDNF or vitamin D serum levels with PHQ-9

Model	PHQ-9 score	BDNF				Vitamin D			
		OR (95% CI)	*p*	χ^2 (*p*)	R^2	OR (95% CI)	*p*	χ^2 (*p*)	R^2
1	<5	1.012 (0.971–1.055)	0.562			1.076 (1.040–1.114)	<0.001		
	5–9	1.026 (0.984–1.070)	0.228			1.063 (1.026–1.100)	<0.001		
	10–14	1.017 (0.971–1.065)	0.479	5.8	0.002	1.054 (1.015–1.093)	0.006	33.9	0.011
	≥15	Reference		(0.120)		Reference		(<0.001)	
		Joint effect	0.120			Joint effect	<0.001		
2	<5	1.020 (0.979–1.064)	0.346			1.068 (1.032–1.106)	<0.001		
	5–9	1.028 (0.985–1.073)	0.199			1.056 (1.020–1.093)	0.002		
	10–14	1.019 (0.972–1.068)	0.431	140.8	0.043	1.048 (1.010–1.087)	0.014	164.6	0.050
	≥15	Reference		(<0.001)		Reference		(<0.001)	
		Joint effect	0.423			Joint effect	<0.001		
3	<5	1.016 (0.972–1.062)	0.488			1.076 (1.037–1.116)	<0.001		
	5–9	1.028 (0.983–1.075)	0.232			1.059 (1.020–1.099)	0.003		
	10–14	1.019 (0.970–1.071)	0.451	152.3	0.047	1.042 (1.001–1.084)	0.045	185.0	0.056
	≥15	Reference		(<0.001)		Reference		(<0.001)	
		Joint effect	0.271			Joint effect	<0.001		

Results from multinomial logistic regression models (n = 3,926). In the multinomial logistic regression models, an increase of 1,000 pg/mL in BDNF and of 1 ng/mL in vitamin D was modeled. The joint effect of the exposure was tested with a Wald χ^2 test. Model 1 = unadjusted; model 2 = adjusted for sex, age, physical activity, and smoking, and for WHR in depression models; model 3 = model 2 + platelets and fibrinogen in BDNF models or + season in vitamin D models. The overall model fit is given by the χ^2 (p) values as well as Nagelkerke's R^2. BDNF, brain-derived neurotrophic factor; PHQ-9, Patient Health Questionnaire.

Table 5. Associations of BDNF or vitamin D serum levels with WHR

Model	BDNF						Vitamin D					
	exposure	β coefficient	SE	*p*	*F* value (*p*)	R^2	exposure	β coefficient	SE	*p*	*F* value (*p*)	R^2
1	BDNF	-4.55×10^{-3}	5.65×10^{-4}	<0.001	42.6	0.021	Vitamin D	1.57×10^{-3}	4.13×10^{-4}	<0.001	31.1	0.015
	BDNF'	9.60×10^{-6}	1.77×10^{-6}	<0.001	(<0.001)		Vitamin D'	-3.84×10^{-6}	6.27×10^{-7}	<0.001	(<0.001)	
	Overall			<0.001			Overall			<0.001		
2	BDNF	-1.74×10^{-3}	3.86×10^{-4}	<0.001	681.3	0.548	Vitamin D	2.73×10^{-4}	2.81×10^{-4}	0.332	685.1	0.550
	BDNF'	5.09×10^{-6}	1.21×10^{-6}	<0.001	(<0.001)		Vitamin D'	-1.30×10^{-6}	4.26×10^{-7}	0.002	(<0.001)	
	Overall			<0.001			Overall			<0.001		
3	BDNF	-1.75×10^{-3}	3.89×10^{-4}	<0.001	546.6	0.556	Vitamin D	1.01×10^{-4}	2.89×10^{-4}	0.728	601.2	0.550
	BDNF'	4.71×10^{-6}	1.20×10^{-6}	<0.001	(<0.001)		Vitamin D'	-1.20×10^{-6}	4.28×10^{-7}	0.005	(<0.001)	
	Overall			<0.001			Overall			<0.001		

Results from linear regression models with restricted cubic splines with 3 knots (n = 3,926). In the regression models, an increase of 1,000 pg/mL in BDNF and of 1 ng/mL in vitamin D was modeled. BDNF' and vitamin D' denote the spline components. The overall effect of the exposure was tested with a Wald χ^2 test. Model 1 = unadjusted; model 2 = adjusted for sex, age, physical activity, and smoking; model 3 = model 2 + platelets and fibrinogen in BDNF models or + season in vitamin D models. The overall model fit is given by the F and p values as well as the adjusted R^2. BDNF, brain-derived neurotrophic factor; WHR, waist-to-hip ratio.

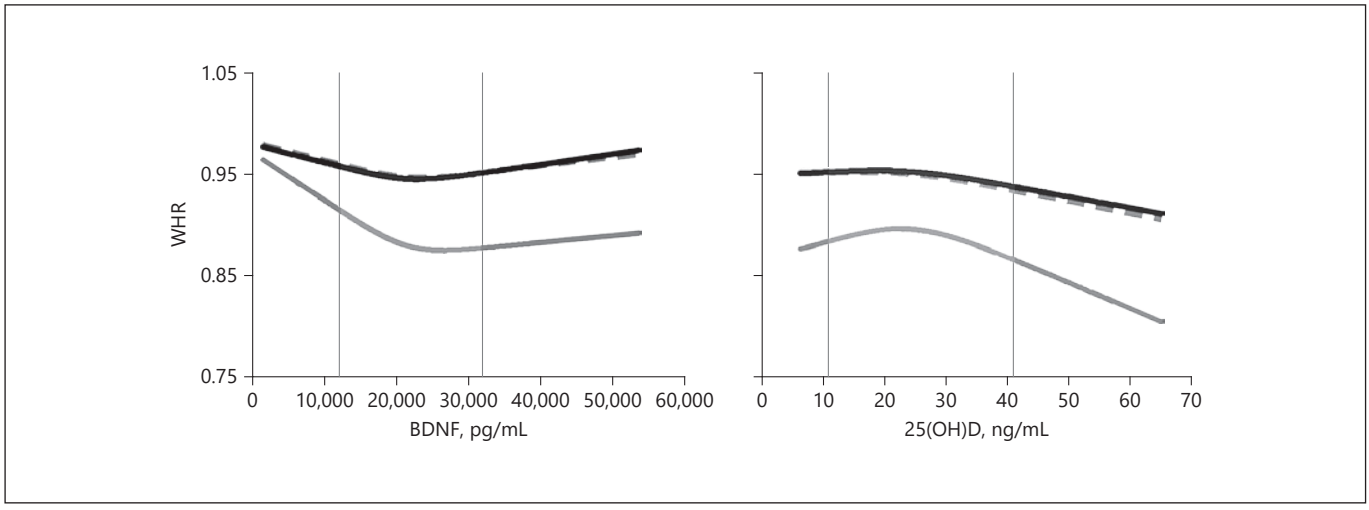

Fig. 1. Associations of brain derived neurotrophic factor (BDNF) or vitamin D with waist-to-hip ratio (WHR). Regression lines from 3 linear regression models with restricted cubic splines (3 knots). The grey solid lines represent the results from model 1 (unadjusted), the grey dashed lines represent the results from model 2 (adjusted for sex, age, physical activity, and smoking status), and the black solid lines represent the results from model 3 (model 2 + platelets and fibrinogen in BDNF models or + season in vitamin D models). The grey shaded areas indicated the central 95% range of the BDNF or vitamin D distribution, respectively. 25(OH)D, 25-hydroxy vitamin D.

Discussion

In the present cross-sectional study we investigated the associations of BDNF and vitamin D with depression, measured with the PHQ-9 [50], and obesity, measured with the WHR [52], in 3,926 adult individuals from the general population. While BDNF was not associated with depression, we revealed a *U*-shaped association of BDNF with WHR. Moreover, inverse associations of vitamin D with depression and obesity were found.

Our hypothesis about an inverse association of BDNF and depression was not confirmed. Indeed, there was no statistically significant association between BDNF and depression or depression severity. A meta-analysis from 2014 [7] reported an inverse association of BDNF and depression but pointed out that evidence for this association was less than initially thought. Besides, these authors questioned the influence of BDNF released from peripheral tissues on the investigated association of BDNF and depression [7]. Similarly, Chacón-Fernández et al. [13] and Serra-Millàs [55] investigated the influence of BDNF released from platelets and megakaryocytes and proposed that alterations in serum BDNF levels result from changes in platelets [13, 55]. However, adjusting for platelet counts did not significantly change the results of this study. Another aspect of this result might be that pro-BDNF, the precursor of mature BDNF, is biologically active, too [56, 57]. Pro-BDNF plays an important role in multiple physiological processes and partially shows effects different from those of mature BDNF [8]. Hence, we recommend distinguishing between pro-BDNF and BDNF for future studies. Another aspect for the lack of an association between BDNF and depression may be found in the small proportion (1.6%) of moderately severe or severely depressed individuals. We cannot exclude that associations between BDNF and PHQ-9 are present in severely ill patients, who are found in clinical samples.

Regarding BDNF, we did not detect an inverse association with WHR, as hypothesized, but we did find a *U*-shaped association. Previously, an inverse association between BMI or body fat mass and BDNF was observed in children after adjusting for platelets, age, and pubertal status [20]. Other studies suggested a positive association between BDNF and BMI: Monteleone et al. [21] investigated this association in women with anorexia nervosa and obesity. Likewise, Nakazato et al. [23] considered 30 young women (age range: 14–34 years) with anorexia nervosa and bulimia nervosa, 10 of whom took antipsychotic drugs [23]. All of these studies [20, 21, 23, 58] did not adjust for platelets, had a small sample size,

and investigated diseased individuals only. Therefore, these results cannot be transferred to the general population or be compared to our results. Huang et al. [58] found no association between BDNF and obesity but they did find one between BDNF and interleukin 6 in 31 obese and non-obese individuals [58]. The authors mention that increasing BDNF levels may have a neuroprotective effect in inflammation caused by obesity [58]. This might be one explanation for our results of high BDNF levels in subjects with a high WHR. A study including 144 individuals found no correlation between BDNF and interleukin 6, and the authors query the findings of Huang et al. [58, 59]. However, the mechanisms of a potential association of BDNF with obesity are not sufficiently determined to date. In rodents, BDNF was found to control appetite by melanocortin signaling, thereby influencing obesity [60]. Additionally an association between BDNF gene variants and obesity was postulated in another study [61]. An fMRI pilot study in 48 healthy individuals found a positive correlation of serum BDNF with connectivity in the (pre-)motor hub. Especially in older adults BDNF levels were associated with physical activity and learning capacities [62]. However, existing results regarding the association of BDNF and physical activity are conflicting, as studies show that endurance training is not related to BDNF levels in elderly persons [63]. Future studies are needed to confirm our results regarding the association of BDNF with obesity and to reveal causation.

Our study revealed a very weak correlation between BDNF and vitamin D. Furthermore we did not find an interaction of BDNF and vitamin D on depression. This is in line with previous studies that found associations of BDNF and vitamin D or vitamin D supplementation with depression but reported no interactions between the measures [64, 65]. In summary, our hypothesis of an interaction between vitamin D and BDNF with obesity was not confirmed.

We revealed an inverse association of vitamin D with depressive symptoms. Thus, our results are consistent with our hypothesis and confirm previous findings [31–33, 66]. Vitamin D is known to be involved in brain functions and passes the blood-brain barrier, and vitamin D receptors are copious distributed in the brain [67]. We saw that a 1 ng/mL increase in vitamin D was related to 6.1% higher odds of being neither depressed nor obese versus being depressed and obese ($p < 0.001$). This might not have clinical relevance at first sight, but Jorde et al. [68] showed in a randomized double blind clinical trial effects in supplementation of vitamin D compared to placebo in obese and overweight depressed patients over a year in decreasing depression scores. Several mechanisms are known to cause this inverse association. Firstly, vitamin D is involved in neuroplasticity [35, 36]. Secondly, depressive disorders are linked to factors that directly influence vitamin D. Persons suffering from depressive disorders often have reduced sun exposure, less engagement in physical activity, and a poorer diet [66]. Thirdly, depressive individuals show a deregulated function of the hypothalamic-pituitary-adrenal axis and sympathoadrenal hyperactivity, combined with elevated levels of inflammatory mediators [69]. Vitamin D has been found to play a role in chronic inflammation [70] and to reduce inflammatory mediators [66]. However, further studies are required to ensure understanding these mechanisms and to determine causation.

In accordance to our hypothesis we found an inverse association of vitamin D and WHR for vitamin D concentrations starting at about 25 ng/mL. Below this threshold the WHR remained constant. These results confirmed previous findings [41–43]. Most obese individuals in our study were postmenopausal women. During menopause a decline in estrogens affects numerous metabolic processes such as changes in body composition [71]. LeBlanc et al. [72] suggested that low vitamin D may be a predisposition for fat accumulation as higher vitamin D levels have been found to be associated with a lower gain in weight.

The main strength of our study is its population-based approach, including the large number of subjects. Data were acquired in a standardized setting. Further, in contrast to previous studies, we were able to adjust for a broad range of confounding factors. On the other hand, its cross-sectional design was a limitation since it does not allow determination of causation in the investigated associations. Additionally, the proportion of subjects who were moderately severely or severely depressed (PHQ-9 score ≥15) was very small (i.e., 1.6%), and thus, we cannot exclude that associations between BDNF and PHQ-9 are present in severely ill patients. Aside from this, there is no information on other psychiatric disorders, e.g., anxiety disorders, obsessive compulsive disorder or psychotic symptoms, and only little information on sleep disorders, which often accompany depressive symptoms. Moreover, all analyses are based on single-occasion measurements, which were taken throughout the year. However season of blood sampling was taken into account in the statistical analyses.

To the best of our knowledge this is one of the first studies to investigate associations of BDNF and vitamin

D with depression and obesity and their interaction in a large population-based sample. The observed *U*-shaped association between BDNF and WHR as well as the marginally significant interaction of BDNF and vitamin D on depression may indicate a potential combined effect of these 2 players in brain development on depression. At the population level, our results support the relevant roles of vitamin D and BDNF in health-related outcomes. Further research focusing on vitamin D and BDNF is necessary to substantiate these results.

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Disclosure Statement

There are no conflicts of interests.

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