Evaluating the role of inflammation on functioning-related outcomes

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Evaluating the role of inflammation on functioning-related outcomes in older adults

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Background

Several cellular and molecular mechanisms contributing to the aging process were identified in the past decades and grouped into a few biological hallmarks of aging. The identification of a few hallmarks of aging paved the basis for the birth of geroscience, an innovative research field aiming to understand the mechanistic interplay between aging and age-related diseases/conditions. The geroscience central hypothesis is that by manipulating the hallmarks of aging, it is possible to prevent and delay the onset of age-related diseases and disability. Chronic inflammation is recognized as one of the hallmarks of aging, tightly intertwined with other aging hallmarks wherein it fuels and is affected by these hallmarks. Chronic inflammation is characterized by persistent, low-grade levels of circulating inflammatory mediators, a phenomenon often observed in older organisms. Previous epidemiological studies showed that age-related chronic inflammation was associated with sarcopenia, physical and cognitive impairments, major chronic diseases, disability, and mortality.

The World Health Organization (WHO) advocates shifting from a negative, disease-based model of aging and frailty towards a positive, function-centered care model of healthy aging. Healthy aging aims at developing and maintaining individuals' functional ability that enables wellbeing in older age. According to the WHO, functional ability (and then, healthy aging) is determined by the individual's intrinsic capacity (IC), the composite of all the physical and mental capacities, the environment and their interaction. IC is composed of five essential domains – cognition, locomotion, psychology, sensory, and vitality, with vitality as a fundamental capacity representing the homeostasis of overall physiological systems and sustaining the other four phenotypic IC domains.

This thesis made a bridge between the fields of geroscience and IC, with a special focus on

inflammation during aging. Among the gaps in the literature this thesis endeavors to fulfill, it can be mentioned: First, there is a lack of consensus on the operational definition of the vitality domain. No study has used a composite of plasma biomarkers to measure vitality, including those that reflect inflammation. Second, there is no existing study that has investigated IC trajectories exclusively on community-dwelling older adults and considering the joint evolution of different domains. Furthermore, it was unknown if IC trajectories have any biological risk factors. Third, prior studies yielded controversial results about the association between high plasma concentrations of inflammatory biomarkers and IC decline, probably owing to their cross-sectional study design and discrete measures of IC levels.

Objectives

This thesis aimed to evaluate how inflammation associates with older adults' IC as a structural component of the IC model and a predictor of longitudinal IC evolution. Our specific objectives for each study were: (I) to investigate the structure and magnitude of the association of vitality with other IC domains and difficulties in basic and instrumental activities of daily living (ADL, IADL) using three operational definitions of vitality, including a composite measure of plasma inflammation-related biomarkers; structural equational modeling (SEM) was applied to compose biomarker-based vitality index and examine the pathways between domains and ADL/IADL difficulties. (II) to identify longitudinal multi-trajectories across IC domains using group-based multi-trajectory modeling in community-dwelling older adults and to examine their associations with plasma biomarkers related to inflammation and mitochondrial dysfunction; (III) to evaluate the cross-sectional and longitudinal associations of plasma inflammation-related biomarkers on IC changes using linear mixed-effect regression.

Methods and main findings

All studies were based on data from the Multidomain Alzheimer Preventive Trial (MAPT), a

randomized controlled trial recruiting 1,679 community-dwelling older individuals aged ≥70 years in France and Monaco. In Study I, we tested three vitality definitions – nutritional status (i.e., Mini Nutritional Assessment [MNA]), handgrip strength, and a combination of five plasma inflammation-related biomarkers – C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor receptor-1 (TNFR-1), monocyte chemoattractant protein-1 (MCP-1), and growth differentiation factor-15 (GDF-15) – to explore the association of vitality with other IC domains, ADL and IADL difficulties. Using a SEM, we observed significant indirect effects of vitality on IADL, mainly through cognitive, locomotor, and psychological domains, regardless of the vitality measurement. At the longitudinal level examined by linear mixed-effect regression, participants with higher vitality had fewer IADL difficulties over four years (MNA score: β [95% CI] = -0.020 [-0.037, -0.003]; handgrip strength: -0.011 [-0.023, 0.000]; plasma biomarker-based index: -0.015 [-0.028, -0.002]). In addition, vitality assessed with the plasma biomarker-based index predicted improved locomotion over time (β [95% CI] = 0.050 [0.010, 0.090]).

In Study II, group-based multi-trajectory modeling was performed to identify clusters of older adults with similar longitudinal patterns across four IC domains: cognition, locomotion, psychology, and vitality. Five IC multi-trajectories were determined, labeled as low in all domains (8.4%), low locomotion (24.6%), low psychological domain (16.7%), robust (i.e., high in all domains except vitality; 28.3%), and robust with high vitality (22.0%). Higher plasma IL-6, TNFR-1, and GDF-15 increased the likelihood of having the "low in all IC domains" trajectory compared to the "robust with high vitality" (IL-6: relative risk ratio [RRR] [95% CI] = 1.42 [1.07 – 1.88]; TNFR-1: 1.46 [1.09 – 1.96]; GDF-15: 1.99 [1.45 – 2.73]). GDF-15 outperformed other biomarkers by showing the highest RRRs and associations with multiple impaired IC patterns.

In Study III, we investigated the cross-sectional and longitudinal association between five

plasma inflammation-related biomarkers (CRP, IL-6, TNFR-1, MCP-1, and GDF-15) and IC using linear mixed-effect regression. IC was assessed over the 4-year follow-up as a score ranging from 0 to 100, derived from four domains: cognition, locomotion, psychology, and vitality. A five-domain IC score (plus sensory) was investigated in a subsample (n = 535) with a 1-year follow-up as an exploratory outcome due to data availability constraint. Increased levels of TNFR-1 and GDF-15 in blood were consistently associated with IC (without including the sensory domain) at both cross-sectional and longitudinal levels (baseline IC levels: TNFR-1: β [95% CI] = -6.86 [-10.25 to -3.47]; GDF-15: -7.07 [-10.02 to -4.12]; IC change over time: TNFR-1: β [95% CI] = -1.28 [-2.29 to -0.27]; GDF-15: -1.42 [-2.26 to -0.58]). However, the longitudinal association between inflammatory biomarkers and IC was not observed in the exploratory analysis using the five-domain IC score (sensory domain included).

Conclusions

The findings of this thesis suggested that inflammation is implicated in IC decline in community-dwelling older adults. How to construct a standard measure of chronic inflammation in humans that better reflects age-related dysregulation in the inflammation network and whether impairments in other hallmarks of aging accompany chronic inflammation to determine the onset of phenotypic aging outcomes (including IC decline) will be the following questions in this field.

RESUME EN FRANÇAIS

Contexte

Plusieurs mécanismes cellulaires et moléculaires contribuant au processus de vieillissement ont été identifiés au cours des dernières décennies et regroupés en quelques caractéristiques biologiques du vieillissement. L'identification de ces caractéristiques a jeté les bases de la géroscience, un domaine de recherche novateur visant à comprendre l'interaction mécanique entre le vieillissement et les maladies/affections liées à l'âge. L'hypothèse centrale de la géroscience est que, en manipulant ces caractéristiques du vieillissement, il est possible de prévenir et de retarder l'apparition des maladies et de l'incapacité liées à l'âge. L'inflammation chronique est reconnue comme l'une des caractéristiques du vieillissement, étroitement liée à d'autres caractéristiques du vieillissement, qui l'affectent et qu'elle alimente. L'inflammation chronique se caractérise par la persistance de niveaux bas et chroniques de médiateurs inflammatoires circulants, un phénomène fréquemment observé chez les individus âgés. Des études épidémiologiques antérieures ont montré que l'inflammation chronique liée à l'âge était associée à la sarcopénie, aux altérations physiques et cognitives, aux principales maladies chroniques, à l'incapacité et à la mortalité.

L'Organisation mondiale de la santé (OMS) préconise de passer d'un modèle négatif basé sur les maladies du vieillissement et la fragilité à un modèle de soins positif axé sur la fonction et le vieillissement en bonne santé. Le vieillissement en bonne santé vise à développer et à maintenir la capacité fonctionnelle des individus, permettant le bien-être à un âge avancé. Selon l'OMS, la capacité intrinsèque (CI) de l'individu, qui est la combinaison de toutes les capacités physiques et mentales, de l'environnement et de leur interaction, détermine la capacité fonctionnelle (et donc le vieillissement en bonne santé). La CI est composée de cinq domaines essentiels : la cognition, la locomotion, la psychologie, les sens et la vitalité ; cette dernière

étant une capacité fondamentale représentant l'homéostasie des systèmes physiologiques globaux et soutenant les quatre autres domaines phénotypiques de la CI.

Cette thèse établit un lien entre les domaines de la géroscience et de la CI, en mettant particulièrement l'accent sur l'inflammation pendant le vieillissement. Parmi les lacunes de la littérature que cette thèse s'efforce de combler, on peut mentionner : premièrement, l'absence de consensus sur la définition opérationnelle du domaine de la vitalité. Aucune étude n'a utilisé un ensemble de biomarqueurs plasmatiques pour mesurer la vitalité, y compris ceux qui reflètent l'inflammation. Deuxièmement, aucune étude existante n'a examiné exclusivement les trajectoires de la CI chez les personnes âgées non institutionnalisées et n'a pris en compte l'évolution conjointe de différents domaines. De plus, on ignore si les trajectoires de la CI sont modifiables par des facteurs de risque biologiques. Troisièmement, les études antérieures ont donné des résultats controversés concernant l'association entre des concentrations plasmatiques élevées de biomarqueurs inflammatoires et le déclin de la CI, probablement en raison de leur design transversal et de mesures discrètes des niveaux de CI.

Objectifs

Cette thèse vise à évaluer comment l'inflammation peut être associée à la CI des personnes âgées en tant que composante structurelle du modèle de la CI et en tant que prédicteur de l'évolution longitudinale de la CI. Nos objectifs spécifiques pour chaque étude étaient les suivants : (I) étudier la structure et l'ampleur de l'association de la vitalité avec les autres domaines de la CI et les difficultés liées aux activités quotidiennes de base (ADL, IADL) en utilisant trois définitions opérationnelles de la vitalité, y compris une mesure composite de biomarqueurs plasmatiques liés à l'inflammation ; la modélisation par équations structurelles (SEM) fut utilisée pour composer un indice de vitalité basé sur les biomarqueurs et examiner les liens entre les domaines et les difficultés ADL/IADL. (II) identifier les différentes

trajectoires longitudinales des domaines de la CI en utilisant la modélisation multi-trajectoire chez les personnes âgées non institutionnalisées et examiner leurs associations avec les biomarqueurs plasmatiques liés à l'inflammation et à la dysfonction mitochondriale ; (III) évaluer les associations transversales et longitudinales entre les biomarqueurs plasmatiques liés à l'inflammation et les changements de la CI en utilisant la régression linéaire à effets mixtes.

Méthodes et principaux résultats

Toutes nos études étaient basées sur les données de l'Essai Multidomaine de Prévention Alzheimer (MAPT), un essai contrôlé randomisé recrutant 1 679 personnes non institutionnalisées âgées de ≥70 ans en France et à Monaco. Dans l'étude I, nous avons testé trois définitions de la vitalité : l'état nutritionnel (c'est-à-dire, la Mini Évaluation Nutritionnelle [MNA]), la force de préhension manuelle et une combinaison de cinq biomarqueurs plasmatiques liés à l'inflammation : la protéine C-réactive (CRP), l'interleukine-6 (IL-6), le récepteur du facteur de nécrose tumorale-1 (TNFR-1), la protéine-1 chimiokine monocytaire (MCP-1) et le facteur de différenciation de croissance-15 (GDF-15) – pour explorer l'association de la vitalité avec les autres domaines de la CI, les difficultés ADL et IADL. En utilisant une SEM, nous avons observé des effets indirects significatifs de la vitalité sur l'IADL, principalement via les domaines cognitif, locomoteur et psychologique, quelle que soit la mesure de la vitalité. Au niveau longitudinal, examiné par régression linéaire à effets mixtes, les participants ayant une vitalité plus élevée présentaient moins de difficultés IADL sur quatre ans (score MNA : β [IC à 95 %] = -0,020 [-0,037, -0,003] ; force de préhension manuelle : -0,011 [-0,023, 0,000] ; indice basé sur les biomarqueurs plasmatiques : -0,015 [-0,028, -0,002]). De plus, la vitalité évaluée avec l'indice basé sur les biomarqueurs plasmatiques prédisait une amélioration de la locomotion au fil du temps (β [IC à 95 %] = 0,050 [0,010, 0,090]).

Dans l'étude II, la modélisation multi-trajectoire a été utilisée pour identifier des groupes de personnes âgées présentant des schémas longitudinaux similaires à travers quatre domaines de la CI : la cognition, la locomotion, la psychologie et la vitalité. Cinq multi-trajectoires de CI ont été déterminées : faible dans tous les domaines (8,4 %), faible en locomotion (24,6 %), faible dans le domaine psychologique (16,7 %), robuste (c'est-à-dire élevées dans tous les domaines sauf la vitalité ; 28,3 %) et robuste avec une vitalité élevée (22,0 %). Des taux plasmatiques plus élevés d'IL-6, de TNFR-1 et de GDF-15 augmentaient la probabilité de présenter la trajectoire "faible dans tous les domaines de la CI" par rapport à la trajectoire "robuste avec une vitalité élevée" (IL-6 : rapports de risques relatifs [RRR] [IC à 95 %] = 1,42 [1,07 – 1,88] ; TNFR-1 : 1,46 [1,09 – 1,96] ; GDF-15 : 1,99 [1,45 – 2,73]). Le GDF-15 surpassait les autres biomarqueurs en affichant les RRR les plus élevés ainsi que des associations avec plusieurs schémas de CI altérée.

Dans l'étude III, nous avons étudié l'association transversale et longitudinale entre cinq biomarqueurs plasmatiques liés à l'inflammation (CRP, IL-6, TNFR-1, MCP-1 et GDF-15) et la CI en utilisant la régression linéaire à effets mixtes. La CI a été évalué sur une période de suivi de 4 ans sous la forme d'un score allant de 0 à 100, dérivé de quatre domaines : la cognition, la locomotion, la psychologie et la vitalité. Un score de CI à cinq domaines (incluant le domaine sensoriel) a été étudié dans un sous-échantillon (n = 535) avec un suivi d'un an en tant que résultat exploratoire en raison de contraintes de disponibilité des données. Des niveaux sanguins accrus de TNFR-1 et de GDF-15 étaient systématiquement associés à la CI (sans inclure le domaine sensoriel) tant au niveau transversal qu'au niveau longitudinal (niveaux de CI initiaux : TNFR-1 : β [IC à 95 %] = -6,86 [-10,25 à -3,47] ; GDF-15 : -7,07 [-10,02 à -4,12] ; changement de la CI au fil du temps : TNFR-1 : β [IC à 95 %] = -1,28 [-2,29 à -0,27] ; GDF-15 : -1,42 [-2,26 à -0,58]). Cependant, aucune association longitudinale entre les biomarqueurs

inflammatoires et le score IC à cinq domaines (avec inclusion du domaine sensoriel) ne fut observée dans l'analyse exploratoire.

Conclusions

Les résultats de cette thèse suggèrent que l'inflammation est impliquée dans le déclin de la CI chez les personnes âgées non institutionnalisées. Construire une mesure standard de l'inflammation chronique chez l'homme reflétant mieux les dérèglements liés à l'âge au sein du réseau inflammatoire et déterminer si les altérations d'autres marques du vieillissement accompagnent l'inflammation chronique pour déterminer l'apparition du vieillissement phénotypique (dont le déclin de la CI) seront les objectifs futurs dans ce domaine.

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LIST OF ABBREVIATIONS

Aβ	Amyloid-beta
AD	Alzheimer's disease
ADL	Activities of daily living
AFAR	The American Federation for Ageing Research
AGFI	Adjusted goodness of fit index
AIC	Akaike information criterion
AL	Allostatic load
AMD	Age-related macular degeneration
APPA	Average posterior probability of assignment
ARHL	Age-related hearing loss
BBB	Blood-brain barrier
BIC	Bayesian information criterion
BMI	Body mass index
CCHA	Clinical Consortium on Healthy Ageing
cGAS-STING	cyclic GMP-AMP synthase-stimulator of interferon genes
CHARLS	China Health and Retirement Longitudinal Study
CI	Confidence interval
CNS	Central nervous system
CFA	Confirmatory factor analysis
CFI	Comparative fit index
CRP	C-reactive protein
CVD	Cardiovascular disease
CXCL9	C-X-C chemokine motif ligand 9
DAMP	Damage-associated molecular pattern
DHA	Docosahexaenoic acid
DHEAS	Dehydroepiandrosterone sulfate
ELSA	English Longitudinal Study on Ageing
EPA	Eicosapentaenoic acid
ET2DS	Edinburgh Type 2 Diabetes Study
FEV	Forced expiratory volume
FOXO3a	Forkhead box O3a
GBTM	Group-based trajectory modeling
GDF-15	Growth differentiation factor-15
GDS	Geriatric Depression Scale
GFI	Goodness of fit index
GFRAL	GDNF family receptor α–like

HHIE-S	Hearing Handicap Inventory for the Elderly – Screening Version
HR	Hazard ratio
IADL	Instrumental activities of daily living
iAge®	inflammatory Age®
IC	Intrinsic capacity
ICAM-1	Intercellular adhesion molecule-1
ICOPE	Integrated Care for Older People
IF1	Inhibitory factor 1
IFITM3	Interferon-induced transmembrane protein 3
IGF-1	Insulin-like growth factor-1
IKKβ	IκB kinase-β
IL-1β	Interleukin-1 beta
IL-1RA	Interleukin-1 receptor antagonist
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-18	Interleukin-18
IQR	Inter-quartile range
JNK	JuN N-terminal kinase
LDL	low-density lipoprotein
LGI	Low-grade inflammation
MAPT	Multidomain Alzheimer Preventive Trial
MCP-1	Monocyte chemoattractant protein-1
MNA	Mini Nutritional Assessment
MMSE	Mini-Mental State Examination
mtDNA	mitochondrial DNA / deoxyribonucleic acid
NF-κB	Nuclear factor-κB
NLRP3	NOD-like receptor protein 3 / nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3
NLR	Neutrophil-to-lymphocyte ratio
NFT	neurofibrillary tangle
OCC	Odds of correct classification
OXPHOS	Oxidative phosphorylation
PAMP	Pathogen-associated molecular pattern
PCA	Principle component analysis
POMP	Percent of maximum possible
POMS	Proportion of maximum scaling

PRR	Pattern recognition receptor
PUFA	Polyunsaturated fatty acid
RAAS	Renin-angiotensin-aldosterone system
RCT	Randomized controlled trial
RMSEA	Root-mean-square error of approximation
ROS	Reactive oxygen species
RRR	Relative risk ratio
SAGE	Study on global AGEing and adult health
SASP	Senescence-associated secretory phenotype
SD	Standard deviation
SEM	Structural equation modeling
SPPB	Short Physical Performance Battery
SRMR	Standardized root mean squared residual
sVCAM-1	soluble vascular cell adhesion molecule-1
T2DM	Type 2 diabetes mellitus
TAME	Targeting Aging with Metformin
TNF-α	Tumor necrosis factor-alpha
TNF-β	Tumor necrosis factor-beta
TNFR-1	Tumor necrosis factor receptor-1
TNFR-2	Tumor necrosis factor receptor-2
VEGF	Vascular endothelial growth factor
WBC	White blood cell
WHO	World Health Organization

LIST OF SCIENTIFIC PAPERS AND ORAL COMMUNICATIONS

The doctoral thesis is based on the following original papers, which will be referred to in the text as Studies I, II, and III.

I. **Lu WH**, Gonzalez-Bautista E, Guyonnet S, Martinez LO, Lucas A, Parini A, Rolland Y, Vellas B, de Souto Barreto P. Investigating three ways of measuring the intrinsic capacity domain of vitality: nutritional status, handgrip strength, and aging biomarkers. *Age Ageing. 2023;52(7):afad133.*

II. **Lu WH**, Guyonnet S, Martinez LO, Lucas A, Parini A, Vellas B, de Souto Barreto P. Association between aging-related biomarkers and longitudinal trajectories of intrinsic capacity in older adults. *Geroscience. 2023;45(6):3409-18.*

III. **Lu WH**, Gonzalez-Bautista E, Guyonnet S, Lucas A, Parini A, Walston JD, Vellas B, de Souto Barreto P. Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults. *J Cachexia Sarcopenia Muscle. 2023;14(2):930-39.*

Oral communications:

- **Lu WH**, Guyonnet S, Martinez LO, Lucas A, Parini A, Vellas B, de Souto Barreto P. Plasma inflammatory markers predict longitudinal trajectories of intrinsic capacity in older adults. *The 13th International Conference on Frailty and Sarcopenia (ICFSR), Toulouse, France, 22-24, March 2023.*
- **Lu WH**, Gonzalez-Bautista E, Vellas B, de Souto Barreto P. Differences in blood-based inflammatory profile according to longitudinal intrinsic capacity trajectories in community-dwelling older adults. *The 2nd Euro-Geroscience Conference, Toulouse, France, 24-25 March 2022.*

Other published papers not included in the thesis:

- **Lu WH**, Rolland Y, Guyonnet S, de Souto Barreto P, Vellas B. Reference centiles for intrinsic capacity throughout adulthood and their association with clinical outcomes: a cross-sectional analysis from the INSPIRE-T cohort. *Nat Aging. 2023;3(12):1521-28.*
- **Lu WH**, Giudici KV, Morley JE, Guyonnet S, Parini A, Aggarwal G, Nguyen AD, Li Y, Bateman RJ, Vellas B and de Souto Barreto P. Investigating the combination of plasma amyloid-beta and geroscience biomarkers on the incidence of clinically meaningful cognitive decline in older adults. *Geroscience. 2022;44(3):1489-1503.*
- **Lu WH**, Giudici KV, Guyonnet S, Aggarwal G, Nguyen AD, Morley JE, Vellas B, and de Souto Barreto P. Associations of plasma neurofilament light chain and progranulin with frailty in older adults. *J Am Geriatr Soc. 2022;70(4):1236-43.*
- **Lu WH**, Giudici KV, Rolland Y, Guyonnet S, Mangin JF, Vellas B, de Souto Barreto P. Associations between nutritional deficits and physical performance in community-dwelling older adults. *Front Nutr. 2021;8:771470.*

CHAPTER 1. INTRODUCTION

1.1 Challenges of population aging

The world population is rapidly aging due to the demographic shift and rising life expectancy, with the global population over 60 years reaching 22% by 2050[1]. In Europe, more than one-fifth (21.1%) of people were aged ≥65 years by 2022[2]. In response to a rising number of dependent people in the aging society, public long-term care spending among European Union countries is estimated to increase from 1.6% to 2.2% of the gross domestic product between 2016 and 2040[3].

In 2015, the World Health Organization (WHO) released the World report on ageing and health[4], whose main findings and recommendations were further summarized and published in The Lancet[5]. The WHO World report conducted a comprehensive review of population aging and recognized several challenges in older adults' care. They found that the health of older people is not keeping up with increasing longevity. Although older adults live longer nowadays, they are not experiencing better health than their parents' generation. Furthermore, current health systems are ineffective and do not meet the needs of older people. The healthcare approach today is designed to predict or respond to a clinically manifest disease, followed by medical treatment to eliminate or limit the consequences of the disease. This disease-oriented care pathway may no longer be adequate and efficient to respond to the rising care demands of the growing number of older people. One clear challenge is that the disease-based care model substantially increases life expectancy in older adults. Still, it seems not very effective to maintain/improve their functions. In addition, conventional care tends to regard older people as passive care recipients and seldom respects their and caregivers' unique needs. Care management is often fragmented across diseases, health professionals, and settings. All this evidence emphasizes that a new framework for older people's care is required.

1.2 Healthy aging and intrinsic capacity (IC)

1.2.1 Definition

The WHO World report further proposed a new healthcare approach centered on "*Healthy aging*", defined as "the process of developing and maintaining the functional ability that enables wellbeing in older age"[4]. Here, *functional ability* is conceptualized as "all the health-related attributes that enable people to be and to do what they have reason to value[6]". It includes (but may not be limited to) five essential abilities for older people: meet basic needs; learn, grow and make decisions; be mobile; build and maintain relationships; and contribute. Functional ability is further determined by the intrinsic capacity (IC) of the individual, surrounding environmental factors, and the interactions between the individual and the environment (**Figure 1.1**).

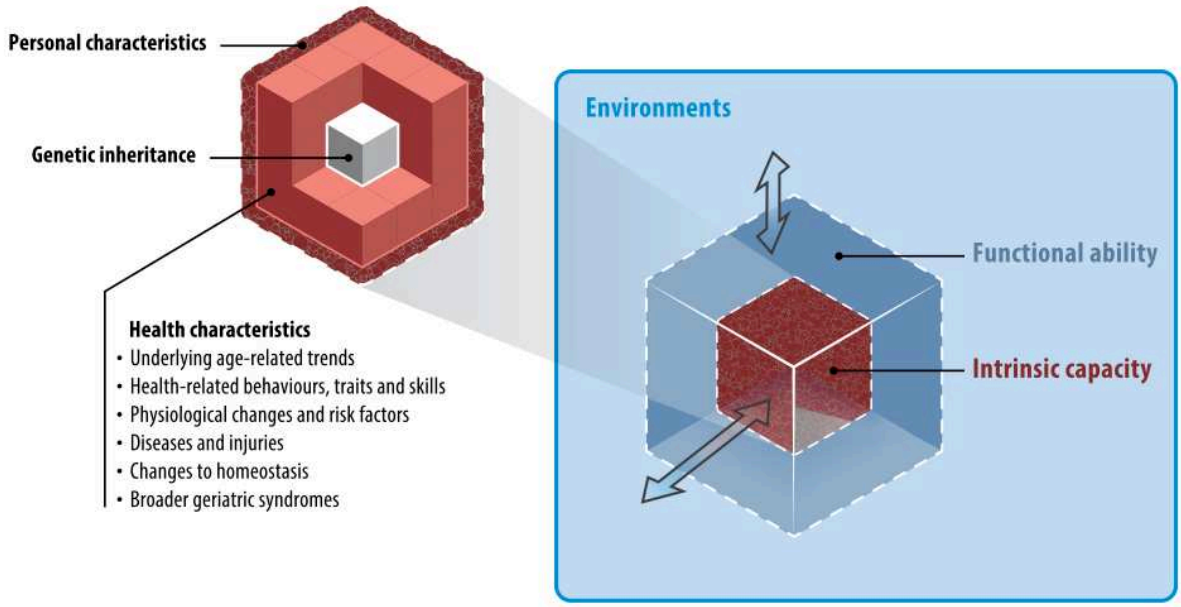

Figure 1.1 The concept of functional ability and intrinsic capacity proposed in the WHO World report on ageing and health in 2015

Data source: *World Health Organization. World Report on Ageing and Health (2015)*

Healthy aging focuses on fostering and maintaining the individual's functional ability rather than the presence or absence of diseases. The healthy aging approach is characterized as longitudinally monitoring an individual's functional trajectory and providing proactive and personalized interventions for enhancing functional ability independently of clinical phenotypes[6]. Under the framework of healthy aging, IC is created as a positive health attribute that reflects a person's functional ability to inform the sequent clinical intervention and public health strategies.

In the World report in 2015, IC is conceptualized as the composite of total physical and mental capacities of an individual[4]. In order to translate the theoretical concept into practice, IC needs decomposition into operational subdomains that can be assessed through objective measurements. The working definition of IC was gradually developed in the later WHO publications and academic research on healthy aging. Currently, IC is often determined by five domains that link to essential body function: cognition, locomotion (physical function), psychology (mental function), vitality (nutrition and energy balance), and sensory (including vision and hearing). More introduction about IC construct and individual domains is provided in Section 1.5.

1.2.2 Integrated Care of Older People (ICOPE)

The WHO World report highlighted the need to redesign the healthcare model for older people to promote healthy aging[4]. In 2017, an innovative healthy aging care approach named the Integrated Care for Older People (ICOPE) was proposed, which aims to maintain and enhance functional ability through intervention on IC[7]. The WHO released ICOPE guidelines, a handbook, and a mobile app, which established clear pathways and concrete recommendations on preventing, slowing, or reversing IC decline in community and primary care settings[7,8]. In brief, the ICOPE approach is composed of five steps, starting from regularly screening older

adults' IC deficits (Step 1) to introducing in-depth functional assessments (Step 2) and personalized care management (Step 3) and coordinating specialized care and community support (Step 4 and 5)[8]. ICOPE reflects a continuum of care, with health and social services being reoriented and centered on each older individual's need. The INSPIRE ICOPE-CARE program, an ongoing study conducted by Gerontopole of Toulouse, applies the ICOPE care approach in the Occitanic region of France to evaluate the large-scale implementation and feasibility of ICOPE in real-life clinical practice. By the end of 2021, more than 10 thousand older adults (and more than 40 thousand currently in September 2023) participate and are continuously monitored by the INSPIRE ICOPE-CARE program[3].

Each year, the WHO holds the Clinical Consortium on Healthy Ageing (CCHA), a forum gathering global experts in the field of aging and the WHO staff to discuss important topics about IC, ICOPE, and other activities on promoting healthy aging. The concept of IC and ICOPE became comprehensive and are continuously advanced by the research, preliminary experiences, and expert opinions shared in the CCHA meeting.

✦

Preventing IC decline is a major goal in achieving a healthy aging process that enables older adults to maintain physical and mental autonomy in their daily lives. IC is composed of key body functions, showing that IC decline is biologically determined and, like other aging-related pathologies, can be early screened through biomarkers and even be manipulated if underlying mechanisms are identified. Thus, understanding the biological mechanisms involved in functional deterioration during aging and identifying effective methods for early detection of these mechanisms would further facilitate the realization of healthy aging.

Based on this perspective, this thesis reviewed the biological mechanisms of aging (Section 1.3). Then, we concentrated on one aging hallmark – chronic inflammation – and its impact on

aging-related functional impairments (Section 1.4). We focused on chronic inflammation because it plays a central role in biological aging by being tightly connected with other hallmarks. Moreover, chronic inflammation has been identified as a significant contributor to aging-related diseases (known as "inflammaging") far before the idea of hallmarks of aging was proposed. Therefore, we think chronic inflammation could be a good entry point to study how biomarkers of aging mechanisms link to IC decline (Section 1.5).

1.3 Biological aging

1.3.1 Biomarkers of aging

At a biological level, aging is associated with the gradual, lifelong accumulation of various molecular and cellular damage[9,10]. Since decades ago, scientists and clinicians have already noticed that chronological age (i.e., the amount of time elapsed since birth) per se is not a good predictor of physiological or functional capacity, particularly at later stages in the lifespan of a person[11,12]. The between-individual variation in the "biological aging" rate helps explain why some adults experience age-related decline faster than their peers[13].

As the National Institute of Health established, biomarker is a feature that can be objectively measured and used to indicate normal biological processes, pathogenic processes, or pharmacologic responses to a therapy[14]. The term "*biomarker of aging*" was mentioned as early as 1988 by Baker and Sprott, who described it as "a biological parameter of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age than will chronological age[11]". In other words, biomarkers of aging attempt to give the actual "biological age" and help predict the residual lifetime and/or onset of age-related diseases/conditions[15].

The American Federation for Ageing Research (AFAR) further recommended more detailed

criteria for a biomarker of aging[16,17]: First, it should predict the rate of aging and, operationally, estimate a person's lifespan better than chronological age alone. Second, it should monitor the primary mechanisms that underlies the aging process, not be the result of disease. Third, it should be easily and repeatedly testable without harming individuals; for example, blood and image examination are appropriate techniques since they can be performed accurately and reproducibly without the need for specialized equipment or techniques[18]. Finally, It should be effective in both humans and experimental animals; this allows a biomarker to be tested preliminary in animals before being validated in humans. Some characteristics are also important for a biomarker of aging, such as being inexpensive to use and causing little pain and stress[17,18].

Since then, the field of aging research has put effort into identifying and validating markers that can provide insights into the aging process and age-related diseases. Although biomarkers that strictly fulfill all AFAR criteria are unlikely to exist, investigating biomarkers of aging would push to identify drugs or interventions that ameliorate the aging process[17].

1.3.2 Hallmarks of aging

In 2013, López-Otín et al. identified nine cellular and molecular hallmarks contributing to the aging process and determining the aging phenotype[19]; they further expanded the aging hallmarks to twelve in 2023 based on the updated evidence: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, disabled macroautophagy, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis (**Figure 1.2**)[20]. Each hallmark fulfills the following criteria: (1) it should manifest during normal aging; (2) its experimental aggravation should accelerate aging; and (3) its experimental amelioration should retard the normal aging process and hence increase healthy lifespan[19,20]. These hallmarks are

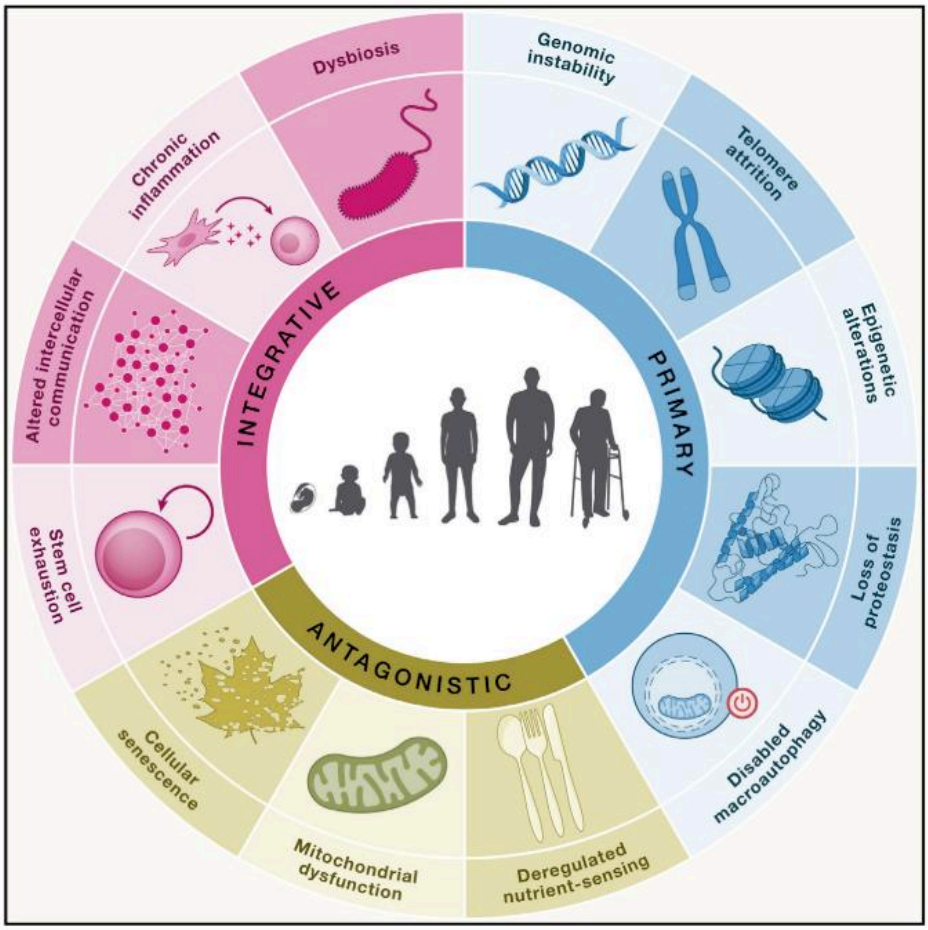

Figure 1.2 Twelve hallmarks of aging

Data source: *López-Otín C, et al. Cell. 2023;186(2):243-278.*

grouped into three categories showing their hierarchical and interconnected relationship: (1) *the primary hallmarks*, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, and disabled macroautophagy, which reflect damages that progressively accumulate with time and unambiguously contribute to the aging process; (2) *the antagonistic hallmarks*, including deregulated nutrient-sensing, mitochondrial dysfunction, and cellular senescence, which reflect responses to damage and may act in opposite roles under different conditions in the aging process; and (3) *the integrative hallmarks*, including stem cell exhaustion, altered intercellular communication, chronic inflammation, and dysbiosis, which appear once the accumulated damage caused by the primary and antagonistic hallmarks cannot

be compensated[20]. The hallmarks of aging provided a contextual framework for future studies on investigating molecular mechanisms of aging and designing interventions to extend human healthspan (i.e., the period of one's life that one is healthy and free from disease)[19].

1.3.3 Geroscience

Geroscience is an emergent research field of aging. It aims to understand the mechanistic links between aging and age-related diseases/conditions and to develop multiple diseases/conditions therapeutic and preventive approaches by targeting the fundamental mechanisms (hallmarks of aging)[21]. The major hypothesis of geroscience is that aging is the leading risk factor for most diseases and conditions that limit healthspan, and manipulating the aging process would delay or reduce the onset or severity of chronic diseases[22]. Based on the geroscience perspective, biological aging is modifiable by a variety of genetic, behavioral, and pharmacological means[23]. Despite its origins in biological science, geroscience seeks to translate what we learn from cell and animal studies to humans, and ultimately to improve clinical care and public health policy[23].

Investigating the effects of geroscience-guided interventions on mortality and chronic disease incidence in humans requires a long follow-up period. Therefore, it is necessary to establish secondary endpoints to evaluate the effect of geroscience interventions or geroprotective agents in clinical trials. One way is using biomarkers of aging as secondary endpoints. For instance, the team of the Targeting Aging with MEtformin (TAME) study, a proposed randomized clinical trial aiming to investigate the metformin's effect on preventing age-related multimorbidity and functional decline, reviewed potential blood-based biomarkers of aging and age-related disease to serve as an intermediate trial endpoint; the biomarker evaluation was carried out in accordance with the AFAR guidelines[24]. They further selected eight biomarkers from 258 candidate biomarkers and composited them into a biomarker index to reflect accelerated

biological aging (**Figure 1.3**). This biomarker index was tested in another randomized trial of caloric restriction and exercise and showed response to interventions and associations with physical capacity[25].

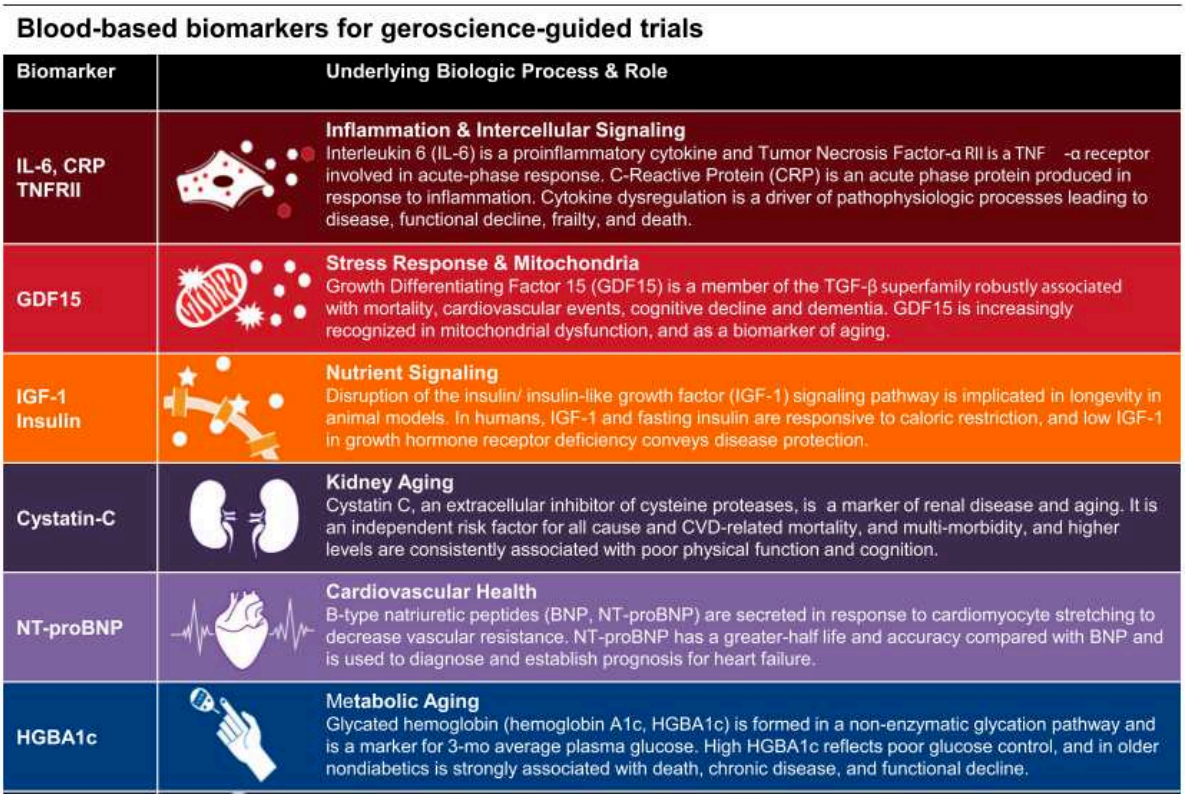

Figure 1.3 TAME biomarker index

Adapted from: *Justice J, et al. GeroScience. 2018;40(5-6):419-436.*

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Franceschi et al. highlighted that tightly networked aging hallmarks converge on chronic inflammation, one of the integrative hallmarks, as impairment of any one hallmark fuels inflammation, which subsequently affects all the other hallmarks[26]. In the next chapter, we will discuss chronic inflammation's definition (Section 1.4.1 and 1.4.2), causes (Section 1.4.3), and effects on age-related outcomes (Section 1.4.4).

1.4 Inflammation

1.4.1 Acute versus chronic inflammation

Inflammation is a response of the immune system to extrinsic or intrinsic stressors that aims to eliminate them and promote tissue healing[27,28]. It is the first line of host defense and a part of the innate immune response, which is non-specific to the stressors and can initiate further specific, adaptive immune responses[27]. Inflammation changes the microcirculation of the tissue, including the plasma exudation and the leukocytes emigration from blood vessels to the site of infection or injury, leading to cardinal clinical signs: redness, warmth, pain, swelling, and loss of tissue function[27,28].

Ruslan Medzhitov proposed that an inflammatory pathway consists of four components: inducers, sensors, mediators, and effectors[29]. Inducers are the stimuli that initiate an inflammatory response and can be classified according to their origins, either exogenous or endogenous (**Figure 1.4**). The non-self-molecular structures belonging to microorganisms (such as bacteria and viruses) are defined as pathogen-associated molecular patterns (PAMPs). Damage-associated molecular patterns (DAMPs) are endogenous agents that are released from stressed, damaged, or dying cells. Inducers are detected by specialized sensors, such as pattern recognition receptors (PRRs) and inflammasomes (the multiprotein complexes assembled by PRRs in the cytoplasm)[30], and then trigger the production of numerous mediators, including vasoactive amines, complement components, cytokines, and chemokines. The mediators, in turn, alter the functional states of tissues and organs (the effectors), allowing them to adapt to the conditions and control homeostasis[29].

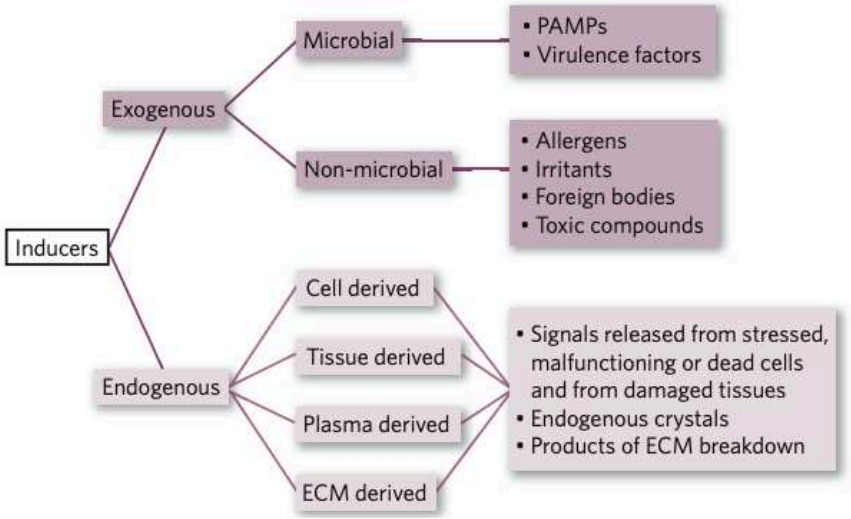

Figure 1.4 Classification of inducers in the inflammatory pathway

ECM, extracellular matrix; PAMP, pathogen-associated molecular pattern. Data source: *Medzhitov R. Nature. 2008;454(7203):428-35.*

Despite the beneficial role of the inflammatory response in provoking pathogen elimination and tissue repair, uncontrolled inflammation can cause additional tissue damage and become detrimental to the body. A normal inflammatory response occurs immediately and transiently, which resolves once the threat has been cleared; this process is often called *acute inflammation*. However, the inflammatory response can be non-resolving and becomes chronic under some circumstances, including the persistence of inflammatory stimuli and the weakening regulatory systems of the immune response. Compared to acute inflammation, this *chronic inflammation* is characterized by a lower degree of immune response, systemic, and typically induced by DAMPs (**Table 1.1**).

Table 1.1 Acute inflammation versus chronic inflammation

	Acute inflammation	Chronic inflammation
Trigger	Infection, injury	Tissue malfunction
Inducer	PAMPs (infection), DAMPs (cellular stress, trauma)	DAMPs (exposome*, metabolic dysfunction, tissue damage)
Duration	Short-term (few days)	Persistent (last for months or years)
Magnitude	High-grade	Low-grade
Outcome	*Beneficial:* healing, trigger removal, tissue repair	*Harmful:* collateral damage to tissues and organs over time
Age-related	No	Yes

*Exposome refers to a person's lifelong exposure to physical, chemical and biological elements, starting from the prenatal period onward. DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern. Data source: *Furman D et al. Nat Med. 2019;25(12):1822-1832*

In the following texts of the thesis, we use the term "inflammation" to denote "chronic, low-grade inflammation (LGI)." Any other acute or infectious inflammatory conditions that do not belong to the category of chronic inflammation will be explicitly elucidated during the descriptions.

1.4.2 Inflammaging: Age-related chronic inflammation

Chronic inflammation was recognized as one of the hallmarks contributing to aging and multiple age-related diseases (Section 1.3.2)[19–21]. This concept can be traced back to 2000, when Claudio Franceschi first proposed the term "*inflammaging*" to describe the persistent, low-grade levels of the circulating pro-inflammatory mediators that he observed in older organisms even in the absence of acute infection and major diseases[31]. Although the etiology remains largely unknown, several mechanisms have been proposed to contribute to age-related chronic inflammation:

- **Immunosenescence**

The term "*immunosenescence*" globally refers to an age-dependent alteration in immune function, featured by an impaired ability to respond to new antigens, unsustained memory responses of immune cells, greater propensity for autoimmune responses, and chronic LGI[32]. Adaptive immunity is generally more susceptible to aging, while innate immunity is relatively preserved and even mild hyperactive[33,34]. Immunosenescence leads to decreased production of anti-inflammatory proteins and an exaggerated inflammatory response, thus resulting in a heightened pro-inflammatory profile in older adults[35]. Chronic, continuous generation of inflammatory factors can exhaust the adaptive immune responses, forming a vicious feedback loop between inflammaging and immunosenescence[34]. Other consequences of immunosenescence include enhanced susceptibility to infectious diseases and some specific diseases (e.g., cancers and autoimmune disorders), and a decreased response to vaccination.

- **Imbalance in production/elimination of self-debris**

An imbalance between the production and elimination of cellular debris (macromolecules released from damaged or dead cells and organelles), misfolded proteins, and misplaced self-molecules has been proposed as the source of inflammatory stimuli[33,36]. Self-debris acts as DAMPs that activate innate immunity and facilitate the release of pro-inflammatory cytokines. For example, in macrophages, DAMPs induce the expression and activation of the NOD-like receptor protein 3 (NLRP3) inflammasome; NLRP3 oligomerization in cytoplasm further contribute to the maturation and secretion of interleukin-1β (IL-1β) and IL-18 through the activation of caspase-1[37]. With age, the production of these degraded self-molecules increases, and their disposal through autophagy and other pathways regulating proteostasis (such as proteasome activity) declines. It results in a progressive accumulation of self-debris and the chronic and maladaptive innate immune response to DAMPs[33,36].

- **Mitochondrial dysfunction**

Mitochondria are bioenergetic organelles found in the cytosol of eukaryotic cells that produce ATP via oxidative phosphorylation (OXPHOS)[38]. An age-related decline in mitochondrial quality and activity can contribute to chronic inflammation through the release of various mitochondrial components and metabolic products, such as mitochondrial DNA (mtDNA), reactive oxygen species (ROS), and cardiolipin (which typically resides in the inner mitochondrial membrane)[33,39]. Accumulation of the mitochondria-derived molecules in the cytosol or the extracellular environment can act as DAMPs and induce several pathways linked to inflammation[40,41]. For example, mtDNA fragments in cytosol have been proposed to trigger inflammation via three distinct signaling pathways: NLRP3 inflammasome signaling (resulting in IL-1β and IL-18), nuclear factor-κB (NF-κB) signaling (resulting in TNF-α, IL-1, IL-6, and IL-8), and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway (resulting in the production of type 1 interferons)[39,41].

- **Cellular senescence and associated secretory phenotype (SASP)**

Cellular senescence is a state of permanent cell proliferation arrest induced by persistent DNA damage and other stress-induced signals. It is a tumor suppressor mechanism that permanently arrests cells at risk for malignant transformation. The accumulation of senescent cells in multiple tissues and organs drives aging and age-related pathologies (one of the aging hallmarks). Persistent senescent cells involve the acquisition of senescence-associated secretory phenotype (SASP), a secretion of a wide range of inflammatory cytokines, chemokines, growth factors, and matrix-degrading proteases. These secretory molecules mainly function in a paracrine fashion that alters the tissue microenvironment and facilitates the development of cellular senescence in neighboring cells, but some of the soluble mediators are released into the circulation and thus contribute to inflammaging[33,42,43].

- **Gut microbiota dysbiosis and increased permeability**

Aging is associated with a reduction in beneficial commensal microorganisms, which is important for inhibiting the expansion of pathogenic microbial communities and maintaining intestinal barrier integrity. The age-related changes in the composition of gut microbiota can lead to the onset of dysbiosis and unresolved intestinal inflammation[36], thereby increasing mucosal barrier permeability and the leakage of bacteria and bacterial-derived inflammatory compounds into the circulation. These compounds are recognized by PRRs in blood as PAMPs and activate the inflammatory process[42].

- **Obesity and age-related change in adipose tissue**

Obesity, in particular visceral obesity, provides a rich reservoir of chronic inflammation. Excess nutrient intake contributes to adipocyte growth and proliferation. Eventual adipocyte hypertrophy (lipid overload) leads to endoplasmic reticulum stress, the activation of resident immune cells in adipose tissue, and the secretion of a variety of adipokine (such as leptin) and pro-inflammatory mediators (such as IL-6) to decrease lipid storage, promote lipolysis and induce insulin resistance[44]. The activation of resident immune cells and infiltrated macrophages also secrete pro-inflammatory cytokines to recruit circulating immune cells into inflammatory adipose tissues and further amplify the pro-inflammatory phenotype[45]. Obesity is also associated with gut dysbiosis and increased intestinal permeability, leading to endotoxemia and systemic inflammation mentioned above[46].

Adipose tissue mass generally increases at old age due to a positive calorie balance, decreased physical activity, and a lower basal metabolic rate[47]. Adipose tissue composition and function also alter with age, including the accumulation of senescent cells, fat redistribution favoring the visceral depots, and declining sex hormone levels, collectively contributing to a chronic inflammatory state[48].

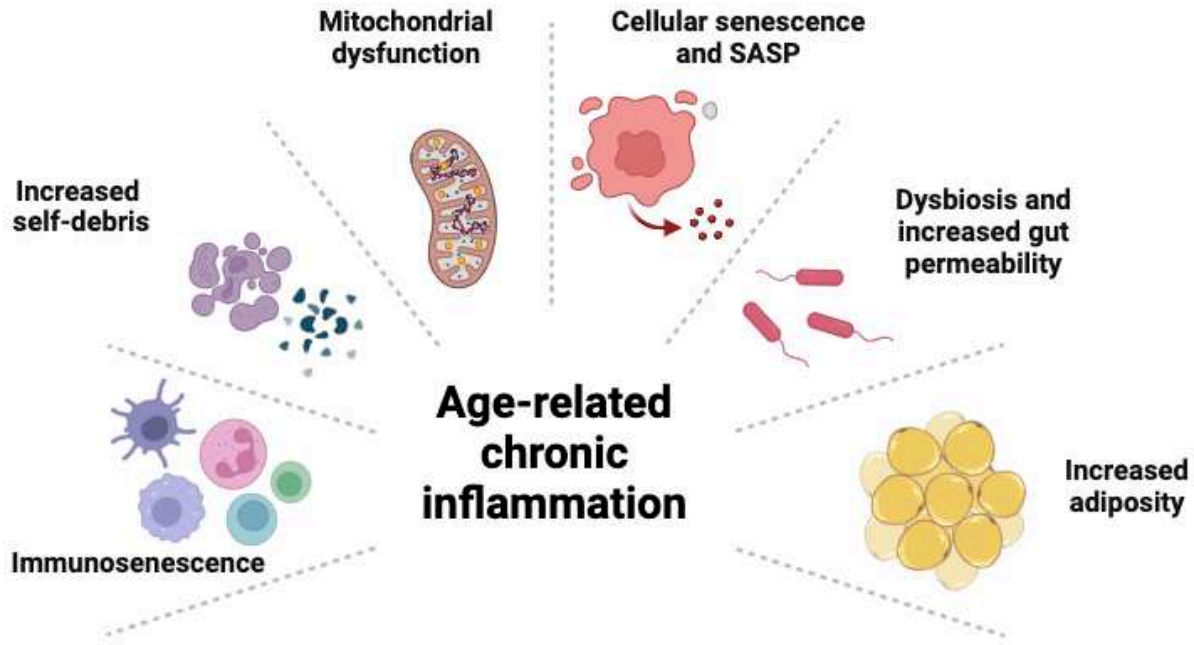

Figure 1.5 Summary of main drivers of chronic inflammation

This figure was created with BioRender.com.

Here, we only summarized the mechanisms commonly proposed to contribute to age-related chronic inflammation in most reviews on inflammaging (**Figure 1.5**). One recent review by Baechle et al. suggested that chronic inflammation somehow links with all other aging hallmarks through intra- and extracellular signaling and collectively develops a vicious cycle that exacerbates cellular functions and induces aging-related pathology[49]. Thus, **Figure 1.5** is far from exhaustive and more potential mechanisms or drivers not covered in this section may also contribute to age-related chronic inflammation.

1.4.3 Measurement of inflammation

Measuring the inflammatory state and its severity in the body enables researchers to investigate its connection with potential adverse outcomes. The inflammatory response involves the activation of immune cells and the release of circulating inflammatory molecules in the peripheral blood. Therefore, the quantity of cells and molecules serves as ideal indicators to

reflect the existence and persistence of the inflammation. For example, an increased neutrophil-to-lymphocyte ratio (NLR) has been used to measure the intensity of the systemic immune-inflammatory reaction in patients with sepsis and cancer[50]; some studies also demonstrated that high NLR predicted future cardiovascular events[51]. The prominent feature of inflammaging is defined as the increased circulating levels of pro-inflammatory mediators. The association of these mediators, particularly interleukins and tumor necrosis factors, with age-related diseases, disabilities, and functional loss has been extensively investigated. Recently, novel markers upregulated under chronic inflammatory conditions have shown potential in measuring inflammaging. Moreover, there is a trend towards utilizing composite approaches and innovative techniques such as omics in measuring inflammation. To date, there has been no standard way of measuring age-related chronic inflammation.

- **Circulating cytokines**

Inflammatory cytokines include interleukins, interferons, tumor necrosis factors (TNF-α and TNF-β), and tumor growth factors. These molecules function as either pro-inflammatory (e.g., IL-6, IL-1β, TNF-α) or anti-inflammatory (e.g., IL-4, IL-10, IL-13) mediators. Cytokine levels are consistently low in younger individuals and typically elevate only in response to physiological stress. With advanced age, the levels of cytokines and their receptors, particularly IL-6 and TNFR-1, were found to increase even without chronic diseases[52,53].

According to the review of Singh et al. in 2011, IL-6 and TNF-α are two inflammatory markers most consistently associated with age-related chronic diseases and disability[54]. In acute inflammation, IL-6 can modulate acute phase proteins (such as C-reactive protein [CRP] and fibrinogen) production in the liver, increase vascular permeability, and promote lymphocyte differentiation and antibody synthesis. IL-6 also has an anti-inflammatory function, which can limit the inflammatory response by suppressing the production of other pro-inflammatory

cytokines (IL-1β and TNF-α) and increasing the synthesis of molecules with anti-inflammatory activity such as IL-1 receptor antagonist (IL-1RA) and soluble TNF receptor (TNFR)[55]. The reason for chronic IL-6 elevation in old age is still unclear: whether elevated levels of IL-6 are aimed at resolving an inappropriately prolonged inflammation or whether a primary dysregulation of IL-6 production is responsible for a chronic pro-inflammatory state remains to be established[55].

TNF-α is secreted mainly by macrophages and signals through two transmembrane receptors in charge of different signaling pathways in cells: (1) type 1 (TNFR-1), which promotes inflammation and cell apoptosis; (2) type 2 (TNFR-2), which promotes tissue homeostasis, cell survival, and proliferation[56]. Both transmembrane receptors can also be cleaved and released as soluble receptors, which compete with the transmembrane forms by binding circulating TNF-α and thereby inhibiting its action, especially in acute inflammatory conditions. However, in proportion to concentrations, TNFR can stabilize TNF-α and thus enhances its cytotoxicity[57]. Since TNF-α is relatively difficult to measure due to its very low circulating levels and short half-life, TNFR seems less prone to fluctuation and is a more reliable marker, and may indicate the body's attempt to control a chronic state of inflammation[58].

- **Acute-phase proteins**

Elevations of acute-phase proteins, particularly CRP, have been widely used to illustrate a chronic inflammatory state in the literature and have been associated with several age-related chronic illnesses[54]. CRP is primarily synthesized by the liver in response to elevations in other cytokines (e.g., IL-6) and can activate the complement system, promoting phagocytosis and facilitating antibody/antigen binding. Its concentration in the circulation can increase by up to 10000-fold during acute responses to serious infection or major tissue damage[59]. Circulating CRP levels higher than 3 mg/L had been used to indicate chronic inflammation in

atherosclerotic studies[60]. On the other hand, CRP >10 mg/L is commonly regarded as caused by acute infection or major trauma, which has been suggested to be ignored when evaluating long-term vascular risk[61]. Previous research on other age-related diseases or outcomes seldom routinely excluded extreme values based on these cut-off values.

- **Other inflammatory markers**

Together with the aforementioned cytokines and acute-phase proteins, other serological markers involved in the inflammatory response are often explored as a panel of inflammation indicators, including chemokines like monocyte chemoattractant protein-1 (MCP-1), soluble adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and E-selectin, and growth factors like vascular endothelial growth factor (VEGF)[62,63]. Recent emerging markers of biological aging, such as growth differentiation factor-15 (GDF-15), also show potential in reflecting the dysregulated balance between pro- and anti-inflammatory mechanisms during aging[64] and become widely used in the studies investigating age-related chronic diseases and disability[64,65].

- **Composite biomarker measure**

To date, no single biomarker has shown to be superior to other markers in characterizing age-related inflammation by demonstrating consistent associations with adverse health outcomes. Most studies only focused on one or two pro-inflammatory markers[66] and assumed that these markers could reflect the broader changes occurring in inflammation[67]. However, evaluating only pro-inflammatory markers fails to consider the feedback regulatory complexity of the inflammation system, the impact of reduced anti-inflammatory activity, and the potential accumulative effect of multiple low-level inflammatory mediators.

To address these limitations, some studies employed a multi-dimensional approach, which can assess multiple inflammatory markers and then combine them into more robust indices

representing the heightened inflammatory status. For example, both Morrisette-Thomas et al.[68] and Hsu et al.[69] applied principle component analysis (PCA) to integrate inflammatory markers, and they identified axes composed of similar markers. Furthermore, Bandeen-Roche et al. summarized seven serum markers into up-regulation and down-regulation scores following the multiple roles of these markers in pro-inflammatory and anti-inflammatory signaling pathways[67]. Finally, Varadhan et al.'s work created an inflammatory index combining IL-6 and TNFR-1, which showed better predictability to all-cause mortality than individual markers[70].

Recent work from Stanford 1000 Immunomes Project developed a novel metric for systemic chronic inflammation called inflammatory Age® (iAge®), derived from a deep-learning method on serum immune markers[71]. Compared to individual biomarkers of inflammation, iAge® better represents the chronic inflammatory load that the body experiences. Interestingly, their work did not identify canonical markers of acute inflammation like IL-6 and TNF-α as the primary components in iAge® in their work. In contrast, they found that C-X-C chemokine motif ligand 9 (CXCL9) was the most significant contributor to iAge, probably owing to its role in age-related endothelial cell senescence and vascular function.

1.4.4 Inflammation and age-related health outcomes

Inflammation is a strong risk factor for multiple diseases that are highly prevalent in older individuals, including (but not limiting) cardiovascular disease (CVD)[42], metabolic disorder[72], chronic renal disease[73], cancer[74,75], depression[76], dementia[77], osteoporosis[78], and sarcopenia[79]. This section will focus on age-related health outcomes closely linked to impaired functional capacity, including CVD, type 2 diabetes mellitus (T2DM), Alzheimer's disease (AD), depression, sarcopenia, frailty, and age-related sensory impairment. Specifically, we will elucidate the potential mechanistic associations between inflammation and these age-related diseases or syndromes, which may further contribute to functional limitations and disability.

- **Cardiovascular disease (CVD)**

Inflammation is intricately involved, through multiple mechanisms[42], in the genesis and progression of atherosclerosis, the dominant cause of CVD[80]. In the initial phase of atherosclerosis, inflammation is triggered by vascular endothelial dysfunction characterized by an increased permeability and accumulation of cholesterol-containing lipoproteins, including low-density lipoprotein (LDL) in the intimal space of arteries[81]. During the endothelial inflammatory response, recruited monocytes migrate into the arterial wall and differentiate into macrophages. Macrophages uptake the oxidized lipoproteins, transform into foam cells, and ultimately coalesce into a lipid-rich necrotic core of the atheroma[80].

Atherosclerosis can aggregate and sustain the pro-inflammatory status[42], leading to more recruited immune cells in the lesion site and exacerbating plaque growth[80]. Large quantities of senescent cells are found in atherosclerotic plaques and express an SASP, which fuels inflammation and destabilizes the plaques[42]. At the final stage, the narrowed arteries and the fragility of plaque lead to rupture or erosion, which triggers thrombus formation and vascular occlusion, leading to cardiovascular events (such as myocardial infarction and stroke)[80].

Strong epidemiological evidence indicates that elevated pro-inflammatory markers predicted cardiovascular events. Using data from the Health, Aging, and Body Composition study, Cesari et al. recruited 2225 participants aged 70 to 79, initially free of CVD, and followed them for an average of 3.6 years[82]. They found that higher circulating CRP, IL-6, and TNF-α predicted future cardiovascular events in older adults, including coronary heart disease, stroke, and congestive heart failure. They also evaluated cardiovascular risks by combining measurements of three inflammatory markers and observed the highest risk in participants with the highest tertile level for all three markers[82]. Circulating CRP is considered a good predictor of future

cardiovascular events for decades[61]. A meta-analysis done by Kaptoge et al. recruited 160,309 adults (mean ± standard deviation [SD] = 60 ± 8 years old) without a history of vascular disease across 54 long-term prospective studies, with 27,769 of them experiencing non-fatal or fatal vascular and non-vascular disease outcomes (median 5.8 years to first outcome). Circulating CRP concentration was log-linearly associated with increased risks for coronary heart disease, stroke, and vascular mortality[83]. Interestingly, they also observed high CRP was associated with non-vascular death from several cancers and lung disease. Still, the relevance of CRP to such a range of disorders is unclear[83].

People with CVD are at a higher risk of cognitive decline[84]. Although the mechanisms are not fully understood, it has been suggested that cognitive dysfunction in CVD patients may be caused by several factors related to inflammation, such as blood-brain barrier (BBB) impairment, dysfunction of brain microglia, and cerebral tissue damage[85]. Notably, amyloid-beta (Aβ) peptide clearance through transporting from the brain to the blood vessels is disturbed when the BBB is damaged, leading to deposition of Aβ in the brain (one of the pathological features of AD)[8687].

Apart from the atherosclerosis-induced systemic inflammation, low cardiac output in CVD may favor a self-perpetuating inflammatory state by reducing renal blood flow and triggering the renin-angiotensin-aldosterone system (RAAS) activation[85]. RAAS can further activate the sympathetic nervous system, consequently releasing ROS and enhancing oxidative stress[88]. The ongoing inflammation further aggravates both CVD and cognitive impairment.

It is still unknown if the cognitive impairment exists in the early stage or long term after the onset of CVD[85]. For instance, Xie et al. investigated 7888 participants from the English Longitudinal Study of Ageing (ELSA; mean ± SD age = 62.1 ± 10.2) and evaluated their cognitive function before and after incident coronary events. They noticed no short-term

cognitive function change after the onset of coronary event. However, in the long term, participants' global cognition, verbal memory, and temporal orientation scores declined significantly faster than before the coronary event occurance[89].

- **Type 2 diabetes mellitus (T2DM)**

T2DM is a complex metabolic disorder, diagnosed by impaired glucose tolerance and hyperglycemia resulting from insulin resistance or deficiency[90]. Insulin resistance is a condition where the three primary insulin-sensitive tissues (skeletal muscle, liver, and adipose tissue) become less responsive to insulin action, resulting in severe failures in blood glucose uptake[91]. Accumulative evidence suggests that insulin resistance is contributed by inflammation[92]. Excessive nutrients, including glucose and free fatty acids, activate IκB kinase-β (IKKβ)/NF-κB and JuN N-terminal kinase (JNK) signaling pathways in adipocytes, hepatocytes, and associated macrophages[92]. Both pathways increased the expression of pro-inflammatory mediators, including TNF-α, IL-6, and IL-1β[93], which directly promote localized insulin resistance by affecting insulin signaling or indirectly by stimulating other inflammatory pathways[94]. The releasing cytokines may also be transported through circulation, leading to systemic insulin resistance[93]. In response to insulin resistance, pancreatic islets increase their cell mass and insulin secretion; once this compensatory mechanism is insufficient to overcome the level of insulin resistance, insulin deficiency will occur[93]. Notably, tissue inflammation has also been detected in the pancreatic islets of T2DM patients, featuring as immune cell infiltration and fibrosis in conjunction with amyloid deposits[93].

Obesity is a crucial risk factor for developing insulin resistance and T2DM. Adipose tissue is one of major sources of chronic inflammation (Section 1.4.2). It produces numerous cytokines, chemokines, and adipokines – hormones produced exclusively by adipocytes (e.g., leptin and adiponectin)[93], all collectively contributing adipose tissue inflammation and promoting

inflammation in other tissues[95]. In addition, quickly expanding adipose tissue may present hypoxia, or eventually lead to adipocyte cell death when expansion beyond oxygen and nutrient requirements; both situations may induce an inflammatory response that aims to recruit macrophages to repair damaged tissue or remove cellular debris[93].

Chronic hyperglycemia associated with diabetes can result in end-organ dysfunction and failure involving the retina, kidneys, nerves, heart, and blood vessels[95]. Inflammation is intricately linked to the development of these macrovascular and microvascular complications[96,97], which contribute to the high burden of functional limitations and disability in T2DM patients[98,99]. People with T2DM are at increased risk of dementia[100]. Anita et al.[101] conducted a meta-analysis using data from 40 studies to compare circulating inflammatory marker concentrations between T2DM patients with and without cognitive impairment (mild cognitive impairment, AD, and vascular cognitive impairment). They observed significant differences in the levels of IL-6, CRP, and soluble vascular cell adhesion molecule-1 (sVCAM-1), suggesting an increased inflammatory-vascular interaction associated with cognitive impairment in T2DM[101]. One recent longitudinal study from Edinburgh Type 2 Diabetes Study (ET2DS) echoed this relationship[102]. ET2DS was a population-based study that recruited 1066 adults aged 60 to 75 years with T2DM. In this analysis, Sluiman et al. analyzed 581 participants followed for 10 years and found that higher baseline IL-6 and CRP levels predicted greater cognitive decline in these individuals[102].

- **Alzheimer's disease (AD)**

AD is the most prevalent type of dementia and one of the most common age-related neurodegenerative disorders[103]. Extracellular Aβ deposition and intracellular neurofibrillary tangles (NFTs; forming by hyperphosphorylated tau protein) are two pathological hallmarks of AD[104]. The presence of Aβ plaques and NFTs activate microglia to produce pro-inflammatory

mediators, including cytokines IL-1β, IL-6, and TNF-α, the chemokines IL-8, macrophage inflammatory protein-1α, and MCP-1[105]. These pro-inflammatory mediators lead to the peripheral monocyte recruitment to pass through the BBB[87,105]; they also activate additional microglia and promote their proliferation, further releasing more inflammatory factors[105]. Aβ deposits also activate astrocytes, which secrete various pro-inflammatory molecules that can attract and activate microglia[105]. Neurons also contribute to the pro-inflammatory molecule production in AD, in which neuronal chemokines act as messengers between neurons and glial cells[105]. The inflammatory process in the central nervous system (CNS), often called neuroinflammation, can result in neuronal damage, leading to neurodegeneration and deterioration of cognitive functions[106].

Although the initial mechanism of cerebral Aβ deposits is not fully clear, inflammation has been hypothesized to be implicated in this process[87107]. It has found that Aβ production can be modulated by neuroinflammation via an interferon-induced transmembrane protein 3 (IFITM3)-γ-secretase complex[108]. Moreover, pro-inflammatory cytokines can inhibit microglial phagocytosis to Aβ and contribute to its accumulation in the brain[109].

It has been noted that AD is more likely to develop in people with chronic inflammatory diseases, including CVD, T2DM, and major depressive disorder[110], leading to a hypothesis that inflammaging is underlined in all these diseases[87]. As stated previously, systemic chronic inflammation can either contribute to amyloidosis or increase BBB permeability, allowing more blood-derived inflammatory mediators and immune cells to infiltrate the CNS[111], leading to AD development. Indeed, a meta-analysis of 175 studies done by Lai et al.[77], which reviewed 51 analytes in 13,344 AD patients and 12,912 healthy controls, suggested AD is accompanied by a peripheral inflammatory response by observing several peripheral pro-inflammatory cytokines and acute-phase proteins significantly elevated in individuals with AD than healthy

controls. However, not everyone with inflammaging suffers from AD or other age-related neurodegenerative diseases, implying that the inflammatory process alone is insufficient for AD development , and a variety of mechanisms participate in neurodegenerative processes and remain to be elucidated[87].

It is worth noting that high peripheral inflammatory markers also predict cognitive decline in non-AD people. Existing studies of community-based cohorts generally suggest associations of peripheral inflammatory markers with cognitive decline in older individuals, especially elevated CRP, IL-6, and TNF-α[112–116]. In a recent study by Gross et al., high TNFR-1 also increased the risk of progression from normal cognition to mild cognitive impairment[117]. However, other studies have not been able to observe similar associations[118–120], probably due to the heterogeneity in cognitive outcome measures, population characteristics, and follow-up period.

- **Depression**

Depression can affect individuals of all ages. Although depression is less prevalent among older adults than at earlier ages, it has more severe consequences and impact on functional capacity[121,122]. Depression in late life, usually defined as age older than 65, is frequently associated with chronic diseases such as CVD and a worse prognosis in these patients[123]. Depression is associated with cognitive impairment[124], and can increase the risk for physical disability[125,126].

The mechanisms of depression are complex and not fully understood. It is worth noting that depression can be induced by biological and psychosocial factors[127], and the effect of biological factors, particularly non-genetic ones, loom large in late life[121]. It has been proposed that late-life depression is the clinical expression of dysfunction in reward, salience, and cognitive control networks, with the extent of dysfunction within these networks accounting for the

severity and heterogeneity of clinical presentations[128]. Age-related and disease-related inflammation may contribute to the etiology of depression in older adults, mediated by increased periphery immune responses, disruption in the periphery-brain immune communication, and a shift of the CNS into a chronic pro-inflammatory state[128,129].

Previous epidemiological findings support the role of inflammation in depression in old age. Martínez-Cengotitabengoa et al.[130] reviewed the longitudinal evidence of six large-sample studies focusing on older adults. In this review, significant associations of future depressive symptoms had been reported with a high level of IL-6, IL-8, and TNF-α. Conversely, a recent work by Luning Prak et al.[131] investigated 119 older individuals with clinically significant depression but not medical conditions associated with systemic inflammation. They found that depressive patients' inflammatory marker concentrations in peripheral and cerebrospinal fluid were not significantly different from their age-matched health controls, implying that depression alone does not result in systemic or intrathecal cytokine elevations.

- **Sarcopenia**

The disturbed balance between muscle protein degradation and synthesis in old age leads to progressive loss of skeletal muscle mass and functionality, referred to as sarcopenia. Sarcopenia is clinically important, as it triggers a downward spiral of physical decline in older individuals from reduced strength, fewer physical activities, further strength decline, functional limitations, disabilities, and ultimately loss of independent living[132]. Among 5036 adults aged ≥65 from the Cardiovascular Health Study, those with severe sarcopenia was associated with a 27% increased risk of developing disability in instrumental activities of daily living (IADL) over eight years compared to their no-sarcopenia counterparts[133].

Inflammation may contribute to age-related muscle wasting through several mechanisms. First, chronic inflammation enhances the activity of the ATP-dependent ubiquitin-proteasome

pathway, which is responsible for muscle protein degradation[132,134]. Proteins awaiting recycling are first conjugated with polypeptide ubiquitin, and then recognized and degraded by the proteasome complex into short peptides. It was suggested that pro-inflammatory cytokines, especially the TNF-α and IL-6, might upregulate this proteolytic pathway through activating Forkhead box O3a (FOXO3a) transcription factor, which regulates the ubiquitin-proteasome system[79,135]. Second, inflammation reduces the regenerative capacity of muscle cells by affecting satellite cell activity and differentiation[79,134]. Satellite cells are undifferentiated myogenic precursors (i.e., muscle stem cells), which are activated and proliferated upon muscle damage to replace and/or repair the injured muscle fibers[79]. It was shown that pro-inflammatory cytokine, particularly TNF-α, might disrupt the regenerative process in skeletal muscle[134]. Third, pro-inflammatory cytokines can impair muscle function by reducing the production and inhibiting activity of insulin-like growth factor-1 (IGF-1)[136,137]. IGF-1 is a growth factor that stimulates cell growth and proliferation, including muscle development. Barbieri et al. observed that high circulating IGF-I was cross-sectionally associated with better muscle function only in subjects with low IL-6 levels in blood, suggesting that the effect of IGF-I on muscle function depends on IL-6 levels[137].

The co-existence of excessive adiposity in the sarcopenic condition (i.e., sarcopenic obesity) may exacerbate systemic inflammation and muscle wasting[134,138]. Notably, aging leads to a decline in total energy expenditure (including reduced resting metabolic rates, physical activity, and adaptive thermogenesis), contributing to gradually increased visceral fat[138]. Sarcopenic obesity is associated with multiple adverse cardiometabolic effects and poor health outcomes[139], including disability[140]. The recent work of Morikawa et al. followed 4197 Japanese older adults (mean ± SD age = 74.6 ± 5.0) for five years and noticed the group of people with sarcopenic obesity had the highest risk of disability onset compared to those with only sarcopenia or obesity[140].

- **Frailty**

Frailty is a geriatric syndrome characterized by a physiologic vulnerability to stressors due to cumulative declines across physiological systems and progressive loss of internal homeostasis[141,142]. Frailty is often assessed by three of five signs and symptoms: unintentional weight loss, fatigue, slow gait speed, low handgrip strength, and physical inactivity[141]. Frailty denotes a high-risk clinical state, which has been validated to predict adverse events including death, disability, falls, and hospitalization[141,143,144]. Frailty can manifest both in the presence and absence of chronic diseases or disability[141], but it is often observed before these health outcomes develop[142]. Frailty is also highly correlated with cognitive dysfunction and potentially shares the underlying mechanisms[145]. Frailty has been associated with faster cognitive decline in older adults[146] and moderated the relationship between AD pathology and dementia manifestation[147].

Fried et al. proposed that five interconnected frailty components form a detrimental cycle (**Figure 1.6**)[141,148]. Inflammation plays a role in advancing this adverse cycle by triggering the onset of the frailty components[149]. As mentioned earlier, inflammation contributes to losing muscle mass and quality[132,134], resulting in a lower handgrip strength. Inflammation-related muscle weakness and peripheral nerve impairment[150] can deteriorate gait speed. Reduced grip strength and slower gait speed further contribute to fatigue and decreased physical activity, perpetuating muscle loss and increased adipose tissue[149]. This, in turn, reinforces systemic inflammation and exacerbates the severity of frailty. Additionally, inflammation can contribute to decreased appetite in old age[151,152] (known as "anorexia of aging"[153]), resulting in weight loss and reinforcing muscle weakness and its associated outcomes.

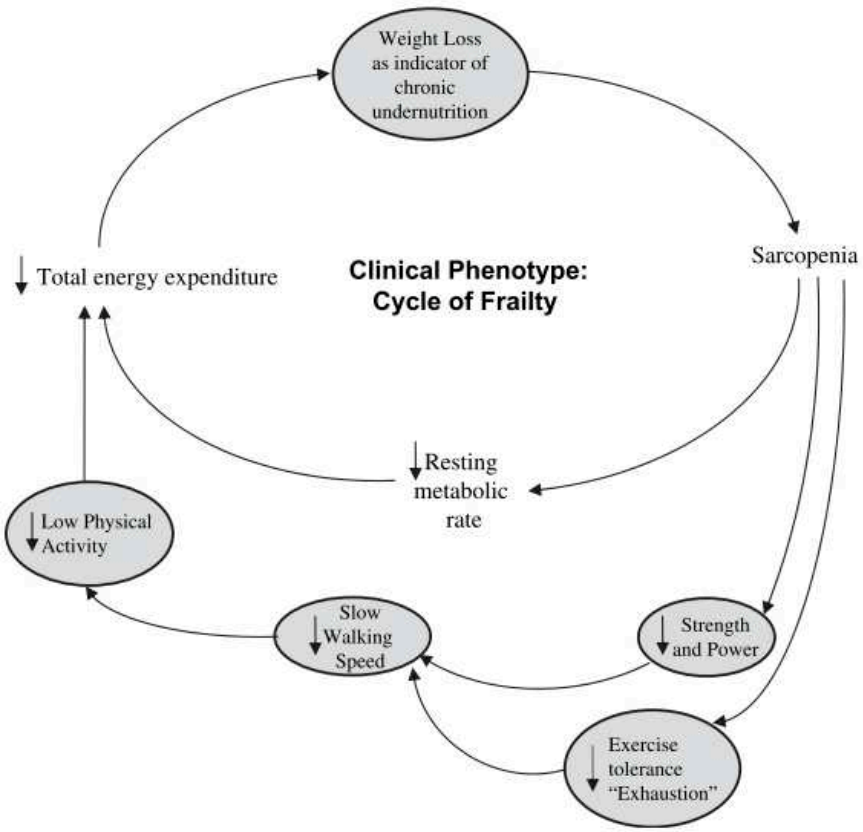

Figure 1.6 Components of frailty phenotype related in an adverse feedforward cycle
Data source: Fried L, et al. Journals Gerontol - Ser A Biol Sci Med Sci. 2009;64(10):1049-1057.

Substantial evidence supported that inflammation marked by upregulated inflammatory markers in the blood is associated with frailty syndrome, including several large populational-based cohorts. Puts et al. had identified that moderately elevated levels of CRP (3-10 mg/L) predicted 3-year incident frailty among individuals aged ≥65 from the Longitudinal Aging Study of Amsterdam[154]. Gale et al. enrolled participants aged ≥60 years from the ELSA and found that high levels of CRP and fibrinogen were predictors of incident frailty in women but not in men; having high levels of both inflammatory markers was more strongly predictive of incident frailty than having a high level of either marker alone[155]. In the Women's Health and Aging Study, white blood cell (WBC) counts and IL-6 levels were significantly higher in frail older women compared to their robust counterparts[156]; similar associations were observed

in neutrophil and monocyte counts[157]. Collerton et al. used cross-sectional data from the Newcastle 85+ Study and confirmed the association of high inflammatory markers (CRP, IL-6, TNF-α, and neutrophil count) with both Fired frailty phenotype and Rockwood frailty index in a very old populations (aged 85)[158]. Finally, in a community-based Atherosclerosis Risk in Communities Study cohort, Walker et al. suggested that midlife systemic inflammation marked by high CRP and a composite index of four inflammatory markers (fibrinogen, von Willebrand factor, Factor VIII, and WBC) was associated with frailty in later life[159].

- **Sensory impairment (hearing and vision)**

Our five classical senses – vision, smell, hearing, touch, and taste – are affected by aging. Hearing and vision impairment are the most prevalent conditions in older adults[160] but are potentially preventable or adaptable through proper aids[161,162]. Decreased vision and hearing have been linked to limitations in other functions. For example, visual impairment increases the risk of events that diminish physical function, such as falls and fractures[163,164]. Older individuals with hearing deficit have an increased risk of cognitive decline[165], possibly due to long-term sensory deprivation or general neurodegeneration[166]. Hearing loss in older adults, whether co-occurring vision loss or not, was associated with higher levels of depressive symptoms, mediated by difficulties in activities of daily living (ADL) and low social activity engagement[167]. Overall, older adults with hearing or vision impairment showed a higher risk of disability and poor quality of life[168–170].

Age-related hearing loss (ARHL), characterized by reduced hearing sensitivity and slowed processing of acoustic information[171], is caused by degenerative changes in the cochlea, including loss of sensory hair cells, cochlear synaptopathy, and reduced vascularization[172]. Inflammation was observed in the cochlea of humans with ARHL[173] and animal models of human-like ARHL[174], suggesting that inflammation may contribute to the development of

ARHL. However, epidemiological studies demonstrated inconsistent findings on the association between ARHL and circulating inflammatory markers. While the cross-sectional work of Verschuur et al. found that increased inflammatory markers (WBC count, neutrophil count, CRP, and IL-6) were associated with the degree of hearing loss in older people[175], Nash et al. only duplicated the similar association with persistently high levels of CRP (defined as >3 mg/L) in older age group (≥60 years) in the longitudinal approach[176]. In another longitudinal study, Lassale et al. investigated 4879 middle-aged and older people from the ELSA. They observed the association of hearing impairment with WBC count, fibrinogen, and CRP independent of age and sex. Still, most associations disappeared after adjusting for cardiovascular risk factors, cognitive function, and depression[177].

Inflammation involves the pathophysiology of several chronic eye diseases that commonly cause age-related vision impairment, such as age-related macular degeneration (AMD)[178,179], glaucoma[180,181], and diabetic retinopathy[182,183]. Clinical evidence further linked systemic inflammatory biomarkers with these eye diseases. For example, Klein et al. observed that, among a random sample of 975 persons without signs of AMD initially, high serum levels of inflammatory markers, including CRP, IL-6, and TNFR-2, were associated with the onset of early AMD over 20 years[184]. Van Hecke et al. investigated a subsample of a 50- to 74-year-old general Caucasian population (n=625) and found that CRP, together with ICAM-1, was cross-sectionally associated with diabetic retinopathy[185].

In summary, inflammation plays an important role in age-related diseases or syndromes and can further impair different aspects of functional ability. Inflammation is linked to the functional domains composed of IC (cognition, psychology, mobility, and sensory), implying that it can also contribute to the decline of IC in older adults. In the next section, we will deeply discuss

how IC is operationalized in the current epidemiological research and its association with health outcomes and biomarkers, including the markers of age-related chronic inflammation.

1.5 Intrinsic capacity (IC)

As mentioned in Section 1.2, IC is an innovative construct of the healthy aging approach. IC reflects an individual's reserves and residual capacities and, together with the environment, determines their functional ability (Section 1.2.1). IC serves as the core element being monitored, maintained, and maximized in the ICOPE program (Section 1.2.2). IC is a concept still in its infancy, with growing research contributing the knowledge on its operationalization (Section 1.5.1 and 1.5.2), longitudinal trajectories (Section 1.5.3), and associations with clinical outcomes (Section 1.5.4) and biomarkers (Section 1.5.5).

1.5.1 IC construct

- **Five-domain structure**

When the IC concept was proposed, there was no clear way to operationalize it. In 2018, Cesari et al. conducted a literature review of the physiological functions of body systems most strongly associated with an increased risk of loss of dependence and autonomy[6]. Their work identified five body functions – cognition, locomotion, psychology, sensory, and vitality – closely related to disability, which supported that IC is a 5-domain construct.[6] (**Figure 1.7**).

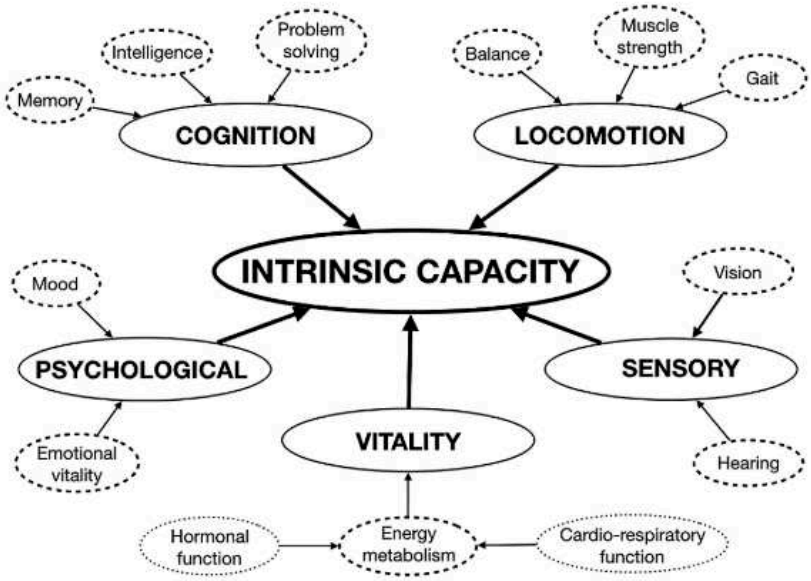

Figure 1.7 The five-domain IC construct with examples of possible subdomains

Data source: *Cesari M, et al. Journals Gerontol A Biol Sci Med Sci 2018;73(12):1653-1660.*

Beard et al. further validated the 5-domain IC construct through structural equation modeling (SEM) by using cohorts from the ELSA and the China Health and Retirement Longitudinal Study (CHRLS), respectively[186,187]. ELSA is an ongoing study of a nationally representative sample of the English population aged ≥50 years. CHARLS recruited participants aged ≥45 across 150 counties/districts and 450 villages/urban communities across China to ensure national representativeness. Both Beard et al.'s works focused on individuals older than 60 years (n=2352 for ELSA and n=7643 for CHARLS) and applied the same series of analyses. First, they included all potential measures that might provide objective estimates of IC domains in the exploratory and confirmatory factor analyses to identify the subfactors (i.e., domains) of IC. Second, they tested the construct validity of the identified IC construct by examining the association between IC factor scores (the methodology is detailed in the next section) and personal characteristics. Finally, the predictive validity of the IC score was assessed in a mediation model that considered the direct and indirect relationships of IC and multimorbidity with incident loss of ADL and IADL. In summary, both ELSA and CHARLS data revealed that

the SEM model with five subfactors was the best-fitting one. The IC score obtained from the 5-domain IC construct predicted declining performance in ADL and IADL after controlling for all personal characteristics. The consistent findings in these two large and different cohorts suggest that the IC construct is robust and likely applicable across populations and settings[187].

There is a lack of standard measurement for each IC domain in the current literature. In 2020, Gonzalez-Bautista et al. performed the first literature review on human studies investigating IC (a total of nine observational studies and one randomized controlled trial [RCT]). By then, they had already recognized the considerable inconsistency in each domain measurement between studies[188]. Another recent review by López-Ortiz et al. published in 2022 also highlighted a high degree of heterogeneity across 33 studies of IC regarding the ways they assessed IC; for example, up to 15 different variables were used for locomotion and 16 variables for cognition[189]. It is probably because most studies on IC were conducted as secondary analyses based on the available variables in each dataset.

- **Bi-factor model**

Based on the results of SEM, Beard et al. proposed that IC can be represented by a bi-factor structure, where IC acts as a general factor directly contributed by all measurements, and the five domains act as group factors explaining the clusters among measurements (**Figure 1.8**)[186]. IC bi-factor construct was found to fit well in the cohort from England[186], China[187], Hong Kong[190], Brazil[191], and US[192]; all studies showed that the bi-factor structure fitted their IC data better than the second-order model, in which IC acts as an umbrella factor covering five domains and all the domain indicators.

Figure 1.8 Bi-factor model of IC construct

Data source: *Beard JR, et al. BMJ Open 2019;9(11)*

- **Vitality as a fundamental capacity**

The bi-factor IC model discovered in Beard et al.'s work also declares another important message. In their first SEM using ELSA data, in which vitality was operationalized by handgrip strength, forced expiratory volume (FEV), circulating levels of dehydroepiandrosterone (DHEAS), hemoglobin and IGF-1, they observed that the vitality domain not only loaded independently to the general factor (i.e., IC) but also interacted strongly with the other subfactors of the bi-factor model (i.e., other four domains)[186,187]. They thus suggested that vitality acts as a core domain of IC, representing the overall variance in the biological systems that sustain life and functioning. On the other hand, other IC domains are overt, easily observable and assessable (i.e., expressed capacities), where their manifestation are driven by the impaired biological systems included in vitality (**Figure 1.9**).

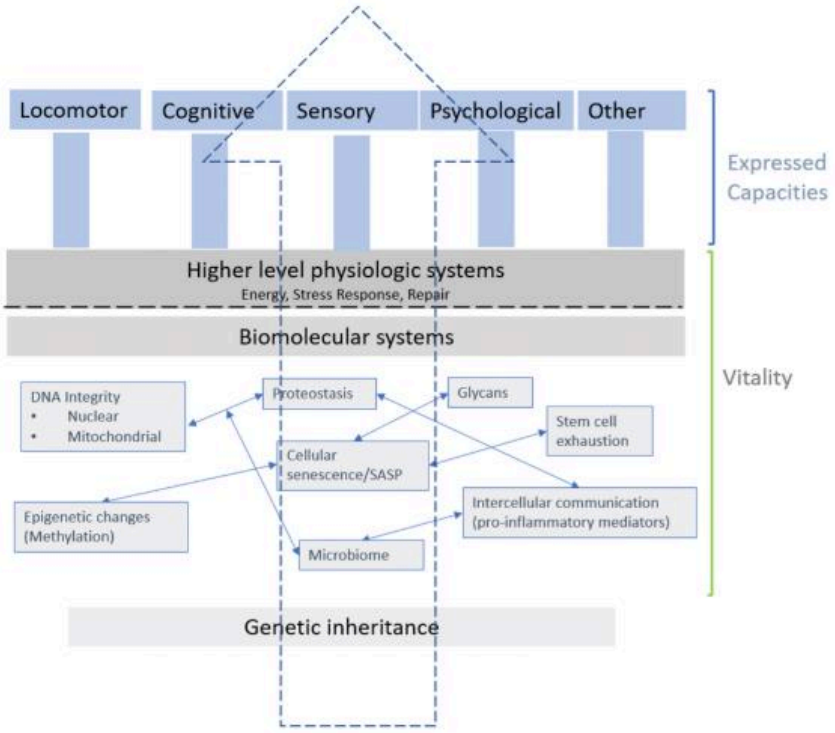

Figure 1.9 Conceptual frame for the IC construct showing the hierarchical relationship between vitality and other IC domains

Data source: *Beard JR, et al. Journals Gerontol Ser A. 2022;77(1):94-100.*

Compared to other domains, the concept of vitality might be the most obscure one. The vitality domain is conceptualized as a capacity to maintain homeostasis for functioning properly[193]; it is related to body functions involving energy metabolism because energy metabolism strongly determines a person's resilience to stressors or challenges for maintaining optimal homeostasis[194]. Indeed, Cesari et al.'s review in 2018[6] echoed this definition by observing that imbalanced energy intake and expenditure (e.g., weight loss, abnormal body mass index [BMI]) strongly predicted of disability in older people[195,196].

The conceptual frame for the IC construct proposed by Beard et al. (**Figure 1.9**) indicates two peculiar characteristics of vitality. First, the role of the vitality domain is similar to "physiological reserve," which affects the ability of an individual to retain their functioning levels. In other words, low vitality can directly lead to impairment of other functions; in addition,

it has a more profound impact on overall functional ability than other domains. Second, all physiological and biological functions involved in maintaining optimal homeostasis of the body and shaping the function of other domains can be covered by vitality. Thus, a full measure of vitality, if any, can be seen as an individual's "biological age"[197], because it is able to reflect the degree of deficit accumulation in cellular and molecular systems as aging.

The WHO ICOPE program suggests using nutritional status assessments, such as the Mini Nutritional Assessment (MNA), to evaluate older individuals' vitality[8], given that "nutrition is one of the key factors in maintaining vitality in older age[193]." However, the peculiar characteristics of vitality imply several potential measures of vitality besides nutritional status. In 2021, the WHO Working Group, together with geriatric experts, reached a consensus working definition of vitality as " a physiological state (due to normal or accelerated biological ageing processes) resulting from the interaction between multiple physiological systems, reflected in (the level of) energy and metabolism, neuromuscular function, and immune and stress response functions of the body.[194]" They summarized several attributes for vitality capacity besides nutritional status. For example, neuromuscular function quantified by handgrip strength was identified, given that it serves as a vital sign of biological age[198] and was a robust predictor of mortality[199]. Biomarkers linked to immune and stress response were also proposed to be promising indicators of vitality. By far, only a few studies used biomarkers as part of vitality measures. These biomarkers often reflect the physiology of the respiratory (FEV[186,187]), hematopoietic (hemoglobin[186,187]), and endocrine systems (DHEAS and IGF-1)[186]. The summary of vitality measures used in previous studies is presented in **Table 1.2**.

Table 1.2 Variables used by previous studies for the vitality assessment

Nutrition	Muscle strength	Biomarkers
MNA[200–203]	Handgrip strength[186,187,192,200,201,206–210]	Hemoglobin[186,187]
Weight loss[204,205]		DHEAS[186]
BMI[200,204,206–209]		IGF-1[186]
Abdominal circumference[200,209]		FEV[186,187]
Mid-upper arm circumference[205]		Peak flow test[192,206]

BMI, body mass index; DHEAS, Dehydroepiandrosterone sulfate; FEV, forced expiratory volume; IGF-1, insulin-like growth factor 1; MNA, Mini Nutritional Assessment.

1.5.2 IC scaling methods

No standard method to scale IC is available in the literature. Previous studies used to integrate the IC domains into a "global" or "composite" score in the following methods:

- **Z-standardization (composite z-score)**

Studies that applied this method included Giudici et al. (based on four domains)[211], Locquet et al. (four domains)[202] and Huang et al. (five domains)[201,212]. The z-score indicates the SDs by which the individual's raw value deviates from the population mean, with a positive z-score indicating that the individual's value is higher than the mean and a negative one showing the opposite[202]. In Huang et al.'s works, some domain indicators were transformed after stratification by sex (i.e., handgrip strength), age and educational level (i.e., cognitive tests) to better indicate an individual's capacity to their same-condition peers; domain indicators with dichotomous responses rather than continuous values had been converted to regression scores before z-standardization[201,212]. To parallelize other domain values, indicators whose higher values showed an impaired function, such as most psychological tests, were weighted as -1 before transformation[211,212]. Previous studies all defined the final IC composite score as the mean of four or five IC domain z-scores[201,202,211,212].

- **IC index**

Studies applied this method included Gutiérrez-Robledo et al. (0-10 scale)[206], Ma et al. (0-10 scale)[213], Meng et al. (0-12 scale)[207], Stolz et al. (0-100 scale)[192], Salinas-Rodríguez et al. (0-100 scale)[208], Lee et al.(0-100 scale)[203], and Liu et al. (0-5 scale)[214]. Most studies created their IC indices as positive metrics, with higher scores indicating higher IC[192,206–208,213]. On the other hand, Liu et al. created their index in the opposite direction by counting the numbers of impaired IC domains[214]. Both Gutiérrez-Robledo et al.[206] and Ma et al.'s works[213] had each domain divided into three categories (intact/optimal, mild impairment, severe impairment), resulting in a total score of IC ranging from 0 to 10. In Meng et al.'s[207] IC index, each domain consisted of two variables and was further weighted according to their degree of association with IADL limitations; chair rise speed and wellbeing were weighted by two points, and other variables were scored with one point. Stolz et al.[192], Salinas-Rodríguez et al.[208] and Lee et al.[203] investigated longitudinal IC measurements across several time points. Stolz et al.[192] and Lee et al.[203] rescaled their variables for each domain using the percent of the maximum possible (POMP) method[215], with a score of 0 equal to the lowest values of all observations (the minimum possible value) and 100 as the highest one (the maximum possible value). Salinas-Rodríguez et al. constructed an IC score through Item Response Theory and a Graded Response Model and then transformed it on a scale of 0–100[208].

- **Structural equation modeling (SEM)**

Six studies generated their IC scores through confirmatory factor analysis (CFA), part of the SEM technique[186,187,190,191,216,217]. CFA investigates the relationship between latent and observed variables in a priori-specified, theory-derived model. In this approach, IC was generated as a latent variable determined by domain indicators, and the IC value can be calculated as a factor score as the sum of indicators weighted by the corresponding factor loadings. While Lu et al.[217] applied a one-factor model for IC, the other studies used a bi-factor

IC model mentioned in the Section 1.4.2[186,187,190,191,216].

1.5.3 Longitudinal trajectory of IC

- **Declining trend along with advanced age**

IC is a dynamic construct that varies across the lifespan. Theoretically, IC fully develops in young adulthood and then gradually declines with age, with the rate of decline becoming steep at the end of life[218]. To date, only a few papers investigated the longitudinal trend of IC decline. A work by Stolz et al. followed 754 older adults aged ≥70 and observed that IC declined progressively, with the IC score (on a scale of 0-100) changing from 77 to 11 points on average over 21 years[192]. They found that IC decline was a heterogeneous process with a wide variety of baseline IC levels and over-time trajectories (**Figure 1.10**).

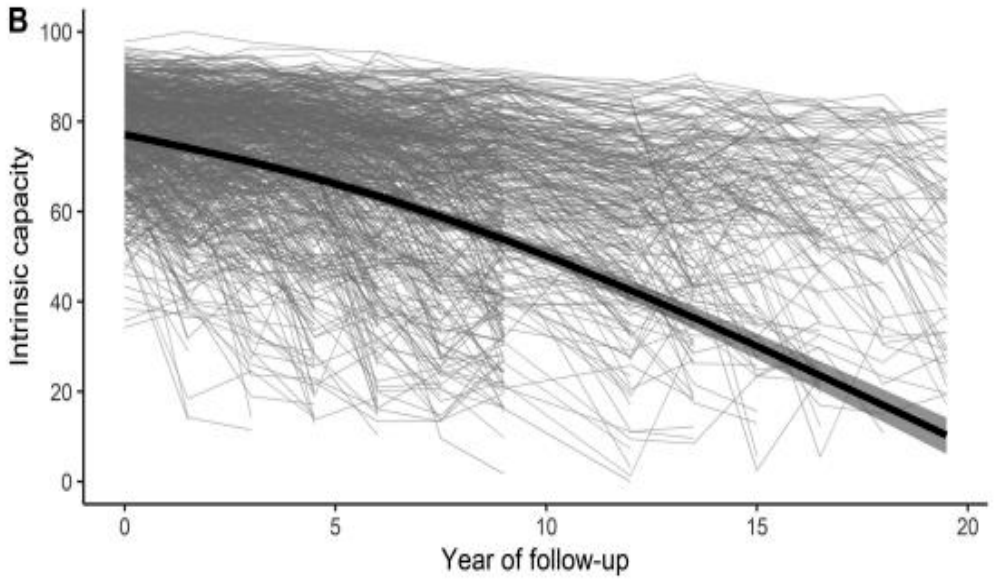

Figure 1.10 Trajectory of IC over time identified in Stolz et al.'s work

Thin gray lines show raw longitudinal observations of IC for 754 participants, and the thick black line indicates the estimated average trajectory of IC. Data source: *Stolz E, et al. Journals Gerontol A Biol Sci Med Sci 2022;77(1):101-105.*

- **Heterogeneous patterns of IC decline**

Two studies have investigated how IC declined in different patterns over time between individuals by using latent class modeling approaches. They assumed the existence of unobserved subpopulations (latent classes) with similar IC patterns and determined the number of classes by identifying the model that best fits the given data. Latent class modeling approaches contrast classic statistical models, which assume all individuals share common population parameters. The measured outcomes are averaged out in the classic statistical models, and only the evolution across the entire study sample or pre-specified observed subgroups is analyzed[219].

A study by Salinas-Rodríguez et al. used the cohort of the WHO Study on global AGEing and adult health (SAGE) in Mexico, which included 2,735 adults aged ≥50 years[208]. In their work, three IC longitudinal trajectories were identified: *low baseline IC with steep decline, medium baseline IC with slight decline,* and *high baseline IC with a moderately increasing trajectory* (**Figure 1.11**). Compared to the steep declining trajectory, people with better trajectories were associated with a higher quality of life and lower functional disability.

Another study with a cohort of 9,448 long-term care recipients in Taiwan identified four classes of individuals with similar IC trends across three years: *high-stable, normal-stable, sensory-dysfunction,* and *all-dysfunction*; the labels of these trajectories were determined by the proportion of impaired domains at baseline[220]. They further found that the all-dysfunction group was associated with an earlier onset of severe dependency in 2.5 years (**Figure 1.12**).

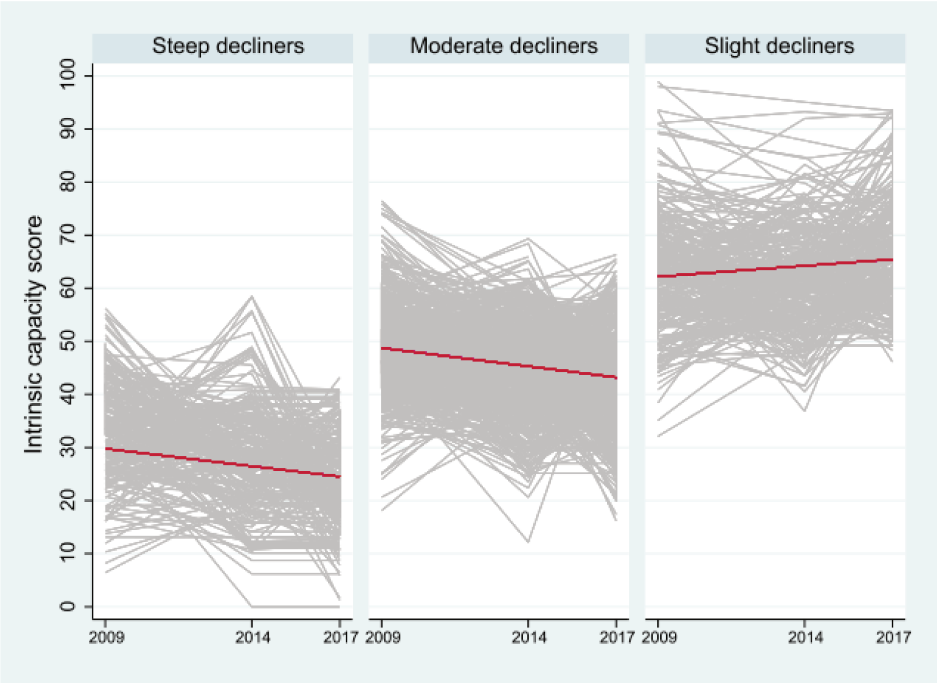

Figure 1.11 Three IC trajectories identified in the SAGE cohort

The percentage of participants in each trajectory: 17% for steep decliners, 50% for moderate decliners, and 33% for slight decliners. Data source: *Salinas-Rodríguez A, et al. Maturitas. 2022;161:49-54.*

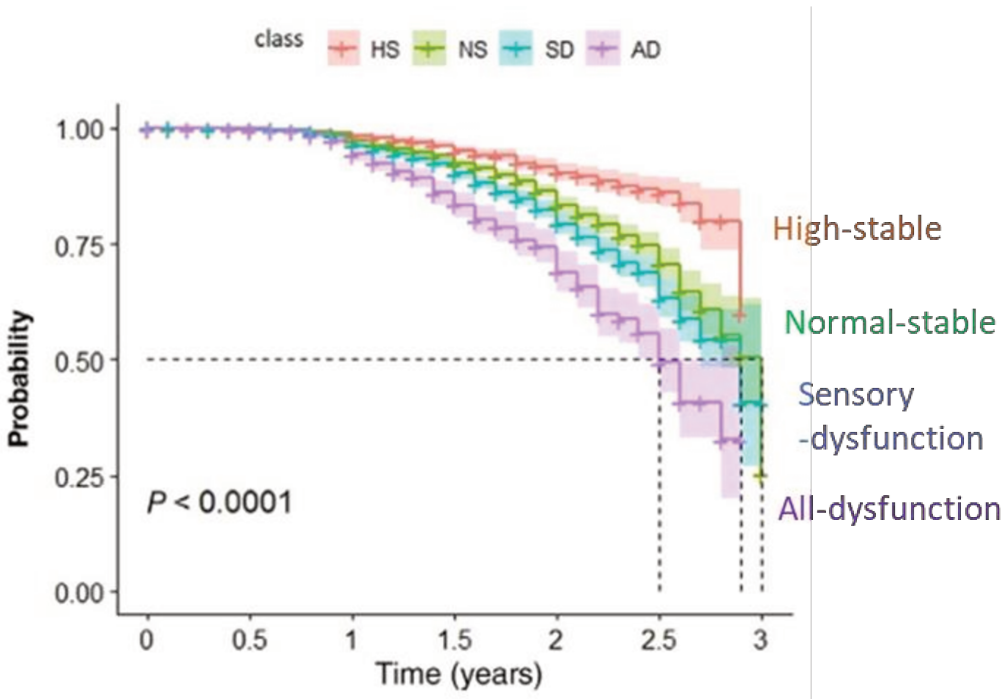

Figure 1.12 The year of onset of a severe dependency across IC trajectories identified in the Taiwanese cohort

Data source: *Chen J, et al. Geriatr Gerontol Int. 2022;22(7):516-522.*

1.5.4 Association between IC decline and health outcomes

It has been known that the impairment in individual IC domains (cognitive, physical, psychological, or sensory function) is linked to adverse health events. Growing evidence has found that composing multiple functional domains into a single IC score is also associated with negative health outcomes, including mortality, disability, and frailty. Most of the studies were performed in older populations.

- **Mortality**

Several studies found that individuals with higher IC had a lower risk of all-cause mortality. The work of Charles et al. followed 604 nursing home residents (mean ± SD age = 82.9 ± 9.1) in Belgium over three years. They found that high baseline locomotion (assessed by balance performance) and vitality (assessed by MNA score) were associated with lower risk of death, after adjusting for age, sex, comorbidities, medication, and education[200]. Locquet et al. investigated 481 community-dwelling participants aged ≥65 years and observed the 5-year mortality risk was decreased by 49% for an increase of one SD in IC composite Z-score[202]. Meng et al. demonstrated similar findings in middle-aged and older individuals by classifying their participants based on IC tertiles and observing a 3-fold mortality risk over four years among participants in the lower IC tertile compared to those in the upper tertile[207]. Stolz et al.'s work on 754 older adults showed a 1-point higher IC value was associated with a 5% decrease in the risk of mortality (HR [95% CI] = 0.95 [0.95-0.96]), after adjusting for age, sex, ethnicity, education, and chronic diseases[192]. Lee et al. observed a similar association among 1839 Taiwanese (63.9 ± 9.3 years old), whose HR for 10-year mortality was 0.95 [0.93-0.97] as a 1-point increased IC value[203]. Ramírez-Vélez et al.'s work used data from 443,130 UK Biobank participants (mean age = 58.4 years; 76.9% was < age 65) and calculated their IC deficit scores based on seven indicators (with a total score ranging from 0 to 7; higher means worse IC).

After a mean follow-up of 10.6 years, they found that people with the highest number of IC deficit score (i.e., 4 points) was associated with an increased incidence of CVD mortality than those without any IC deficits (HR [95% CI] = 2.10 [1.81–2.43] in men and 2.29 [1.85–2.84] in women)[221].

- **Disability and frailty**

IC is also a good predictor of disability and frailty. Gonzalez-Bautista et al. evaluated the association of incident disability with the number of impaired IC domains among 759 non-demented older participants aged ≥70 years. They found that each additional domain impairment increased the 5-year risk of incident IADL disability by 27%, the risk of ADL disability by 23%, and the risk of frailty by 47%[222]. Tay et al. found that adults aged >55 with higher baseline IC were less likely to progress to pre-frailty / frailty over one year[223]. The work of Stolz et al. also demonstrated that a 1-point higher IC value was associated with a 7% decrease in the risk of ADL disability[192].

1.5.5 Association between IC and biomarkers

Various types and origins of biomarkers, including blood-based, tissue-related, and imaging markers, serve as valuable tools for predicting functional impairment. However, current research on the association between IC and biomarkers in humans predominantly focuses on blood-derived biomarkers (**Table 1.3**), with limited studies available. Indeed, plasma biomarkers offer the advantage of low invasiveness and easy accessibility, making them more suitable for clinical applications. Notably, researchers are exploring a wider array of IC biomarkers. For example, the INSPIRE program is investigating digital markers as IC decline predictors[224]. Given the multidimensional nature of IC, Rivero-Segura et al. have proposed that combining biomarkers from multi-omics approaches (e.g., genomics, epigenomics, proteomics, and metabolomics) holds promise as a means to characterize IC[225].

Among all plasma biomarkers, inflammation-related markers are the most often studied, but their associations with IC are inconsistent. In the work of Giudici et al., persistently high CRP levels (defined as two consecutive CRP measures between 3 and 10 mg/L within one year) were associated with faster IC decline over time, but the association attenuated after controlling for demographic covariates and BMI. It is worth noting that people with combined high CRP and hyperhomocysteinemia (>15 μM/L) condition were significantly associated with IC decline in the adjusted model[211]. Lee et al. also observed the association of high CRP (≥ 0.3 mg/L) with low baseline IC but not with the rapidly deteriorated IC, defined as having an IC score decline more than 10% over seven years (equal to one SD of mean IC decline)[203]. Other inflammatory markers were investigated in a cross-sectional approach. Meng et al. found an association between high IL-6 and low IC in middle-aged and older populations, but the associations did not replicate in the levels of soluble IL-6 receptor[207]. In the work of Ma et al., people with increased TNFR-1 levels were more likely to have at least one impaired IC domain[204]. Lee et al. observed that individuals with an upper tertile of NLR tended to have lower baseline IC compared to those with NLRs in the lower tertile[203].

Some nutritional and endocrine markers also showed the association with IC. Lee et al.[203] observed that serum 25-OH vitamin D <10 ng/mL (defined as vitamin D deficiency) was related to rapid IC deterioration over seven years. Meng et al.[207] found that individuals with low serum albumin and low folate were less likely to be in the high IC group than those with low IC. DHEAS, showed individual association with IC in the work of Lee et al.[203] but not in Meng et al.'s[207]. In contrast, growth hormone and IGF-1 failed to demonstrate significant association with baseline IC levels and IC evolution in Lee et al.'s study[203]. Meng et al.[207] and Gutiérrez-Robledo et al.[206] demonstrated that poor IC was associated with increased allostatic load (AL). AL refers to the cumulative effect of chronic stresses and life events through measuring several markers of stress response systems[226], including cardiometabolism, neuroendocrine function,

and inflammation (in Meng et al.'s work[207] only). Each biomarker was dichotomized into high and low risk based on appropriate cut-off; AL was calculated as the count of biomarkers falling in the high-risk category. The link between AL and IC supports the involvement of persistent inflammation, one potential response of chronic stresses, in deteriorated IC.

Table 1.3 Studies evaluated the association between biomarkers and IC

Authors (journal)	Design	Population (number of subjects)	Biomarkers	IC operationalization	Results
Giudici KV. (Exp Gerontol. 2019)[211]	Longitudinal (5-year follow-up)	Community-dwelling, aged ≥70 (n=1516)	CRP, homocysteine	A composite IC Z-score is based on four domains: locomotion (SPPB), cognition (CCS), psychological (GDS), and vitality (handgrip strength); a higher value means better capacity.	People with both persistently high CRP (3-10 mg/L) and hyperhomocysteinemia (>15 μM) had faster IC decline over time compared to those with normal CRP and homocysteine levels.
Gutiérrez-Robledo LM. (J Nutr Heal Aging. 2019)[206]	Cross-sectional	Community-dwelling, aged ≥60 (n=1888)	Allostatic load (AL), including ten biomarkers related to cardiovascular, metabolism, and neuroendocrine.	An IC index (ranging from 0-10; higher is better) is measured through 5 domains: locomotion (chair rise time, 3-meter usual gait speed, pick pencil test), cognition (modified MMSE), psychological (GDS, self-reported life satisfaction, locus of control, social participation), vitality (peak flow test, handgrip strength, BMI), sensory (self-reported visual and hearing abilities).	Compared to people with 0-1 abnormal biomarker of AL, the possibility of having greater IC decreased by 33% in the AL category of 2-3 abnormal biomarkers of AL (p=0.008) and by 44% for the ≥4 abnormal biomarkers category (p=0.002), after controlling demographic covariates and chronic diseases.
Ma L. (J Nutr Heal Aging. 2021)[204]	Cross-sectional	Aged ≥60 (n=130)	TNFR-1	IC was measured through 5 domains: locomotion (SPPB), cognition (MMSE), psychological (GDS),	Increased TNFR-1 was associated with having at least one IC domain deficit (adjusted

Authors (journal)	Design	Population (number of subjects)	Biomarkers	IC operationalization	Results
				vitality (weight loss, BMI), and sensory (self-reported visual and hearing impairments). The authors did not report the cutoff used to define the deficit for each domain.	OR=1.013, p=0.038).
Ma L. (J Nutr Heal Aging. 2021)[227]	Cross-sectional	Aged ≥60 (n=283)	CRP, NT-proBNP	IC domain deficits were measured according to the ICOPE screening tools; the authors did not report the detailed instruments for each domain.	NT-proBNP was associated with having at least one IC domain deficit (adjusted OR [95% CI] = 1.005 [1.000-1.010]). CRP was not associated with IC after adjusting for clinical covariates.
Meng LC. (Front Med. 2022)[207]	Cross-sectional	Community-dwelling, aged ≥50 (n=839)	Plasma biomarkers related to cardiometabolic, neuroendocrine, inflammation, and diseases; allostatic load; genetic markers	A 12-point IC scoring system based on five domains (higher is better): locomotion (gait speed, chair stand), cognition (SPMSQ, MMSE), psychological (CES-D-10, PSS-10), vitality (BMI, grip strength), sensory (visual acuity, hearing loss). Subjects were further classified into three IC tertiles: high IC, medium IC, and low IC).	High IL-6, high E-selectin, low serum albumin, and low folate were associated with low IC in the entire sample. In the subgroup aged ≥60, high IL-6, low serum albumin, low folate, high allostatic load, and APOE ε4 genotype were associated with low IC.

Authors (journal)	Design	Population (number of subjects)	Biomarkers	IC operationalization	Results
Lee WJ. (Aging 2023)[203]	Longitudinal (7-year follow-up)	Community-dwelling, aged ≥50 (n=1019)	Serum biomarkers related to cardiometabolism, neuroendocrine, inflammation, sex hormone, and nutrients. Inflammatory markers included NLR, platelet-to-lymphocyte ratio, CRP, and homocysteine.	A mean score of 5 IC domains (ranging from 0-100; higher is better): locomotion (6-meter usual gait speed), cognition (MMSE), psychological (CESD), vitality (MNA), sensory (self-reported visual and hearing impairments).	Hyperglycemia, low DHEAS, high CRP, and NLR were associated with low baseline IC. Low DHEAS and vitamin D deficiency were associated with rapid deterioration of IC.

BMI, body mass index; CCS, composite cognitive score; CESD, Center for Epidemiologic Studies - Depression scale; CRP, C-reactive protein; GDS, Geriatric Depression Scale; ICOPE, Integrated Care for Older People; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; LGI, low-grade inflammation; MMSE, Mini-Mental State Examination; NLR, neutrophil-to-lymphocyte ratio; NT-proBNP, N-terminal pro-B-type natriuretic peptide; sIL-6R, soluble interleukin-6 receptor; SPPB, Short Physical Performance Battery; WBC, white blood cell.

1.6 Current knowledge gaps

Several knowledge gaps on IC and aging-related biomarkers remain to be investigated:

- **The most appropriate operational definition of vitality remains unknown**

The various attributes of vitality lead to different suggested ways of measuring it (**Table 1.2**), which results in the lack of agreement on operational definition in the literature. Nutritional status, representing the balance of energy intake and expenditure of the body, is used as vitality assessment in the WHO ICOPE program[8]. The SEM results from Beard et al.'s works[186,187] highlighted that vitality could be understood as the sum of all functions of physiological and biomolecular systems that resist the challenges and determine lifespan and healthspan, which is similar to "physiological reserve." This supports the use of handgrip strength, a vital sign of physiological reserve, to measure vitality in many previous studies on IC, particularly in the works applying the bi-factor IC construct[190,191]. Finally, the recent proposal regarding vitality as biological age[197] implies that a composite biomarker measure can be an ideal vitality marker, which had never been investigated before.

Directly comparing the performance of different vitality operationalizations on predicting functional disability can contribute to the knowledge of appropriate vitality definition. This investigation should consider the role of vitality as a fundamental component of the IC model, as suggested by the bi-factor results, which may directly link to other IC domains and contribute to functional disability through the mediation of other IC domains.

- **Lack of evidence on IC trajectories and their biological determinants**

IC is a dynamic metric that varies over time and within individuals. In the work of Stolz et al., the pattern of IC change was heterogeneous, with a wide variety of baseline IC levels and over-time trajectories between study participants (**Figure 1.10**)[192]. Potential longitudinal IC

trajectories in older adults are still poorly recognized, with only two studies exploring this topic[208,220]. In particular, no study has investigated IC trajectories exclusively on community-dwelling older adults and considering the joint evolution of different domains. IC trajectories can inform clinical care and public health actions for promoting healthy aging. Understanding the nature of IC trajectories allows clinicians to identify older adults who deviate from normality before the onset of clinical manifestations and introduce an early intervention plan. For public health authorities, poor and declining IC in specific regions or populations is a critical sign for attention and resource rearrangement[228]. Furthermore, it was unknown if IC trajectories have any biological risk factors.

- **Methodological limitations in previous studies**

The controversial results about plasma inflammatory biomarkers and IC decline (**Table 1.3**) may be due to several methodological limitations. First, only a few studies investigated the association between plasma biomarkers and IC in a longitudinal approach[203,211]. Furthermore, there is a lack of consistent IC operationalization regarding domain instruments and scoring systems. Some studies examined IC as an index by first categorizing the degree of impairment in each domain and then summarizing all domains into an overall score[206,207]. This approach was similar to the screening phase in the ICOPE (i.e., ICOPE Step 1), which may be easily applicable in clinical practice. However, it may lose information on individual variances in IC.

- **Novel biomarkers are poorly studied**

As aforementioned, the association of IC with recently emerging biomarkers linked to hallmarks of aging remains poorly investigated. For instance, plasma GDF-15 had demonstrated a strong prediction in mortality and functional decline[65,229], but its association with IC has yet to be elucidated. Various tissues or organs express and secrete GDF-15, such as the adipose tissue, the skeletal muscle, and the liver, and its expression is upregulated in various

age-related pathological conditions[64,230]. GDF-15 is also involved in regulating energy intake and body weight change via the GDNF family receptor α–like (GFRAL)[230]. Secretion of small amount of GDF-15 during specific periods of life is adaptive as it participates to restore/maintain homeostasis through regulating inflammatory and metabolic signals. However, homeostatic mechanisms become impaired with age and GDF- 15 elevation prolongs, leading to an overall detrimental effect on body function. GDF-15 levels can be induced by several aging hallmarks, including chronic inflammation, mitochondrial dysfunction, and cellular senescence[64]. The TAME study identified GDF-15, along with two pro-inflammatory cytokines, CRP and other markers, as potential biomarkers of accelerated biological aging (**Figure 1.3**)[24]. Therefore, it is imperative to understand the relationship between GDF-15 and IC, as IC is a major determinant of functional ability.

CHAPTER 2. AIMS

General aim

To evaluate how inflammation associates with older adults' IC, as both a structural component of the IC model and a predictor of longitudinal IC evolution.

Specific aims

STUDY I. To test different operational measures of the vitality domain of IC, including a new approach through a composite biomarker measure, by evaluating the pathways that connect vitality, the overt IC domains, and functional disability. Moreover, the longitudinal association of different vitality operational definitions with the overt IC domains and functional disability would be examined.

STUDY II. To identify how different domains of IC changed jointly over time in community-dwelling older adults using group-based trajectory modeling (GBTM) approach. In addition, the association between these determined IC trajectories and biological risk factors would be investigated.

STUDY III. To evaluate how five plasma inflammation-related biomarkers were individually associated with IC scores at cross-sectional and longitudinal levels using linear mixed-effect regression. IC would be operationalized as a continuous score with two scaling methods (POMP and Z-standardization).

CHAPTER 3. GENERAL MATERIALS AND METHODS

3.1 Study source

3.1.1 The MAPT Study

This thesis is based on data from the Multidomain Alzheimer Preventive Trial (MAPT; ClinicalTrials.gov [NCT00672685])[231]. The MAPT Study was a three-year, multicenter RCT investigating the preventive effect of interventions on cognitive function among community-dwelling older adults. The MAPT interventions included omega-3 polyunsaturated fatty acid (PUFA) supplementation (two capsules per day, providing a total daily dose of 800 mg docosahexaenoic acid [DHA] and 225 mg eicosapentaenoic acid [EPA]) and a multidomain intervention (totally 43 group sessions integrating cognitive training, physical activity counselling, and nutritional advice plus three preventive consultations with a physician within three years). Participants were randomly assigned (1:1:1:1) to the combined intervention (i.e., omega-3 PUFA plus the multidomain intervention), omega-3 PUFA alone, the multidomain intervention plus placebo, or placebo alone.

The MAPT Study recruited individuals aged 70 years or older, community-dwelling, non-demented, and met at least one of three criteria: spontaneous memory complaints expressed to their physicians, limitation in one IADL[232], or slow walking speed (≤0.8 m/s). Individuals with a Mini-Mental State Examination (MMSE) score <24 or a diagnosis of dementia, or those with any difficulties in the ADL[233], and those taking PUFA supplementation at baseline were excluded. A total of 1,680 participants were enrolled from 13 memory clinics in France and Monaco between May 30th, 2008 and February 24th, 2011, with one participant in the PUFA group withdrawing consent. Finally, 1679 participants comprised the study cohort (mean ± SD age = 75.3 ± 4.4 years; 64.7% female). Among 1679 participants, 99.2% presented spontaneous memory complaints at the study enrollment; 11.2% had limitation in one IADL, and 11.9%

showed slow walking speed. 16.2% of participants met two inclusion criteria, and 3.0% met all three conditions.

The primary outcome of the MAPT Study was the cognitive change from baseline to 36 months in a composite z-score combining four cognitive tests, including free and total recall of the Free and Cued Selective Reminding test, ten MMSE orientation items, Digit Symbol Substitution Test, and Category Naming Test. After the three-year intervention, the omega-3 PUFA supplementation and multidomain intervention, either in combination or alone, had no significant effects on cognitive decline after controlling for multiple comparisons[234]. Several secondary analyses also found no beneficial effect of the MAPT intervention on physical performance and handgrip strength[235], and IC (in a four-domain measure)[236]. After the three-year intervention period, an additional two-year observation period was applied, leading to an overall five years of follow-up.

3.1.2 Data collection in MAPT

In this section, we describe the time points of data collection in the MAPT Study. The MAPT variables used for this thesis are summarized in **Table 3.1**. The procedure of each assessment is detailed in the next section.

In MAPT, participants underwent clinical assessments at baseline and 6, 12, 24, 36, 48, and 60 months after the study enrollment. Assessments contained: (1) neuropsychological tests, including MMSE, Free and Cued Selective Reminding test, Digit Symbol Substitution Test, Category Naming Test, Trail Making Test (motor activity and selective attention) and Clinical Dementia Rating Scale; (2) depressive symptoms, measured by the 15-item Geriatric Depression Scale (GDS); (3) physical performance (assessed by Short Physical Performance Battery (SPPB); (4) frailty syndrome evaluating by Fried's frailty criteria, which including fatigue, involuntary weight loss, grip strength, walking speed, and physical activity. In this

thesis, data on IC domains (cognition, locomotion, psychology, and handgrip strength) was mainly retrieved from these assessments.

Participants assigned to the multidomain intervention groups (combined with omega-3 PUFA or placebo) were assessed for visual acuity (n=769), hearing ability (n=769), and nutritional status (assessed by MNA; n=769) during the yearly preventive consultations (baseline, 12, and 24 months) with a physician. In other words, sensory and nutritional variables were only available for half of the MAPT population at baseline, 12, and 24 months.

Fasting blood samples of individuals were collected at baseline and 12 months to analyze fatty acid composition in erythrocytes (one of the secondary outcomes of the MAPT). Several biomarkers were additionally measured from the blood sample of a sub-population at the 12-month visit, including CRP (n=1097), IL-6 (n=1096), TNFR-1 (n=1097), MCP-1 (n=1097), GDF-15 (n=1096), and ATPase inhibitory factor 1 (IF1) (n=1097).

Regarding functional ability, all participants were assessed for ADL and IADL at baseline for evaluating inclusion/exclusion criteria. Nine hundred eighty participants followed for ADL at 48 and 60 months, and 1286 participants for IADL at 36, 48, and 60 months.

Table 3.1 Variables of interest used in this thesis; time points refer to the visits at which the indicator variable was collected in the MAPT Study

Variable	M0	M6	M12	M24	M36	M48	M60
IC-related indicators							
MMSE			Y	Y	Y	Y	Y
SPPB			Y	Y	Y	Y	Y
GDS			Y	Y	Y	Y	Y
Visual acuity (Monoyer)			Y*	Y*			
HHIE-S			Y*	Y*			
MNA			Y*	Y*			
Handgrip strength			Y	Y	Y	Y	Y
Plasma biomarkers			Y				
Functional outcome							
ADL	Y					Y	Y
IADL	Y				Y	Y	Y
Demographics							
Age	Y**						
Sex	Y						
MAPT group assignment	Y						
Education	Y						
Health status							
Chronic diseases	Y						
Frailty	Y	Y	Y	Y	Y	Y	Y

*Data only collected in the multidomain intervention groups (half MAPT population); **Participants' age was re-calculated based on the date of the 12-month visit (starting point of follow-up) in all analyses of this thesis; M indicates the month and Y indicates yes (data was collected); ADL, activities of daily living; GDS, Geriatric Depression Scale; HHIE-S, Hearing Handicap Inventory for the Elderly – the screening version; IC, intrinsic capacity; IADL, instrumental activities of daily living; MMSE, Mini-Mental State Examination; MNA, Mini Nutritional Assessment; SPPB, Short Physical Performance Battery.

3.2 Study design of the thesis

All studies included in the present thesis have a longitudinal design. We used data from the 12-month visit (the time point of the plasma biomarker assessment) to the 60-month visit of MAPT. All three studies defined the 12-month visit as the starting point of follow-up (referred to as "baseline" in the following text), with a maximum follow-up period of 4 years (**Figure 3.1**).

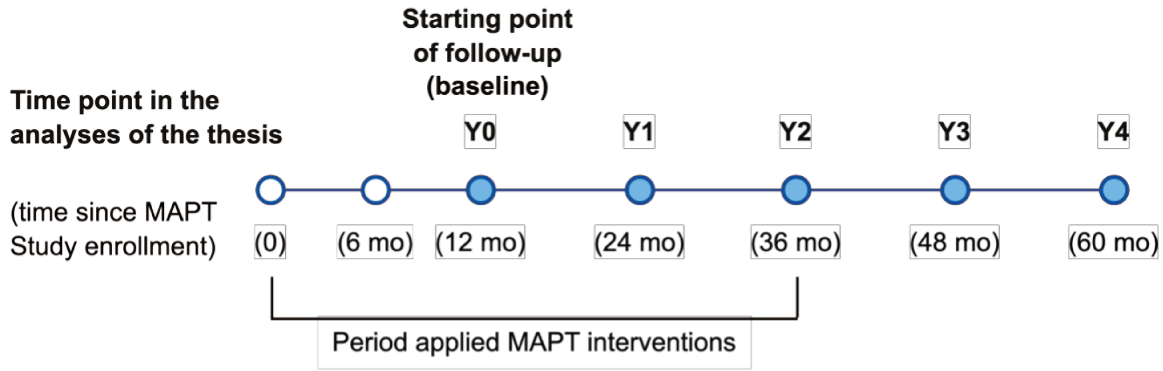

Figure 3.1 Timeline of the MAPT Study and how data were organized for the present thesis; the symbol Y denotes the year.

3.3 Variable measurements

This section describes how the MAPT variables used in this thesis (**Table 3.1**) were measured. Chapter 3 to Chapter 5 will detail the rationale of variable selection in each study.

3.3.1 Plasma biomarkers

C-reactive protein (CRP) concentration was measured by immunoturbidity according to standard protocols (unit: mg/L). Interleukin-6 (IL-6), tumor necrosis factor receptor-1 (TNFR-1), monocyte chemoattractant protein-1 (MCP-1), and growth differentiation factor-15 (GDF-15) were assessed using the fully automated immunoassay platform, Ella (ProteinSimple/Bio-techne, San Jose, CA, USA). Proteins were quantified using a single disposable microfluidic SimplePlexTM cartridge. The plasma samples were thawed on ice, diluted 1:4 in sample diluent

(SD 13), and loaded into cartridges with relevant high and low control concentrates. Each plasma sample was divided into four unique microfluidic parallel channels within the cartridge, specific for each of the four proteins being analyzed. Each protein channel contains three analyte-specific glass nanoreactors (GNRs), allowing each plasma sample to be run in triplicates for each of the four protein samples. Cartridges included a built-in lot-specific standard curve for each defined protein. The instrument automatically conducted all the procedure steps without user activity. The obtained protein concentrations were calculated by the internal instrument software (unit: pg/mL).

ATPase inhibitory factor 1 (IF1) is a crucial molecule involved in mitochondrial biogenesis, and its concentrations was proposed to be a marker of mitochondrial dysfunction[237]. Plasma IF1 levels were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as detailed elsewhere[238]. Briefly, IF1 was quantified in 40- aliquot (EDTA plasma) using trypsin proteolysis and the subsequent analysis of a proteotypic peptide. Intra-assay and inter-assay variability never exceeded 14.2 %.

3.3.2 IC domain indicators

Indicators of each IC domain were determined in reference to the WHO ICOPE Step 2 recommendation and previous IC research. All three studies comprising this thesis used identical measurements for IC domains, except vitality, which was evaluated using three different approaches in Study I to answer the research question.

The cognitive domain was measured by the MMSE[239]. The MMSE is composed of 11 questions, overall 30 items related to the cognitive aspects of mental functions. The MMSE divides into two parts. The first part of MMSE tests temporal and spatial orientation, registration, attention and calculation, and remote memory, which only require a verbal response from a subject. The second part examines the ability to name two objects, follow verbal and written commands,

write a sentence spontaneously, and copy a complex diagram (two crossed pentagons). A summarized score from the two sections ranged from 0 to 30, with a higher value indicating a better cognitive function.

The locomotion was assessed by the SPPB[240]. The SPPB evaluates a subject's gait speed, repeated chair stands, and standing balance performance. For the gait speed, participants were asked to walk 4 meters at their usual pace using walking aids if needed; two walks were performed, and the faster one was recorded. The chair stand test asked participants to stand up from a sitting position with arms folded across their chests and repeat this movement five times as quickly as possible. The time to complete five stands was collected. Balance tests involved three standing positions: tandem, semi-tandem, and side-by-side stands, each held for 10 seconds. Each participant began with a semi-tandem stance, where one foot's heel was placed next to the first toe of the other foot. Participants who failed to maintain a semi-tandem posture for 10 seconds were evaluated with their feet standing side-by-side. Those who passed the semi-tandem test were further assessed with the feet in full-tandem position. For each task, timing stopped when participants lost balance (moved their feet or grasped the interviewer for support) or after 10 seconds. Each task performance was set up with time cut-points and scored a subject on a scale of 0 (the worst performance) to 4 (the best performance). The total SPPB score ranged from 0 to 12, with higher being better.

Psychological domain was examined by the 15-item GDS[241], a screening instrument for depression that was designed exclusively for use with older adults who are more likely to express unique symptoms such as somatic symptoms and cognitive change. The GDS aims to improve the ease of implementation by extracting 15 questions from the long-form GDS-30, covering dysphoric mood, withdrawal and apathy behaviors, anxiety, and cognitive impairment. Of the 15 items, 10 are indicative of depression when answered positively, while the remaining 5 items indicate depression when answered negatively. The total score of GDS ranges from 0

to 15, with lower values meaning lower levels of depressive symptoms.

The sensory domain comprised vision and hearing capacities, which were applied only in the IC measure in Study I and III. Vision capacity was assessed by the distance visual acuity using a Monoyer chart. The Monoyer chart was comprised of 10 rows of letters that decreased in size towards the top; each row represents a different diopter (from 0.1 to 1 in decimal notation). The chart was placed 5 meters away, and participants were asked to isolate each eye and read the letters on each row, staring from the bottom (the largest row), with glasses if needed. Visual acuity for each eye was recorded as a value ranging from 0 (failed to distinguish the largest row) to 10 (correctly discerned the smallest row). The average value of both eyes was used for the analysis. Hearing capacity was evaluated by the Hearing Handicap Inventory for the Elderly – Screening Version (HHIE-S)[242]. The HHIE-S was designed to detect the difficulty and emotional impact of hearing impairment on older adults in daily life activities or social situations. It consisted of 10 questions; each question scored participants 0 points if they perceived no difficulty, 2 if they reported having difficulties sometimes, and 4 if they answered: "yes (having difficulty)." The overall HHIE-S score ranged from 0 to 40; a higher score indicates a more significant hearing handicap.

In this thesis, both nutritional status and handgrip strength were used to assess the vitality domain (detailed explanations are provided in the following chapters). Nutritional status was evaluated by MNA, which comprised 18 items related to anthropometric measurements (BMI, weight loss, mid-arm, and calf circumferences), global assessment (lifestyle, medication use, mobility, and presence of specific diseases), dietary questionnaire (number of meals, consumption of protein, fruit/vegetable, and fluid, the autonomy of feeding and appetite loss), as well as self-perception of health and nutritional conditions. The sum of the MNA score ranged from 0 to 30, with a higher value indicating better nutritional status[243]. The handgrip strength of the participant's dominant hand was measured using a hydraulic hand dynamometer

(Jamar®; Sammons Preston, Bolingbrook, IL, USA). Participants were requested to stand with their arms straight down and fully extended and then hold and squeeze the dynamometer as hard as possible. The maximum value of three attempts was collected (unit: kilograms [kg]).

3.3.3 Functional ability

In Study I, we measured the number of difficulties in ADL and IADL. We used the ADL scale developed by Katz et al., which consisted of six basic activities: bathing, dressing, using the toilet, transferring, continence, and self-feeding[233]; participants were considered as having difficulties in ADL if they were fully dependent on at least one of these activities (cannot perform the activity at all without the help of a third person). IADL was assessed according to the Lawton-Brody IADL Scale, which evaluated an older individual's ability to perform eight complex tasks in their daily lives: telephoning, shopping, food preparation, housekeeping, laundering, use of transportation, managing own medication and finances[232]; those reporting to be incapable of doing at least one of these activities were defined as having difficulties.

As summarized in **Table 3.1**, ADL was evaluated at the study enrollment, 48 and 60 months in the original MAPT study, while IADL was assessed at the study enrollment, 36, 48, and 60 months. Due to the lack of ADL and IADL data at 12 months (the start point of the follow-up of this study), we assumed that our participants maintained the same ADL and IADL performances between the study enrollment and the 12-month visit. It is worth noting that all study participants, as per the inclusion/exclusion criteria of MAPT (section 3.1.1), were fully independent in ADLs (i.e., no difficulty in ADLs) at study enrollment. In other words, all study participants were assumed to be free of ADL difficulty at 12 months in Study I.

3.3.4 Covariates

Age at 12 months was used in a continuous, integral (without decimal) value (unit: year). Sex was coded as a dichotomous variable (female/male). Education was categorized into five levels:

no diploma, primary school certificate, secondary education, high school diploma, and university level. MAPT group allocation was recorded on an intention-to-treat basis (i.e., all participants were labeled as the same group assigned at the study enrollment no matter the length of the follow-up and dropping-out reasons). The number of chronic diseases was measured at the MAPT study enrollment according to the participant-reported diagnosis of the following conditions: asthma/chronic obstructive pulmonary disease, stroke, ischemic heart disease, diabetes, hypertension, hypercholesterolemia, heart failure, and active cancer. These chronic diseases were selected and verified by physicians independent of this thesis considering the high prevalence of these diseases in older adults.

3.4 Ethical considerations

The MAPT Study [no.: NCT00672685] was approved by the French Ethical Committee in Toulouse (CPP SOOM II) and authorized by the French Health Authority. All participants signed informed consent.

CHAPTER 4. STUDY I: Investigating three ways of measuring the intrinsic capacity domain of vitality: nutritional status, handgrip strength, and aging biomarkers.

The elements of this chapter came from a published paper: *Lu WH, Gonzalez-Bautista E, Guyonnet S, Martinez LO, Lucas A, Parini A, Rolland Y, Vellas B, de Souto Barreto P. Investigating three ways of measuring the intrinsic capacity domain of vitality: nutritional status, handgrip strength, and aging biomarkers. Age Ageing. 2023;52(7):afad133.* This original article is provided in the Appendix of this thesis.

4.1 Rationale for this study

Vitality is conceptually considered as a fundamental capacity influencing the manifestations of the other more overtly expressed IC domains – cognition, locomotion, psychological, and vitality (Section 1.5.1). In other words, how vitality contributes to overall functional capacity is partially by modulating other IC domains.

There is a lack of consensus on measuring the IC domain of vitality in the literature (Section 1.6). Several variables had been used to assess the vitality domains, including nutrition and handgrip strength (**Table 1.2**). It is worth highlighting that only a few studies used biomarkers, mainly blood-derived ones, to measure the vitality domain. We further proposed that biomarkers related to aging mechanisms at the biomolecular level can be promising indicators of vitality, considering they reflect homeostatic dysregulation since the subclinical stage.

To our knowledge, no research had used a combination of plasma biomarkers to operationalize vitality. Furthermore, the most appropriate operational definition of vitality remains unknown since no investigation directly compared the performance of different vitality operationalizations, especially using vitality as an underlying domain and examining their association with other IC domains and functional ability.

Considering the knowledge gaps in the literature, this study aimed to investigate the pathways that connect vitality with disability in ADL and IADL, directly and indirectly through the overt IC domains (cognition, locomotion, psychological, sensory [vision and hearing]) in older adults. Three vitality measurements reflecting energy balance (nutritional status), physiological reserve (handgrip strength), and biology of aging (a combination of plasma aging-related biomarkers) were examined. Moreover, we evaluated the association of vitality with longitudinal changes in other IC domains, ADL and IADL.

4.2 Methods

Study source and population

This study is a secondary analysis of MAPT, whose detailed information is provided in Section 3.1. We included 1389 MAPT participants with data on at least one vitality measurement at the 12-month visit (i.e., the baseline of this study). Participants with complete information on three vitality measurements, other IC indicators, and functional outcomes were further included in the path analysis (n=220 for exploring ADL and n=280 for IADL; **Figure 4.1**).

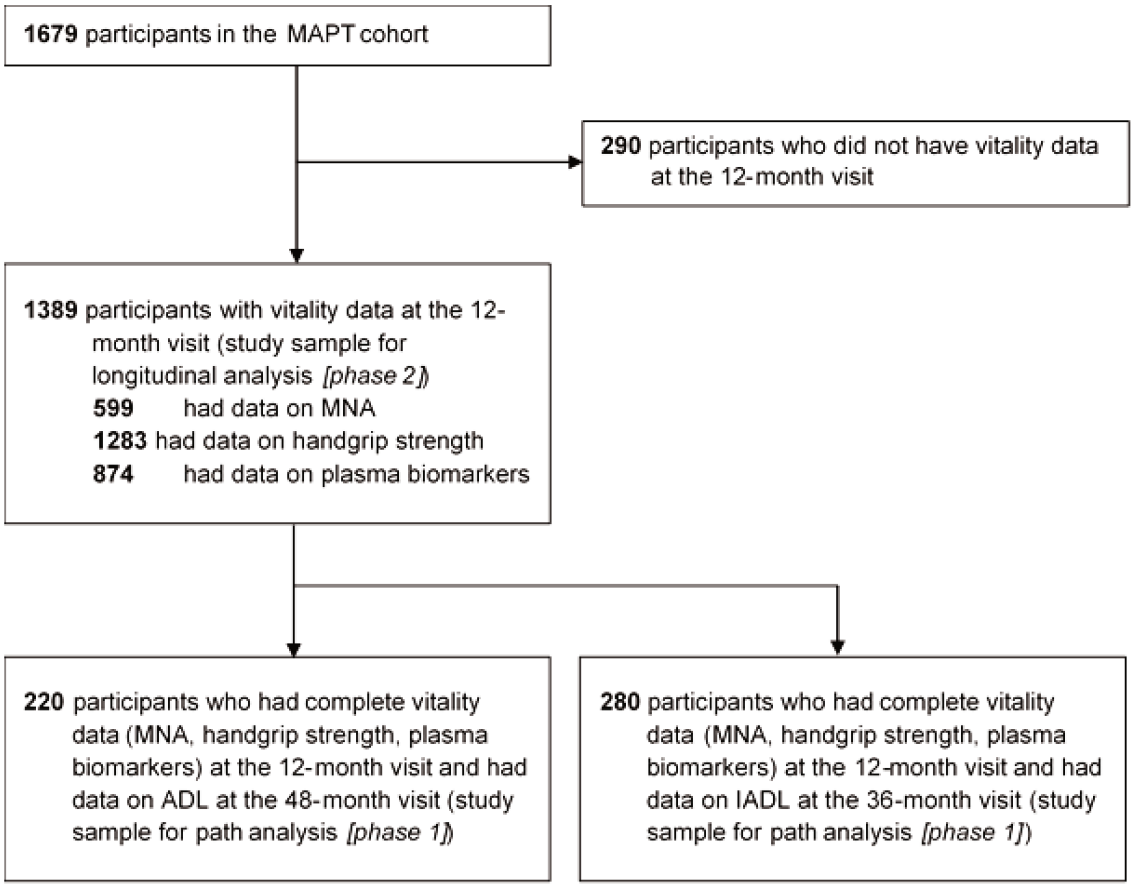

Figure 4.1 Flowchart of cohort identification in Study I

Study design

Study I comprised two phases. Phase 1 explored the structural association between vitality, other IC domains, and functional disabilities. Three definitions of vitality (i.e., MNA, handgrip strength, and plasma biomarkers) and two types of functional difficulties (ADL and IADL) were tested. This phase focused on participants with complete data on three vitality measurements and other IC domain indicators at 12 months and with two measurements of functional disabilities – at 12 and 48 months for ADL (leading to a sample with 220 subjects) or at 12 and 36 months for IADL (leading to a sample with 280 subjects). In phase 2, we examined the longitudinal association of vitality with individual IC domains, ADL and IADL over four years. Three definitions of vitality were tested separately, resulting in different sample sizes in each

analysis (599 subjects for MNA, 1283 for handgrip strength, and 874 for plasma biomarkers) (**Figure 4.1**).

Measurement of vitality

The vitality domain was measured according to three different definitions: (I) nutritional status, evaluated by MNA; (II) handgrip strength; (III) the composite of plasma biomarkers. We selected plasma biomarkers to compose the vitality domain based on data availability and if they met one of the criteria: (1) being related to the hallmarks of aging[19–21] or (2) having been used to measure vitality in prior studies[186,187]. Seven plasma biomarkers were further chosen: hemoglobin, CRP, IL-6, TNFR-1, MCP-1, GDF-15, and ATPase IF1.

Hemoglobin has been applied previously as a vitality indicator of the IC construct[186,187], given that its levels determine the efficiency of the body's energy metabolism. CRP, IL-6, TNFR-1, and MCP-1 are proinflammatory markers and have been explored in various papers on chronic inflammation (Section 1.3.6). In addition, IL-6[244], TNFR-1[245], and MCP-1[246] had been identified as the components of SASP during cellular senescence (another aging hallmark). GDF-15 elevation can be attributed to various stresses linked to the hallmarks of aging, including chronic inflammation, mitochondrial dysfunction, and cellular senescence[64,247]. IF1 is an endogenous inhibitor of ATP synthase that regulates mitochondrial bioenergetics. During the normoxia state (i.e., when the tissue oxygen levels are sufficient and stable), IF1 can inhibit the synthetic activity of ATP synthase and reduce ATP production, leading the mitochondrial energy metabolism toward enhanced aerobic glycolysis, which favors mitochondrial dysfunction[237]. Lower IF1 levels in the circulation indicate higher intracellular concentrations of IF1, which may serve as a marker of mitochondrial impairment.

The detailed measurements for MNA and handgrip strength have been described in Section 3.3.2. All plasma biomarkers were measured according to standard protocols provided in

Section 3.3.1.

Measurement of other IC domains

The cognitive domain was evaluated by the 30-item MMSE. Locomotion was based on the SPPB. The psychological domain was measured by the GDS. Visual acuity of the left and right eyes was examined by a Monoyer chart; the average score of both eyes was applied for the analysis. Hearing capacity was assessed by the HHIE-S. The detailed measurements for MMSE, SPPB, GDS, Monoyer chart and HHIE-S had been described in Section 3.3.

MMSE, SPPB, and GDS were measured annually from 12 to 60 months for the whole population, while visual acuity and HHIE-S assessments were only available at 12 and 24 months for the subjects who received the multidomain intervention (**Table 3.1**).

Measurement of functional ability

The outcomes of interest were the number of difficulties in ADL and IADL, whose detailed components are provided in Section 3.3.3. Participants were considered functional disability if they reported difficulties or were fully dependent on these activities. In the original MAPT Study, ADL was evaluated at the study enrollment, 48 and 60 months, while IADL was assessed at the study enrollment, 36, 48, and 60 months (**Table 3.1**). Due to the lack of ADL and IADL data at 12 months (the baseline in this study), we assumed that our participants maintained the same ADL and IADL performances between the study enrollment and the 12-month visit. It is worth highlighting that all study participants, as per inclusion/exclusion criteria, were fully independent in ADL (i.e., no difficulty in ADL) at study enrollment.

Measurement of other variables

Covariates used for the analyses were age, sex, MAPT group allocation, and education. We also collected information for eight self-reported chronic diseases (Section 3.3.4). Frailty was

assessed based on the Fried frailty criteria[141].

Statistical analysis

As aforementioned, Study I consisted of two phases of analysis. For Phase 1, we applied a statistical technique called SEM, which refers to a family of statistical procedures involving factor analysis and path analysis[248]. SEM involves the construction of a conceptual model(s) to represent the theoretical relationship between the variables of interest, and examining whether the observed data is consistent with this postulated model. Sometimes, multiple models reflecting the research hypothesis can be specified prior. In this case, the SEM technique identifies the model(s) with the best acceptable correspondence to the observed data. When an initial, hypothetical model does not fit the data, the deviations from the estimated values derived from the data can guide modification to generate a better model.

In SEM, variables can be either observed variables (which can be directly observed and collected into the dataset) or latent variables. Latent variables are also known as "hypothetical constructs" or "factors," which reflect a continuum that cannot be directly observed or measured by a single, definitive measurement. SEM also includes residual/error terms of observed and latent variables, representing unexplained variance due to measurement errors or score unreliability.

- ***Phase 1***

In Phase 1, we applied two specific analyses in the family of SEM: CFA and path analysis. CFA is a basic form of SEM that only involves the measurements of latent variables (i.e., measurement models), while path analysis is a causal modeling approach to explore the correlation among observed/latent variables (i.e., path models). Our SEM analysis involved three steps:

First, we hypothesized a biological substrate that represented the overall capacity to maintain homeostasis at the cellular and molecular levels, which was a latent variable measured via multiple plasma biomarkers. Using CFA, we developed a measurement model that included one factor called "biological substrate" and seven plasma biomarkers linked to aging mechanisms as observed variables (**Figure 4.2**). The values of plasma biomarkers were log-transformed, and the outliers (defined as values above or below four SDs from the population mean) were excluded. The model fit and factor loadings of the measurement model were evaluated, and the biomarkers without significant factor loadings to the "biological substrate" factor were removed. This "biological substrate" factor further served as one vitality measurement in the next step of the analysis.

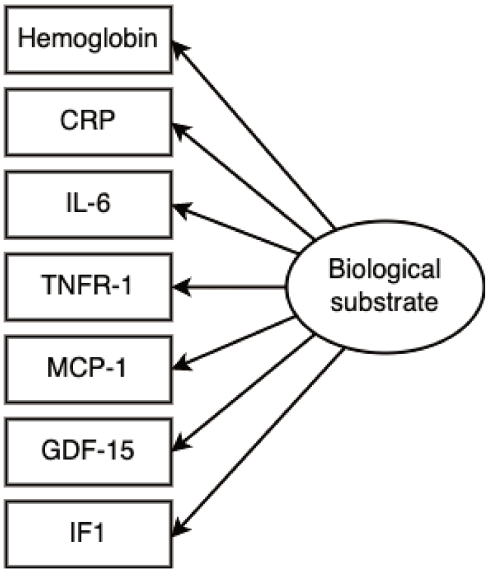

Figure 4.2 Measurement model for biological substrate

In the second step, we hypothesized that decreasing vitality levels could directly contribute to disability or indirectly through the influence on other IC domains. To test this hypothesis, we built path models that included six pathways: one direct effect on changes in ADL /IADL difficulties from the vitality, and five indirect effects with other IC domains (cognition, locomotion, psychology, vision, and hearing) acting as mediators (**Figure 4.3**). Six path

analyses were tested overall since we had three different definitions of vitality (MNA, handgrip strength, and the biological substrate) and two types of functional ability (ADL and IADL).

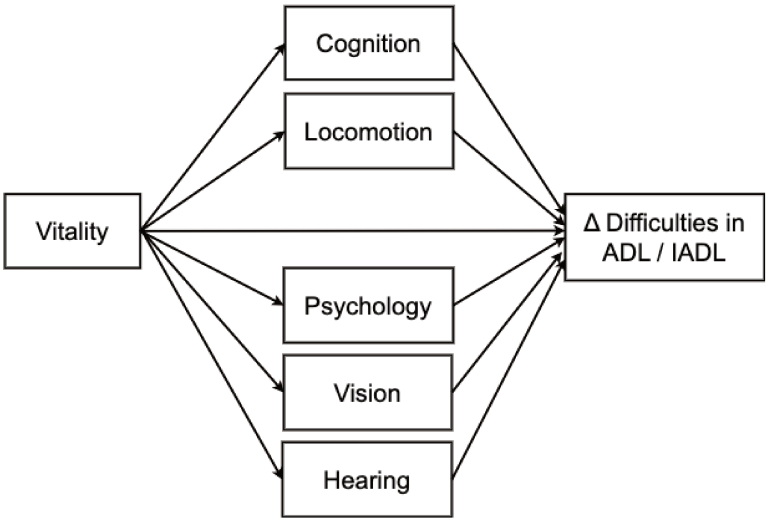

Figure 4.3 Diagram of mediation path analysis

In both steps, the parameters were estimated using maximum likelihood estimation with robust standard errors because some observed variables were not normally distributed. Model fit statistics were performed (1) to assess whether the MAPT Study data support the proposed CFA and path models and (2) to compare whether any of the three path models outperform the others. Several fit indices were reported, including the robust model chi-square (X^2_M) test, root-mean-square error of approximation (RMSEA), the goodness of fit index (GFI), adjusted GFI (AGFI), comparative fit index (CFI), standardized root mean squared residual (SRMR), Akaike information criterion (AIC), and Bayesian information criterion (BIC). We determined the "acceptable" model fit if X^2_M was insignificant, GFI/AGFI ≥ 0.90, CFI ≥ 0.95, RMSEA < 0.08, or SRMR < 0.08[248]. It is important to note that the $\chi 2$ test of significance may be overpowered in large sample sizes. In addition, when X^2_M < the degree of freedom (df_M), one should not evaluate CFI (which will be equal to 1)[248].

In the third step, we created a composite biomarker score (named "bio-vitality index") to

represent the biological substrate of vitality. The weights of each plasma biomarker were derived from the standardized factor loadings of the "biological substrate" (latent variable) in the path analysis on ADL. We referred to the results from ADL rather than IADL because the former is more related to care dependency, the end-point to be prevented in older adults, which is in accordance with the WHO's definition[193] and the WHO ICOPE program. A bio-vitality index derived from IADL analysis was performed as a sensitivity analysis. We summed up the weighted biomarker levels and rescaled the value into a score ranging from 0 to 1 using the proportion of maximum scaling (POMS) method[215]. The largest observed value was defined as 1 (the maximum possible vitality, i.e., best vitality), and the smallest observed value was defined as 0 (the minimum possible vitality, i.e., worst vitality). Other values were transformed into the scores by subtracting the smallest value and dividing by the range. A higher bio-vitality index represented lower overall levels of plasma biomarkers and better vitality capacity. The construct validity of the bio-vitality index was further tested by examining if its differences across age, sex, education, number of chronic diseases, and frailty phenotype; a multivariate linear model was performed with bio-vitality index as a dependent variable and clinical characteristics as independent variables following previous studies' approach[186,187].

- ***Phase 2***

In Phase 2, we used linear mixed-effect regression to investigate how the levels of vitality longitudinally associated with the change of other IC domains and functional difficulties. Each of the three vitality definitions was tested in separate models. Linear mixed-effect regression can model an individual's repeated measurements over time by including the random intercept and the random slope on time. The outcomes were the measurement of other IC domains (cognition, locomotion, psychology, vision, and hearing) and functional difficulties (examined in separate linear mixed-effect models). The mixed models for cognitive, locomotor, and physiological capacities include a random effect at the participant level and a random slope on

time, while the mixed models for vision, hearing, ADL, and IADL include a random effect at the participant level (no random slope on time was used due to the insignificant random effect on time). The independent variables included vitality, time (the year after baseline), the interaction terms between vitality and time, and demographic covariates (age, sex, education, and MAPT group allocation); the coefficients of vitality-time interaction terms described how the degree of outcome change varied with each unit increase in vitality.

- ***Sensitivity analysis***

Theoretically, the IC domains interreact, with one impaired domain that may subsequently induce the deficit in the other ones. However, technically, considering the covariances between two mediators a priori would require estimating additional six parameters for the coefficients of covariances. Under this condition, we would obtain a saturated model (degree of freedom = 0) in the path models examining MNA and handgrip strength, leading to most of the fit indices uninterpretable. Therefore, we first assumed no covariances between four expressed IC domains in the path models (the main analysis). Based on these models, we further evaluated the standardized covariance residual of each path model to see if covariance residuals for the pair of IC domains (mediators) were >2.58[249] (which indicated the correlations between these two domains should not be omitted). Then we reran the path analysis included significant mediator covariance as a sensitivity analysis to see if the main results changed.

For the second sensitivity analysis, we created a bio-vitality index based on the SEM results for IADL and examined if the IADL-based bio-vitality index provided similar construct validity and longitudinal association with outcomes as the ADL-based index.

4.3 Results

Characteristics of the study population

Among the overall study population (n=1389), the mean ± SD age was 76.2 ± 4.4 years, with 63.8% being women. The characteristics of participants at the 12-month visit (the baseline of this study) included in Phase 1 (path analysis) and Phase 2 (longitudinal analysis) were presented in **Table 4.1**.

Table 4.1 Characteristics at the 12-month visit of the study participants included in the longitudinal and path analyses

	Study sample for longitudinal analysis (n=1389)	Study sample for path analysis	
		ADL (n=220)	IADL (n=280)
Age, *years*	76.2 (4.4)	75.4 (4.0)	75.9 (4.3)
Female	886 (63.8%)	145 (65.9%)	177 (63.2%)
MAPT group			
Omega-3 + MI	353 (25.4%)	106 (48.2%)	141 (50.4%)
Omega-3	337 (24.3%)	0 (0%)	0 (0%)
MI	351 (25.3%)	114 (51.8%)	139 (49.6%)
Placebo	348 (25.1%)	0 (0%)	0 (0%)
Education (n=1371)			
No diploma	66 (4.8%)	7 (3.2%)	9 (3.2%)
Primary school certificate	232 (16.9%)	29 (13.3%)	46 (16.6%)
Secondary education	465 (33.9%)	73 (33.5%)	92 (33.1%)
High school diploma	203 (14.8%)	29 (13.3%)	40 (14.4%)
University level	405 (29.5%)	80 (36.7%)	91 (32.7%)
Multimorbidity [a]			
None	398 (28.7%)	78 (35.5%)	95 (33.9%)
1 chronic disease	535 (38.5%)	76 (34.6%)	100 (35.7%)
2 chronic diseases	340 (24.5%)	54 (24.6%)	68 (24.3%)
≥3 chronic diseases	116 (8.4%)	12 (5.5%)	17 (6.1%)
Frailty (n=1243)			
Robust	658 (52.9%)	128 (59.8%)	161 (59.0%)
Prefrail	545 (43.9%)	82 (38.3%)	106 (38.8%)
Frail	40 (3.2%)	4 (1.9%)	6 (2.2%)
IC indicators			
Cognition: MMSE, *0-30* (n=1376)	28.0 (1.9)	28.4 (1.6)	28.2 (1.7)
Locomotion: SPPB, *0-12* (n=1359)	10.6 (1.8)	10.8 (1.6)	10.8 (1.7)
Psychological: GDS, *0-15* (n=1370)	3.1 (2.6)	2.7 (2.3)	2.8 (2.5)
Vision: Monoyer score, *0-10* (n=571)	6.9 (2.2)	7.0 (2.0)	7.0 (2.0)
Hearing: HHIE-S, *0-40* (n=599)	7.0 (7.9)	6.8 (7.8)	6.8 (7.8)

Vitality:			
MNA, *0-30* (n=599)	27.5 (2.0)	27.9 (1.5)	27.8 (1.8)
Handgrip strength, *kg* (n=1283)	26.7 (9.8)	26.8 (9.6)	27.4 (9.9)
Plasma biomarkers			
CRP, *mg/L* (n=1090)	3.3 (5.2)	2.8 (3.4)	2.8 (3.4)
IL-6, *pg/mL* (n=1087)	3.9 (12.2)	3.1 (2.0)	3.1 (2.0)
TNFR-1, *pg/mL* (n=1088)	1225.5 (440.9)	1141.7 (288.5)	1165.1 (306.3)
MCP-1, *pg/mL* (n=1088)	221.7 (85.7)	216.9 (73.4)	218.9 (75.9)
GDF-15, *pg/mL* (n=1087)	1124.4 (501.4)	1031.3 (384.0)	1062.8 (404.8)

Data presented in this table was retrieved from the 12-month visit of the MAPT study; values are presented in frequency (percentage) for categorical variables or mean (standard deviation) for continuous variables. ADL, activities of daily living; CRP, C-reactive protein; GDF-15, Growth differentiation factor-15; GDS, Geriatric Depression Scale; HHIE-S, Hearing Handicap Inventory for the Elderly – the screening version; IADL, instrumental activities of daily living; IC, intrinsic capacity; IL-6, interleukin-6; MAPT, Multidomain Alzheimer Preventive Trial; MCP-1, Monocyte chemoattractant protein-1; MI, multidomain intervention; MMSE, Mini-Mental State Examination; MNA, Mini Nutritional Assessment; SPPB, Short Physical Performance Battery; TNFR-1, Tumor necrosis factor receptor-1.

[a] Chronic disease included asthma/chronic obstructive pulmonary disease, stroke, ischemic heart disease, diabetes, hypertension, hypercholesterolemia, heart failure and active cancer.

Baseline characteristics between MAPT participants included in each analysis and those not included were compared; subjects with MNA were relatively younger, and subjects with plasma biomarker data had higher educational levels. No significant difference in characteristics was found in other study samples.

Confirmatory factor analysis (CFA) for plasma biomarkers

The CFA in Study I showed that CRP, IL-6, TNFR-1, MCP-1, and GDF-15 were statistically correlated with the latent variable "biological substrate," whose standardized factor loadings ranged from 0.27 to 0.78. On the other hand, the standardized factor loadings of IF1 and hemoglobin were insignificant and lower than 0.10, suggesting these two biomarkers contributed little information to the latent variable (**Table 4.2**). Thus, we did not include IF1

and hemoglobin in the following path analysis. The model fit indices before and after excluding IF1 and hemoglobin did not change significantly (**Table 4.2**).

Table 4.2 Confirmatory factor analysis for biological substrate

Plasma biomarkers	Model 1		Model 2	
	Standardized factor loadings (SE)	p	Standardized factor loadings (SE)	p
CRP	0.27 (0.06)	<0.001	0.26 (0.06)	<0.001
IL-6	0.55 (0.05)	<0.001	0.55 (0.05)	<0.001
TNFR-1	0.78 (0.05)	<0.001	0.77 (0.05)	<0.001
MCP-1	0.37 (0.07)	<0.001	0.37 (0.07)	<0.001
GDF-15	0.74 (0.04)	<0.001	0.75 (0.04)	<0.001
ATPase IF1	-0.07 (0.07)	0.320	-	-
Hemoglobin	0.09 (0.07)	0.182	-	-
Model fit statistics				
Robust Model $\chi 2$	58.861		48.293	
Robust $\chi 2$ df	14		5	
Robust $\chi 2$ p-value	<0.001		<0.001	
RMSEA (90% CI)	0.104 (0.078, 0.133)		0.172 (0.130, 0.217)	
GFI	0.946		0.941	
AGFI	0.893		0.824	
CFI	0.833		0.840	
SRMR	0.068		0.079	
AIC	86.861		68.293	
BIC	138.479		105.163	

Model 1 is a one-factor CFA model that included seven plasma biomarkers as indicators; model 2 is a one-factor CFA model that included five plasma biomarkers as indicators; AIC, Akaike information criterion; AGFI, adjusted goodness of fit index; BIC, Bayesian information criterion; CI, confidence interval; CFI, comparative fit index; CRP, C-reactive protein; df, degrees of freedom; GDF-15, Growth differentiation factor-15; GFI, goodness of fit index; IF1, Inhibitory Factor 1; IL-6, interleukin-6; MCP-1, Monocyte chemoattractant protein-1; SE, standard error; RMSEA, root mean squared error of approximation; SRMR, standardized root mean squared residual; TNFR-1, Tumor necrosis factor receptor-1.

Path analysis for vitality, other IC domains, and functional difficulties

Figure 4.4 illustrates the path diagrams from vitality to ADL/IADL difficulties; vitality was measured by MNA score (**Figure a and d**), handgrip strength (**Figure b and e**), and the "biological substrate" loaded by five plasma biomarkers (**Figure c and f**), respectively. **Table 4.3** displays the model fit statistics. Path models using three vitality definitions demonstrated similar and adequate model fits with SRMR around 0.08, implying that none of the three vitality measurements outperformed the others.

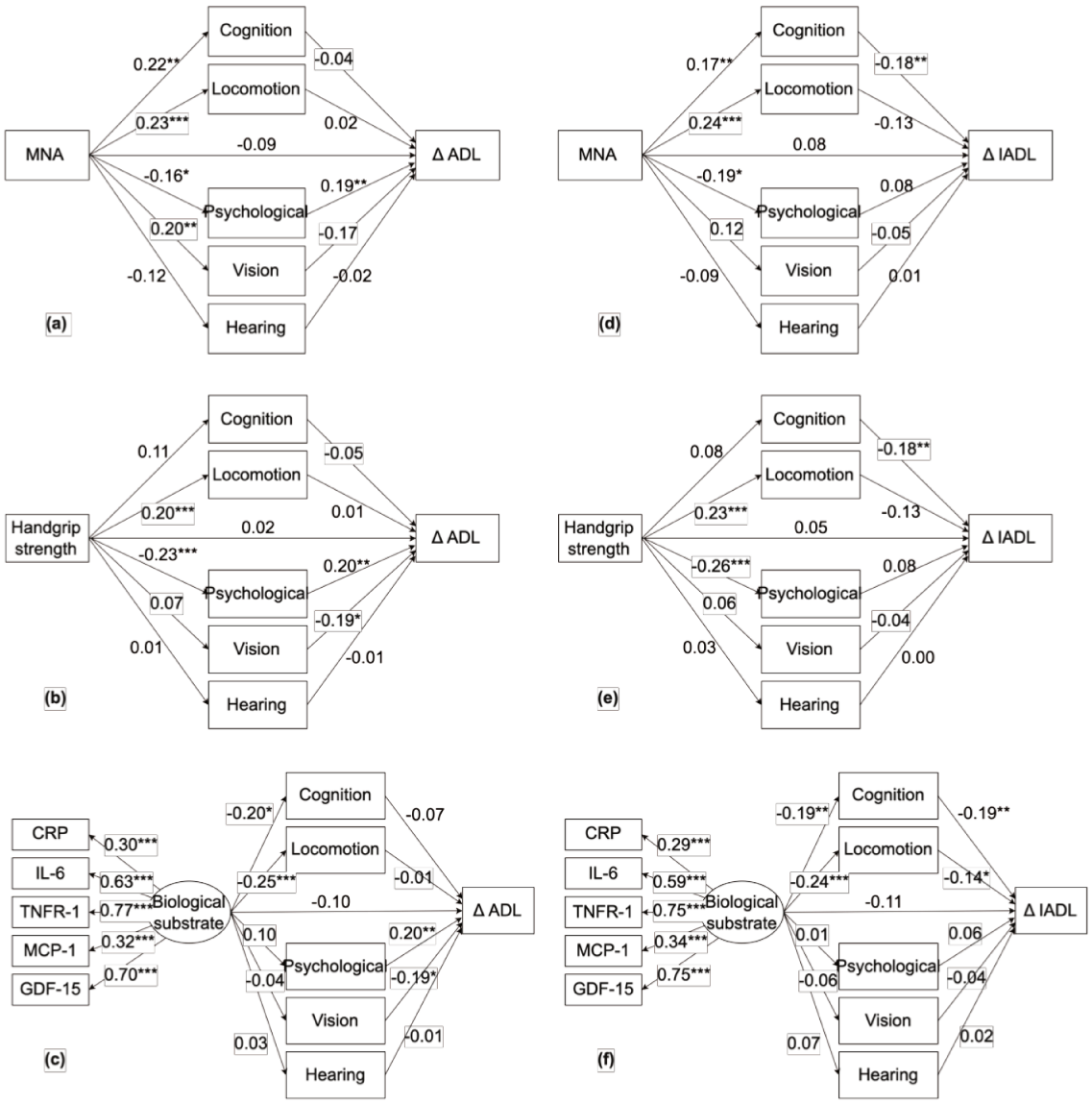

Figure 4.4 Direct and indirect effects of vitality on change in ADL and IADL difficulties

a, b, c: path models for ADL difficulties. d, e, f: for IADL difficulties. *p< .05; **p < .01; ***p < .001.

Table 4.3 Fit statistics from the path models

	Vitality measurement		
	MNA score	**Handgrip strength**	**Plasma biomarkers**
Outcome: ΔADL difficulties			
Robust Model χ2	31.857	34.722	106.005
Robust χ2 df	10	10	39
Robust χ2 p-value	<0.001	<0.001	<0.001
RMSEA (90% CI)	0.100 (0.062, 0.140)	0.106 (0.069, 0.146)	0.089 (0.069, 0.109)
GFI	0.951	0.943	0.907
AGFI	0.862	0.841	0.842
CFI	0.579	0.461	0.712
SRMR	0.079	0.087	0.081
AIC	67.857	70.722	160.005
BIC	128.943	131.808	251.633
Outcome: ΔIADL difficulties			
Robust Model χ2	31.617	36.322	107.591
Robust χ2 df	10	10	39
Robust χ2 p-value	<0.001	<0.001	<0.001
RMSEA (90% CI)	0.088 (0.055, 0.124)	0.097 (0.064, 0.132)	0.079 (0.062, 0.098)
GFI	0.959	0.953	0.924
AGFI	0.886	0.868	0.871
CFI	0.573	0.577	0.749
SRMR	0.073	0.078	0.074
AIC	67.617	72.322	161.591
BIC	133.043	137.748	259.731

ADL, activities of daily living; AIC, Akaike information criterion; AGFI, adjusted goodness of fit index; BIC, Bayesian information criterion; CI, confidence interval; CFI, comparative fit index; df, degrees of freedom; GFI, goodness of fit index; IADL, instrumental activities of daily living; MNA, Mini Nutritional Assessment; RMSEA, root mean squared error of approximation; SRMR, standardized root mean squared residual.

The total, direct and indirect standardized effects from vitality to change in ADL and IADL are summarized in **Table 4.4**. Only MNA showed a marginally significant total effect on ΔADL (standardized coefficient [standard error] = -0.152 [0.074]; p=0.040); however, the direct and total indirect effects were insignificant. All three vitality measurements showed significant indirect effects on ΔIADL (**Table 4.4**), mainly through cognitive, psychological, and locomotor domains (**Figure 4.4**).

The results of the path models considered significant covariances between other IC domains are displayed in **Table 4.5 and 4.6**; the overall model fit was improved after considering important mediator covariance, but the indirect effects of vitality on ΔIADL remain unchanged. All models showed improved model fit after considering important mediator covariance; the indirect effects of vitality on ΔIADL remained unchanged.

Table 4.4 Total, direct and indirect standardized effects from vitality to change in ADL and IADL

	Vitality measurement					
	MNA score		**Handgrip strength**		**Plasma biomarkers**	
	Standardized coefficient (SE)	**p**	**Standardized coefficient (SE)**	**p**	**Standardized coefficient (SE)**	**p**
Outcome: ΔADL difficulties						
Total effect	-0.152 (0.074)	0.040	-0.042 (0.049)	0.392	-0.058 (0.057)	0.303
Total indirect effect	-0.067 (0.044)	0.133	-0.064 (0.035)	0.069	0.043 (0.041)	0.300
Direct effect	-0.085 (0.074)	0.250	0.021 (0.050)	0.670	-0.101 (0.068)	0.136
Outcome: ΔIADL difficulties						
Total effect	-0.004 (0.059)	0.941	-0.014 (0.058)	0.814	-0.032 (0.095)	0.737
Total indirect effect	-0.085 (0.025)	0.001	-0.068 (0.027)	0.011	0.077 (0.033)	0.021
Direct effect	0.081 (0.071)	0.255	0.054 (0.050)	0.278	-0.108 (0.093)	0.242

ADL, activities of daily living; IADL, instrumental activities of daily living; MNA, Mini Nutritional Assessment; SE, standard error.

Table 4.5 Modified path model included significant mediator covariance (outcome: change in ADL difficulties)

	Vitality measurement					
	MNA score		**Handgrip strength**		**Plasma biomarkers**	
Outcome: ΔADL difficulties	**Standardized coefficient (SE)**	**p**	**Standardized coefficient (SE)**	**p**	**Standardized coefficient (SE)**	**p**
Total effect	-0.152 (0.074)	0.040	-0.042 (0.049)	0.392	-0.069 (0.055)	0.215
Total indirect effect	-0.085 (0.071)	0.228	-0.064 (0.029)	0.028	0.031 (0.034)	0.365
Direct effect	-0.067 (0.037)	0.071	0.021 (0.048)	0.653	-0.100 (0.059)	0.093
Vitality to IC domains						
Vitality → Cognitive	0.220 (0.074)	0.003	0.109 (0.062)	0.077	-0.175 (0.081)	0.031
Vitality → Locomotor	0.234 (0.060)	<0.001	0.195 (0.053)	<0.001	-0.224 (0.073)	0.002
Vitality → Psychological	-0.161 (0.067)	0.017	-0.228 (0.065)	<0.001	0.059 (0.092)	0.523
Vitality → Vision	0.203 (0.062)	0.001	0.068 (0.064)	0.289	-0.030 (0.071)	0.669
Vitality → Hearing	-0.122 (0.069)	0.075	0.006 (0.074)	0.933	0.027 (0.085)	0.749
IC domain to ΔADL						
Cognitive → ΔADL	-0.038 (0.096)	0.691	-0.052 (0.100)	0.601	-0.065 (0.102)	0.523
Locomotor → ΔADL	0.024 (0.079)	0.758	0.006 (0.077)	0.942	-0.011 (0.073)	0.877
Psychological → ΔADL	0.195 (0.076)	0.010	0.202 (0.075)	0.007	0.195 (0.074)	0.008
Vision → ΔADL	-0.173 (0.092)	0.060	-0.189 (0.094)	0.044	-0.190 (0.092)	0.040
Hearing → ΔADL	-0.021 (0.065)	0.750	-0.013 (0.067)	0.848	-0.008 (0.064)	0.896
Covariance between IC domains						
Cognitive ⇔ Locomotor	0.175 (0.068)	0.011	0.205 (0.065)	0.002	0.187 (0.069)	0.007

	Vitality measurement					
	MNA score		Handgrip strength		Plasma biomarkers	
Outcome: ΔADL difficulties	Standardized coefficient (SE)	p	Standardized coefficient (SE)	p	Standardized coefficient (SE)	p
Cognitive ⇔ Psychological	-0.207 (0.064)	0.001	-0.212 (0.069)	0.002	-0.228 (0.070)	0.001
Locomotor ⇔ Psychological	-0.205 (0.054)	<0.001	-0.192 (0.053)	<0.001	-0.225 (0.056)	<0.001
Psychological ⇔ Hearing	0.218 (0.073)	0.003	0.229 (0.073)	0.002	0.228 (0.073)	0.002
Model fit statistics						
Robust Model χ2	2.539		3.152		70.242	
Robust χ2 df	6		6		35	
Robust χ2 p-value	0.864		0.790		<0.001	
RMSEA (90% CI)	0 (0, 0.046)		0 (0, 0.058)		0.068 (0.045, 0.091)	
GFI	0.996		0.995		0.939	
AGFI	0.982		0.977		0.885	
CFI	1.000		1.000		0.849	
SRMR	0.023		0.029		0.062	
AIC	46.539		47.152		132.242	
BIC	121.198		121.812		237.444	

ADL, activities of daily living; AIC, Akaike information criterion; AGFI, adjusted goodness of fit index; BIC, Bayesian information criterion; CFI, comparative fit index; df, degrees of freedom; GFI, goodness of fit index; IC, intrinsic capacity; MNA, Mini Nutritional Assessment; RMSEA, root mean squared error of approximation; SE, standard error; SRMR, standardized root mean squared residual.

Table 4.6 Modified path model included significant mediator covariance (outcome: change in IADL difficulties)

	Vitality measurement					
	MNA score		**Handgrip strength**		**Plasma biomarkers**	
Outcome: ΔIADL difficulties	**Standardized coefficient (SE)**	**p**	**Standardized coefficient (SE)**	**p**	**Standardized coefficient (SE)**	**p**
Total effect	-0.004 (0.059)	0.941	-0.014 (0.058)	0.814	-0.038 (0.094)	0.684
Total indirect effect	-0.084 (0.022)	<0.001	-0.068 (0.024)	0.004	0.069 (0.031)	0.027
Direct effect	0.080 (0.068)	0.242	0.054 (0.049)	0.272	-0.107 (0.091)	0.242
Vitality to IC domains						
Vitality → Cognitive	0.166 (0.062)	0.008	0.082 (0.056)	0.143	-0.174 (0.066)	0.009
Vitality → Locomotor	0.240 (0.069)	<0.001	0.230 (0.046)	<0.001	-0.230 (0.063)	<0.001
Vitality → Psychological	-0.191 (0.079)	0.015	-0.261 (0.055)	<0.001	-0.009 (0.074)	0.906
Vitality → Vision	0.117 (0.069)	0.091	0.055 (0.059)	0.347	-0.056 (0.067)	0.401
Vitality → Hearing	-0.086 (0.065)	0.186	0.031 (0.063)	0.619	0.067 (0.076)	0.379
IC domain to ΔIADL						
Cognitive → ΔIADL	-0.184 (0.069)	0.008	-0.177 (0.068)	0.009	-0.191 (0.068)	0.005
Locomotor → ΔIADL	-0.133 (0.074)	0.075	-0.127 (0.069)	0.065	-0.141 (0.065)	0.030
Psychological → ΔIADL	0.080 (0.063)	0.200	0.083 (0.061)	0.174	0.061 (0.065)	0.346
Vision → ΔIADL	-0.047 (0.076)	0.532	-0.042 (0.078)	0.584	-0.044 (0.079)	0.576
Hearing → ΔIADL	0.013 (0.062)	0.833	0.004 (0.059)	0.944	0.017 (0.064)	0.792
Covariance between IC domains						
Cognitive ⇔ Locomotor	0.177 (0.067)	0.009	0.199 (0.064)	0.002	0.166 (0.063)	0.008

	Vitality measurement					
	MNA score		Handgrip strength		Plasma biomarkers	
Outcome: ΔIADL difficulties	Standardized coefficient (SE)	p	Standardized coefficient (SE)	p	Standardized coefficient (SE)	p
Locomotor ⇔ Psychological	-0.157 (0.049)	0.001	-0.135 (0.050)	0.007	-0.201 (0.055)	<0.001
Psychological ⇔ Hearing	0.174 (0.064)	0.007	0.194 (0.063)	0.002	0.191 (0.065)	0.003
Model fit statistics						
Robust Model χ2	9.132		10.750		78.503	
Robust χ2 df	7		7		36	
Robust χ2 p-value	0.243		0.150		<0.001	
RMSEA (90% CI)	0.033 (0, 0.085)		0.044 (0, 0.093)		0.065 (0.045, 0.085)	
GFI	0.989		0.986		0.945	
AGFI	0.955		0.946		0.900	
CFI	0.958		0.940		0.845	
SRMR	0.040		0.045		0.060	
AIC	51.132		52.750		138.503	
BIC	127.462		129.081		247.547	

AIC, Akaike information criterion; AGFI, adjusted goodness of fit index; BIC, Bayesian information criterion; CFI, comparative fit index; df, degrees of freedom; GFI, goodness of fit index; IADL, instrumental activities of daily living; IC, intrinsic capacity; MNA, Mini Nutritional Assessment; RMSEA, root mean squared error of approximation; SE, standard error; SRMR, standardized root mean squared residual.

Construct validity of the bio-vitality index

We calculated a bio-vitality index for the study sample of longitudinal analysis based on the weights derived from ADL path analysis (**Table 4.7**). A higher bio-vitality index indicates a better vitality capacity. Among these participants, a lower bio-vitality index was associated with increasing age, having ≥3 chronic diseases compared to no chronic disease, and being prefrail and frail compared to robust. Females tended to have a higher bio-vitality index than males (**Figure 4.5**). In the sensitivity analysis, the IADL-based bio-vitality index showed similar construct validity.

Table 4.7 Weighting coefficients of plasma biomarkers for bio-vitality index

Variable	Standardized factor loadings from the ADL path analysis	Weight
Log(CRP)	0.30	-0.30
Log(IL-6)	0.63	-0.63
Log(TNFR-1)	0.77	-0.77
Log(MCP-1)	0.32	-0.32
Log(GDF-15)	0.70	-0.70

ADL, activities of daily living; CRP, C-reactive protein; GDF-15, Growth differentiation factor-15; IL-6, interleukin-6; MCP-1, Monocyte chemoattractant protein-1; TNFR-1, Tumor necrosis factor receptor-1.

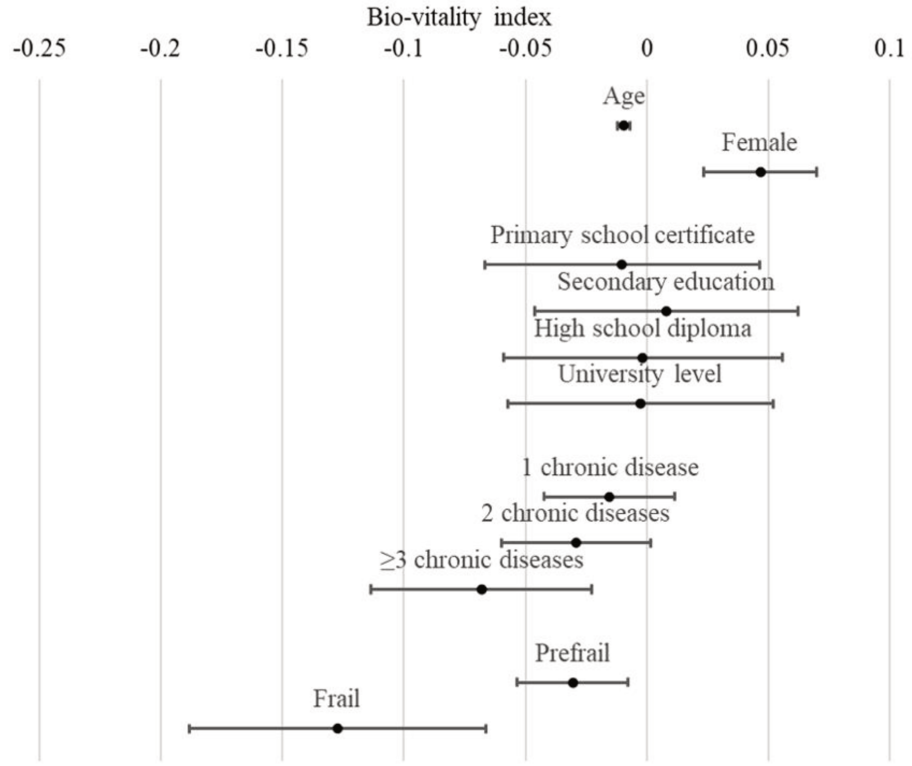

Figure 4.5 Construct validity of the bio-vitality index

Results from the multivariate linear regression with clinical characteristics as the independent variables and bio-vitality index as the dependent variable. Black dots and horizontal bars represent the coefficients (β) and confidence intervals (CIs).

Variation in overtly expressed IC domains, ADL, and IADL according to vitality measurements

Using linear mixed-effect regression, we found that a higher bio-vitality index (β [95% CI] = 0.050 [0.010, 0.090]; p=0.015) was associated with improved locomotor capacity over time (increased SPPB score), after adjusting for age, sex, education, and MAPT group allocation. In contrast, neither MNA nor handgrip strength was longitudinally associated with other IC domains (**Table 4.8**). In sensitivity analysis, the association of the bio-vitality index with the changes in locomotion remains significant by using the IADL-based index (β [95% CI] = 0.051 [0.011, 0.091]; p=0.012).

Table 4.8 Linear mixed-effect regression examining longitudinal association of vitality with other IC domains, ADL and IADL

	Vitality measurement								
	MNA score (N=599)			Handgrip strength (N=1283)			Bio-vitality index (N=874)		
	n	β (95% CI)	p	n	β (95% CI)	p	n	β (95% CI)	p
Outcome: other IC domains									
Cognition: MMSE	590	-0.015 (-0.061, 0.032)	0.535	1258	0.004 (-0.025, 0.033)	0.799	855	-0.023 (-0.058, 0.010)	0.173
Locomotion: SPPB	591	0.037 (-0.013, 0.086)	0.143	1264	0.018 (-0.013, 0.049)	0.262	855	0.050 (0.010, 0.090)	0.015
Psychological: GDS	590	-0.009 (-0.066, 0.049)	0.771	1257	0.027 (-0.010, 0.064)	0.154	855	-0.030 (-0.075, 0.015)	0.190
Vision: Monoyer score	584	0.035 (-0.132, 0.202)	0.680	570	-0.091 (-0.266, 0.084)	0.307	381	0.035 (-0.143, 0.214)	0.698
Hearing: HHIE-S	593	-0.100 (-0.544, 0.345)	0.660	579	0.189 (-0.284, 0.663)	0.433	390	0.138 (-0.412, 0.689)	0.622
Outcome: functional difficulties									
ADL difficulties	595	-0.017 (-0.026, -0.008)	<0.001	1267	-0.008 (-0.014, -0.002)	0.009	859	-0.004 (-0.011, 0.003)	0.271
IADL difficulties	595	-0.020 (-0.037, -0.003)	0.018	1267	-0.011 (-0.023, 0.000)	0.047	859	-0.015 (-0.028, -0.002)	0.027

All models were adjusted for age, sex, education, and MAPT group allocation; the coefficient (β) indicates the annual change from 12 months in each outcome (IC domain, ADL, or IADL) per SD increment in vitality measurement; higher MMSE, SPPB, and Monoyer values indicate better function, while higher GDS, HHIE-S, ADL difficulties and IADL difficulties indicate worse function; ADL, activities of daily living; CI, confidence interval; GDS, Geriatric Depression Scale; HHIE-S, Hearing Handicap Inventory for the Elderly – the screening version; IC, intrinsic capacity; IADL, instrumental activities of daily living; MMSE, Mini-Mental State Examination; MNA, Mini Nutritional Assessment; SPPB, Short Physical Performance Battery.

According to the results from linear mixed-effect regression, lower vitality capacity was significantly associated with increased ADL difficulties over time when vitality was measured as MNA or handgrip strength: *MNA score:* β [95% CI] = -0.017 [-0.026, -0.008]; p<0.001; *handgrip strength:* -0.008 [-0.014, -0.002]; p=0.009. On the other hand, significant associations existed between better vitality and decreased IADL difficulties across all vitality measurements: *MNA score:* β [95% CI] = -0.020 [-0.037, -0.003]; p=0.018; *handgrip strength:* -0.011 [-0.023, 0.000]; p=0.047; *bio-vitality index:* -0.015 [-0.028, -0.002]; p=0.027) (**Table 4.8**). In sensitivity analysis, the association of the bio-vitality index with the changes in IADL remains significant by using the IADL-based index (β [95% CI] = -0.015 [-0.029, -0.002]; p=0.022).

4.4 Interpretation of main findings

This work showed that the vitality domain of IC was indirectly associated with IADL when using all three operational definitions: MNA, handgrip strength, and a combination of plasma biomarkers. None of the vitality measurements fit the MAPT data better than the other. For longitudinal analysis, participants with better vitality demonstrated fewer IADL difficulties over the follow-up across all vitality definitions. Higher vitality was associated with a lower decline in ADL when operationalizing vitality as MNA and handgrip strength. Finally, higher vitality assessed with plasma biomarkers was associated with increased locomotion over time.

The vitality framework proposed in Beard et al.'s study[186,187] is supported by our path analysis, which showed that other IC domains mediated the association between vitality and change in IADL. The reasons we did not obtain the same results for ADL could be related to the smaller sample size and relatively fit population, with unchanged ADL performance for most participants (i.e., 95.5%) between 12 and 48 months. Regardless of the measurement, vitality showed no direct effect on ADL and IADL difficulties. Taken together, our data suggest that vitality is associated with disability through its potential influences on other IC domains.

Our study found that none of the vitality measurements fitted the MAPT data better than the other. This result suggests that each vitality operationalization only partially reflected the aggregate of biological mechanisms involved in homeostasis regulation. The advantage of using plasma biomarkers over the nutritional assessment or handgrip strength for vitality is that the latter still relies on clinical manifestation. In other words, abnormal plasma biomarker levels might indicate subclinical dysregulation in the body even before symptoms of malnutrition (e.g., loss of appetite, involuntary weight loss) and muscle weakness appear. Indeed, in this study, only the bio-vitality index predicted the variation in expressed IC domain (i.e., locomotion), indicating that biomarkers can identify individuals at risk of locomotion decline several years in advance.

Interestingly, all plasma biomarkers retained in the bio-vitality index are related to inflammation, which is also an underlying mechanism of malnutrition[250] and low muscle strength[251]. Thus, despite all these measurements being closely related to homeostasis, we might say that plasma biomarkers describe the biomolecular (intracellular/intercellular) changes, while nutritional status and handgrip strength reflect variations at the physiologic level (organs/systems). Further exploration of the application of plasma biomarkers for quantifying vitality capacity is provided in the integrative discussion chapter.

4.5 Strengths and limitations

This is the first study to test the hierarchical IC structure that vitality underlies all other capacities and contributes to disability through multiple pathways. We compared three vitality approaches in a sample of community-dwelling older people and provided evidence for both structure and magnitude of the cross-sectional and longitudinal association.

Some limitations should be noted. First, this is a secondary analysis of a randomized controlled study that enrolled older individuals with either spontaneous memory complaints or limitation

in one IADL or slow walking speed. Furthermore, due to the data available in the MAPT Study, we used different sample sizes to explore vitality definitions. It is worth mentioning that, in some analyses, study subjects had slightly different characteristics compared to the rest of the MAPT population. Finally, this study only included plasma biomarkers related to inflammation and energy metabolism.

CHAPTER 5. STUDY II: Association between aging-related biomarkers and longitudinal trajectories of intrinsic capacity in older adults.

The elements of this chapter came from a published paper: *Lu WH, Guyonnet S, Martinez LO, Lucas A, Parini A, Vellas B, de Souto Barreto P. Association between aging-related biomarkers and longitudinal trajectories of intrinsic capacity in older adults. Geroscience. 2023;45(6):3409-18.* This original article is provided in the Appendix of this thesis.

5.1 Rationale for this study

Previous literature suggests that IC is a dynamic construct that varies across the lifespan, becoming lower and fast declining at an advanced age (Section 1.5.3). As mentioned in Section 1.5.3, two studies from Mexico[208] and Taiwan[220] identified at least three IC trajectories among the middle-aged and older populations. In their works, people with worse IC trajectories tended to be older, more female, with lower educational levels, single/widowed, physically inactive, current smokers or alcohol consumers, and suffer from chronic diseases. However, it was unknown if IC trajectories have any biological risk factors.

When we started this thesis, no study had investigated IC trajectories exclusively on community-dwelling older adults and considered the joint evolution of different domains (Section 1.6). Therefore, this study aimed to identify longitudinal IC trajectories over four years in a cohort of community-dwelling older individuals using GBTM approach. The multi-trajectories considered the joint evolution of four IC domains – cognition, locomotion, psychology, and vitality – were investigated as the primary outcome. The trajectories of global IC, a summarized score of four IC domains, were also examined as an exploratory outcome. We further evaluated if the identified IC trajectories were associated with plasma biomarkers representing key biological aging mechanisms, mainly inflammation and mitochondrial dysfunction. We hypothesized that distinct IC trajectories existed among older individuals, and

people with impaired IC trajectories were more likely to exhibit abnormal levels of plasma biomarkers.

5.2 Methods

Study source and population

This study used data from the MAPT Study. Among 1679 MAPT participants, we excluded 279 subjects without IC data during the 4-year follow-up. Another 129 subjects who only had one time point of IC data in the follow-up period were excluded because it is incapable of identifying their longitudinal trends of IC. Finally, 1271 participants were included in the analysis (**Figure 5.1**).

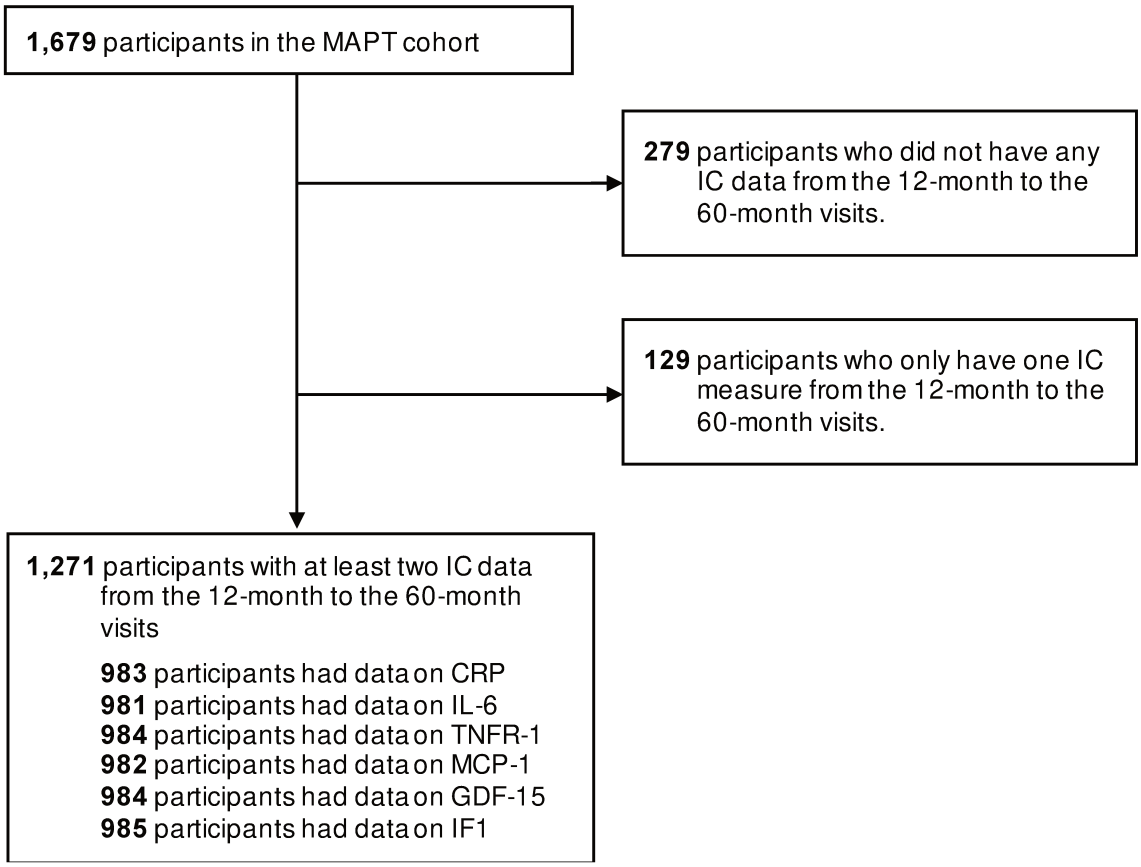

Figure 5.1 Flowchart of cohort identification in Study II

Intrinsic capacity (IC)

Four IC domains were evaluated in the present study: cognition (assessed by MMSE), locomotion (evaluated by SPPB), psychology (measured by GDS), and vitality (operationalized as handgrip strength). Four IC domains were annually evaluated from the 12-month visit to the 60-month visit (a total of four years of follow-up).

We only investigated four IC domains and operationalized vitality as handgrip strength because the trajectory modeling needed the same length of the data period for all domains. As mentioned in the Section 3.1.3, MNA (another vitality operational definition that often used in the literature), vision and hearing data were available only in half of the population at the 12-month and 24-month visits of the MAPT Study (**Table 3.1**). GBTM typically requires multiple outcome measures (usually more than three), whereas MNA and sensory data only available for two time points in the thesis.

Variables for each IC domain were transformed to a 0-to-100 scale; higher is better. Values representing the best performance in the original instruments (30 for MMSE, 12 for SPPB, 0 for GDS) were rescaled as 100 points, while values indicating the worst performance (0 for MMSE and SPPB, 15 for GDS) were rescaled as 0 point. Regarding the vitality domain, we defined 100 points based on the maximum value of handgrip strength among all the observations during the follow-up period (i.e., the maximum possible value achieved by our study cohort); a different maximum value was applied for each sex (i.e., 51 kg for women and 72 kg for men).

We rescaled each domain measurement from 0 to 100 points according to the modified POMP method. Two prior studies used the POMP method to create the IC index (Section 1.5.2). It defined the lowest value of a variable across all observational periods (i.e., the minimum possible value) as a score of 0 and the highest value (i.e., the maximum possible value) as 100

points[215]. However, we noticed a potential issue with the POMP method to rescale MMSE values (an indicator of the cognitive domain) in the MAPT participants. The MAPT participants were included to have initial MMSE ≥24, and all our study subjects had MMSE ≥15 during the follow-up. But suppose we defined people with MMSE=15 as having a score of 0 for the cognitive domain of IC. In that case, we may underestimate our participants' cognitive capacity and make result interpretation and direct comparisons with other studies difficult and potentially misleading. Therefore, we modified the POMP method by setting the lowest and highest possible scores in the measurement tools as 0 and 100. To be specific, 100 points indicated the best performance in the original instruments (i.e., 30 for MMSE, 12 for SPPB, 0 for GDS), while 0 indicated the worst performance (i.e., 0 for MMSE, 0 for SPPB, 15 for GDS). Other values were transformed by subtracting the value of the worst performance and then dividing by the range of the instrument:

$$y = \frac{(x - \text{the value of the worst performance})}{(\text{the value of the best performance} - \text{the value of the worst performance})} * 100 \quad \text{(Equation 5.1)}$$

where x is the value recorded by the measurement tool, and y is the transformed score ranging from 0 to 100. After transformation, all values ranged from 0 to 100 points with higher meaning a better capacity.

For handgrip strength, 100 points were defined as the maximum observed values in our cohort during the follow-up period; we used the sex-stratified maximum observed values (i.e., 51 kg for women and 72 kg for men) due to the significant sex differences in handgrip strength. Zero points referred to 0 kg, and the remaining values were transformed based on Equation 5.1.

Plasma biomarkers

Multiple plasma biomarkers in the MAPT Study were selected if they showed direct links with the hallmarks of aging[19–21]: CRP, IL-6, TNFR-1, MCP-1, GDF-15, and ATPase IF1.

CRP, IL-6, TNFR-1, and MCP-1 act as markers of chronic inflammation[54,252]. In addition, IL-6, TNFR-1, and MCP-1 were identified as the SASP components[244–246]. GDF-15 is a stress-response cytokine upregulated during inflammation, mitochondrial dysfunction, and cellular senescence[64,230]. IF1 is an endogenous inhibitor of ATP synthase that regulates mitochondrial bioenergetics; a lower circulating IF1 level has been proposed to indicate higher intracellular concentrations of IF1, which may serve as a marker of mitochondrial impairment[237].

All biomarkers were measured from the blood samples collected at the 12-month visit (the baseline of this study). To compare the effect of biomarkers with different units and distributions, we log-transformed and standardized biomarker values before statistical analysis. The outlier values, defined as above or below 4 SDs from the population mean, were removed from the analysis.

Statistical analysis

GBTM was used to determine IC trajectories over four years. GBTM is designed to identify a finite number of groups of individuals who follow similar trajectories of a single indicator[253]. Trajectories of multiple indicators can be explored using an extension of univariate GBTM called multi-trajectory modeling, which describes the joint evolution of the indicators. In other words, group-based multi-trajectory modeling determines separate trajectories for each indicator and then groups individuals presenting similar patterns across all indicators[254].

In this study, we applied multi-trajectory modeling to determine the joint trajectories across four separate domains – cognition, locomotion, psychology, and vitality (referred to as “IC

multi-trajectories"). As aforementioned, multiple time points of indicator measures are recommended in GBTM to determine the optimal number and shape of trajectory groups, so we decided to focus on four IC domains instead of five. The scores of IC domains were modeled as following the censored normal distribution. The multi-trajectory modeling approach followed a two-step procedure in Nagin's work[253,255]. In brief, we specified a basic model with the polynomial order as 3 (i.e., cubic terms). We started a basic model with a single trajectory (the number of trajectory groups as 1), then reran the basic models with an increasing number of trajectory groups until the best-fitting model was established. Then we dropped the insignificant cubic and quadratic terms from the best model determined in the first step and evaluated whether BIC continued to improve. The linear terms remained in the final model even if they were insignificant[256].

The best-fit model was selected based on statistical criteria[257], including BIC (higher means better goodness of fit), the average posterior probability of assignment (APPA; >0.7 in all groups), odds of correct classification (OCC; >5 in all groups) and group proportions (≥5% of the total sample in each group). Nagin and colleagues suggested that the choice of the best model is not simply based on a single test statistic; instead, one should also consider if the selected model is able to summarize the distinctive features of the data in the most parsimonious and practical way[253]. Therefore, when several trajectory models generated from our data met the statistical fit criteria, we chose the model that followed the principle of parsimony and fit clinical interpretation, that is, having substantive and clinically meaningful differences between each trajectory. In other words, if an additional group did not capture a distinctive trajectory from the other groups, we preferred a model using a smaller number of groups in the interest of parsimony. As sensitive analyses, group-based multi-trajectory modeling were performed in women and men separately to see if we could obtain trajectory group membership similar to the primary analysis (on the entire cohort).

After the IC multi-trajectories were determined, the association between multi-trajectory groups and plasma biomarkers was analyzed using multinomial logistic regression, adjusting for age, sex, education, MAPT group allocation, and the number of chronic diseases. We calculated relative risk ratios (RRRs) and 95% confidence intervals (CIs) for each trajectory group per SD increment in plasma biomarker concentrations; plasma biomarker levels were log-transformed and standardized for better comparison. Sensitivity analysis with biomarker outliers included was performed.

- ***Exploratory analyses***

We were interested if associations between plasma biomarkers and IC trajectories remained when composing IC domains into a single value. This analysis was exploratory since only four components were included in the IC construct instead of six (with vision and hearing). Following the prior studies[202,236], the composite IC score was calculated by averaging the performance of the four domains; in other words, each domain was weighted equally, and the composite IC score also ranged from 0 to 100. The trajectories of the composite IC score (referred to as "global IC trajectories" hereafter) were identified by using the univariate GBTM and following the two-step model selection procedure aforementioned. Sensitivity analyses on women and men separately were performed. Multinomial logistic regression also examined the association of global IC trajectories with plasma biomarkers (with and without biomarker outliers included).

Another exploratory analysis was performed by additionally adjusting for the baseline IC levels in the multinomial logistic models. When exploring IC multi-trajectory groups as dependent variables, baseline levels of each IC domain (cognition, locomotion, psychology, and vitality) were used as covariates; when exploring global IC trajectory groups as dependent variables, baseline composite IC score was used as a covariate. This exploratory analysis was proposed

by one reviewer during our paper submission. IC trajectories contained information on initial IC levels and the slope of IC decline. Thus, significant associations of plasma biomarkers with IC trajectories, if any, can be either due to the correlation between biomarkers and initial IC levels, the correlation between biomarkers and overtime declining IC trend, or both. By controlling baseline IC scores in regression models, we could further distinguish the biomarkers' association with the slope of IC decline. This analysis was exploratory since evaluating how biomarkers affect the slope of IC over time was not our main objective.

5.3 Results

Identification of multi-trajectories across four IC domains

Group-based multi-trajectory modeling began with one group, fixing at cubic function, then increased the number of trajectory groups to six (**Table 5.1**). The 6-group cubic model had higher BIC than the 5-group cubic model, and its fit indices reached the recommended values. However, the 6-group model did not include a group that was substantively different from those in the five-group model. In the interest of parsimony, we preferred a multi-trajectory model that included 5 groups.

We further dropped insignificant cubic and quadratic terms of each trajectory. The 5-group model with one quadratic psychological trajectory and one cubic psychological trajectory showed better BIC value, and it had APPA >0.7 and OCC >5 for all groups (values shown in bold in **Table 5.1**). Thus, we selected it as the final multi-trajectory model.

The five IC multi-trajectory groups identified in our population are labeled as *low in all domains* (8.4%), *low locomotion* (24.6%); *low psychological domain* (16.7%), *robust* (i.e., high in all domains except vitality; 28.3%), and *robust with high vitality* (22.0%) (**Figure 5.1**). Five trajectories were replicated in the sensitivity analysis on females and males, with slightly

different percentages in each trajectory group (**Figure 5.2 and 5.3**).

Table 5.2 displays the participants' baseline characteristics according to their IC multi-trajectories. The oldest age, the highest percentages of low educational levels, and the highest number of chronic diseases were observed in the "low in all domain" group.

Table 5.1. Fit indices for group-based multi-trajectory modeling of four IC domains

Number of trajectory groups	Polynomial function order*	BIC	APPA		OCC		Proportions in each trajectory group (%)					
			Mean	The lowest value across group(s)	Mean	The lowest value across group(s)	1	2	3	4	5	6
1	Cognition (3) Locomotion (3) Psychological (3) Vitality (3)	-75268.99	1	1	NA	NA	100	-	-	-	-	-
2	Cognition (3, 3) Locomotion (3, 3) Psychological (3, 3) Vitality (3, 3)	-73654.37	0.954	0.947	21.59	16.06	39.7	60.3	-	-	-	-
3	Cognition (3, 3, 3) Locomotion (3, 3, 3) Psychological (3, 3, 3) Vitality (3, 3, 3)	-73100.21	0.928	0.905	30.66	18.02	20.8	34.7	44.5	-	-	-
4	Cognition (3, 3, 3, 3) Locomotion (3, 3, 3, 3) Psychological (3, 3, 3, 3) Vitality (3, 3, 3, 3)	-72751.76	0.914	0.892	39.74	14.44	15.7	17.2	36.3	30.8	-	-
5	Cognition (3, 3, 3, 3, 3) Locomotion (3, 3, 3, 3, 3) Psychological (3, 3, 3, 3, 3) Vitality (3, 3, 3, 3, 3)	-72445.42	0.905	0.895	48.18	21.91	8.3	25.0	16.5	28.1	22.0	-

Number of trajectory groups	Polynomial function order*	BIC	APPA		OCC		Proportions in each trajectory group (%)					
			Mean	The lowest value across group(s)	Mean	The lowest value across group(s)	1	2	3	4	5	6
5	Cognition (2, 2, 2, 2, 2) Locomotion (2, 2, 2, 2, 2) Psychological (2, 2, 3, 2, 2) Vitality (2, 2, 2, 2, 2)	-72381.55	0.905	0.895	48.18	21.91	8.3	25.0	16.5	28.1	22.0	-
5	**Cognition (1, 1, 1, 1, 1)** **Locomotion (1, 1, 1, 1, 1)** **Psychological (2, 1, 3, 1, 1)** **Vitality (1, 1, 1, 1, 1)**	**-72324.88**	**0.904**	**0.889**	**47.14**	**20.34**	**8.4**	**24.6**	**16.7**	**28.3**	**22.0**	**-**
6	Cognition (3, 3, 3, 3, 3, 3) Locomotion (3, 3, 3, 3, 3, 3) Psychological (3, 3, 3, 3, 3, 3) Vitality (3, 3, 3, 3, 3, 3)	-72215.31	0.900	0.863	66.37	20.29	7.7	10.3	14.3	20.1	27.7	19.8

The model that best fit our data is shown in bold. *Polynomial function order: 1 = linear, 2 = quadratic, 3 = cubic.

APPA, average posterior probability of assignment; BIC, Bayesian information criterion; IC, intrinsic capacity; NA, not applicable; OCC, odds of correct classification.

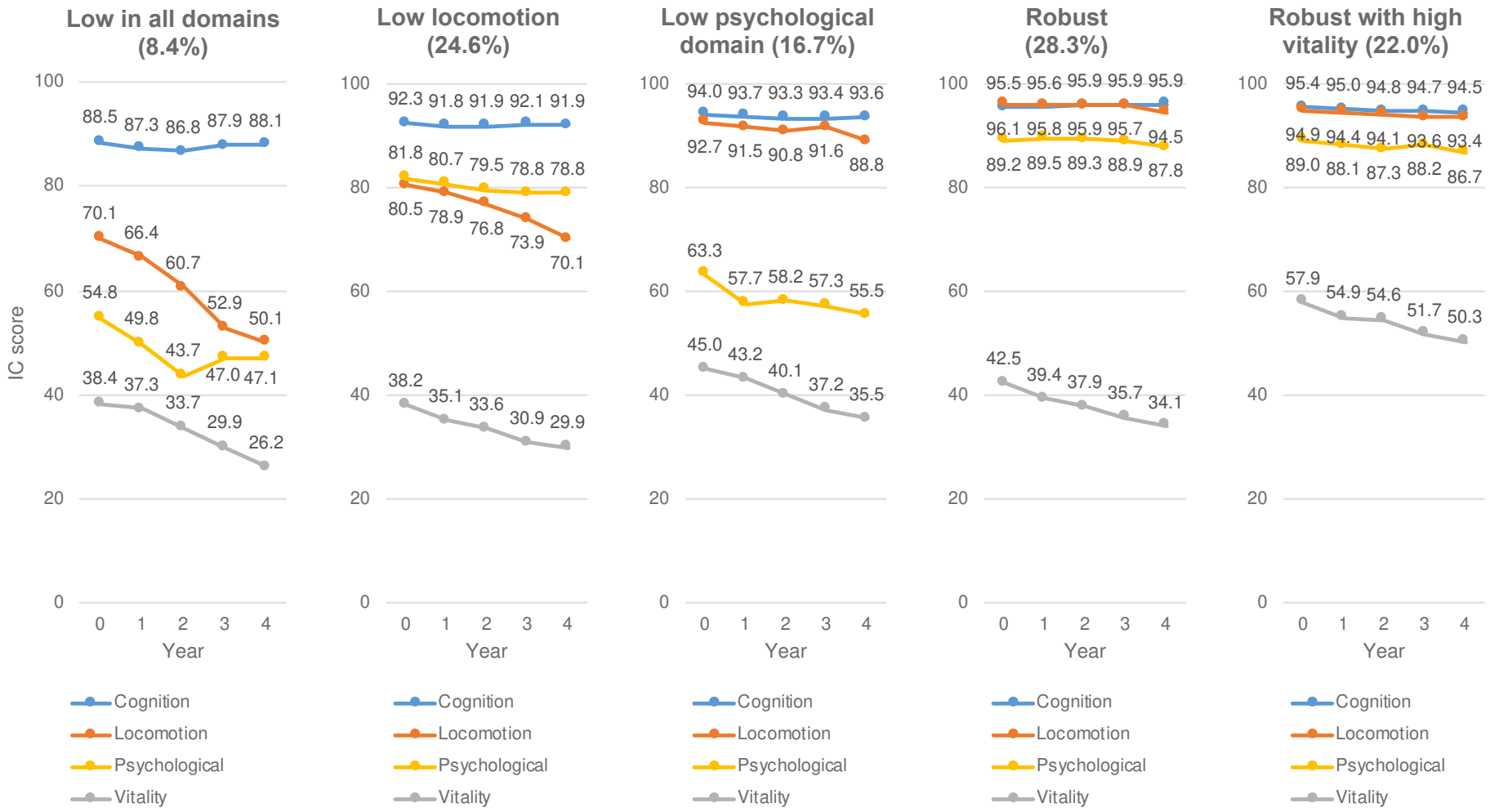

Figure 5.1 Multi-trajectories across four IC domains

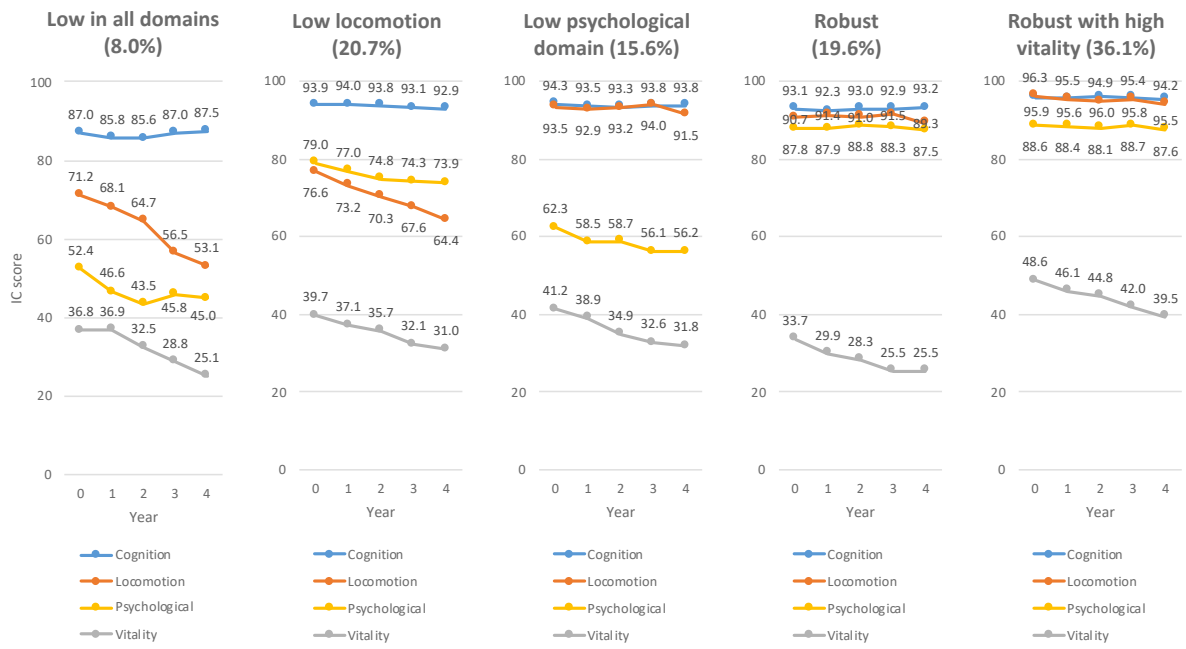

Figure 5.2 IC multi-trajectories in female

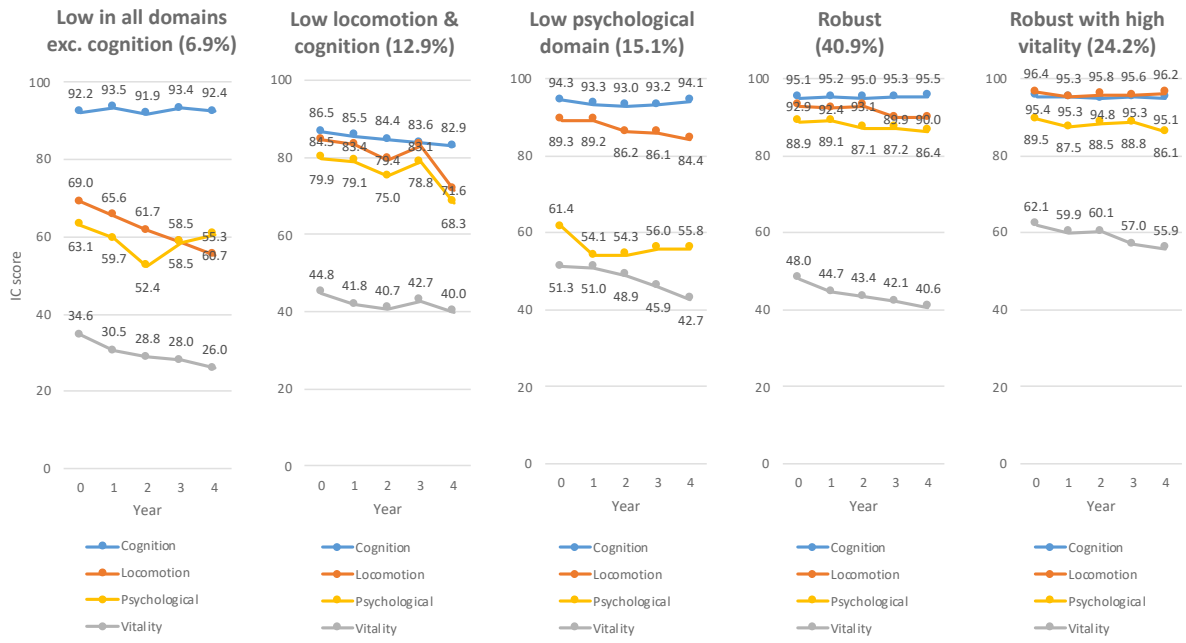

Figure 5.3 IC multi-trajectories in male

Table 5.2 Baseline characteristics of the study population

	Multi-trajectory group membership					
	Low in all domains (n=107 [8.4%])	**Low Locomotion (n=313 [24.6%])**	**Low psychological domain (n=212 [16.7%])**	**Robust (n=360 [28.3%])**	**Robust with high vitality (n=279 [22.0%])**	**p**
Age (years)	78.6 (4.6)[a,b,c]	78.0 (4.7)[d,e,f]	76.1 (4.1)[a,d,g,h]	74.8 (3.6)[b,e,g]	74.3 (3.1)[c,f,h]	<0.001
Female	76 (71.0%)[a]	233 (74.4%)[b]	135 (63.7%)[c,d]	281 (78.1%)[c,e]	96 (34.4%)[a,b,d,e]	<0.001
MAPT group						
Multidomain intervention + omega-3	27 (25.2%)	75 (24.0%)	49 (23.1%)	99 (27.5%)	69 (24.7%)	0.835
Omega-3	34 (31.8%)	72 (23.0%)	54 (25.5%)	81 (22.5%)	72 (25.8%)	
Multidomain intervention	25 (23.4%)	82 (26.2%)	54 (25.5%)	92 (25.6%)	65 (23.3%)	
Placebo	21 (19.6%)	84 (26.8%)	55 (25.9%)	88 (24.4%)	73 (26.2%)	
Education						
No diploma	10 (9.4%)[a,b,c,d]	23 (7.4%)[a,e]	7 (3.3%)[b]	9 (2.5%)[c,e]	11 (4.0%)[d]	<0.001
Primary school certificate	39 (36.8%)	66 (21.4%)	38 (17.9%)	39 (11.0%)	39 (14.3%)	
Secondary education	36 (34.0%)	101 (32.7%)	74 (34.9%)	128 (36.0%)	83 (30.5%)	
High school diploma	6 (5.7%)	46 (14.9%)	27 (12.7%)	58 (16.3%)	41 (15.1%)	
University level	15 (14.2%)	73 (23.6%)	66 (31.1%)	122 (34.3%)	98 (36.0%)	
Number of chronic diseases	1.4 (1.1)[a,b]	1.2 (1.0)	1.1 (1.0)	1.0 (0.9)[a]	1.1 (1.0)[b]	0.003
Global IC score, 0-100	62.9 (7.8)[a,b,c,d]	73.1 (5.7)[a,e,f]	74.3 (5.5)[b,g,h]	80.9 (3.7)[c,e,g,i]	84.4 (4.1)[d,f,h,i]	<0.001
IC indicators						
Cognitive: MMSE, 0-30	26.5 (2.3)[a,b,c,d]	27.7 (1.9)[a,e,f,g]	28.2 (1.6)[b,e,h,i]	28.6 (1.4)[c,f,h]	28.6 (1.3)[d,g,i]	<0.001

	Multi-trajectory group membership					
	Low in all domains (n=107 [8.4%])	**Low Locomotion (n=313 [24.6%])**	**Low psychological domain (n=212 [16.7%])**	**Robust (n=360 [28.3%])**	**Robust with high vitality (n=279 [22.0%])**	**p**
Locomotion: SPPB, 0-12	8.4 (2.2)[a,b,c,d]	9.6 (1.7)[a,e,f,g]	11.1 (1.1)[b,e,h]	11.5 (1.0)[c,f,h]	11.4 (0.9) [d,g]	<0.001
Psychological: GDS, 0-15	6.8 (2.7)[a,b,c,d]	2.7 (1.7)[a,e,f,g]	5.5 (2.4)[b,e,h,i]	1.6 (1.5)[c,f,h]	1.6 (1.4)[d,g,i]	<0.001
Vitality: Handgrip strength, kg	22.1 (8.0)[a,b]	22.0 (7.8)[c,d,e]	26.8 (8.1)[a,c,f,g]	23.7 (6.4)[d,f,h]	37.9 (8.5)[b,e,g,h]	<0.001
Plasma biomarkers						
CRP, mg/L	4.9 (8.9)[a]	3.6 (5.1)	3.0 (5.0)	3.2 (5.4)	2.8 (3.5)[a]	0.032
IL-6, pg/mL	3.9 (3.4)	3.6 (2.9)	3.5 (3.9)	3.1 (2.7)	3.0 (3.1)	0.045
TNFR-1, pg/mL	1372.6 (497.5)[a,b,c]	1276.8 (420.1)[d,e]	1192.5 (365.6)[a]	1141.9 (371.1)[b,d]	1176.1 (354.7)[c,e]	<0.001
MCP-1, pg/mL	224.3 (75.0)	219.2 (77.6)	229.5 (80.6)	217.9 (86.6)	218.4 (84.9)	0.610
GDF-15, pg/mL	1330.4 (507.6)[a,b,c]	1200.5 (545.0)[d,e]	1106.8 (465.6)[a]	1011.3 (378.2)[b,d]	1045.9 (456.0)[c,e]	<0.001
ATPase IF1, ng/mL	586.5 (279.1)	579.3 (274.5)	606.5 (292.6)	581.0 (281.7)	563.9 (258.9)	0.674

Continuous variables are reported as mean (SD), and categorical variables as number (%). p-value based on ANOVA or Chi-square test across groups. Bonferroni-corrected threshold for significance were applied for pairwise comparisons in Chi-square test. Post-hoc pairwise comparisons were performed using the Tukey–Kramer test for continuous variables. Same letters were assigned to the groups showing significant difference based on the Tukey–Kramer test or Chi-square test. Data were missing for some participants for education (n=16), CRP (n=288), IL-6 (n=290), TNFR-1 (n=287), MCP-1 (n=289), GDF-15 (n=287), and IF1 (n=286). Values for global IC score and each IC indicator are based on data at baseline, which were missing for some participants for MMSE (n=18), SPPB (n=35), GDS (n=22), and handgrip strength (n=116). CRP, C-reactive protein; GDF-15, growth differentiation factor-15; GDS, Geriatric Depression Scale; IC, intrinsic capacity; IF1, inhibitory factor 1; IL-6, interleukin-6; MAPT, Multidomain Alzheimer Preventive Trial; MCP-1, monocyte chemoattractant protein-1; MMSE, Mini-Mental State Examination; SPPB, Short Physical Performance Battery; TNFR-1, tumor necrosis factor receptor-1.

Association of IC multi-trajectories with plasma biomarkers

We compared the plasma biomarker levels across five multi-trajectory groups using multinomial logistic regression (one model per biomarker) (**Table 5.3**). Older individuals with higher plasma IL-6, TNFR-1, and GDF-15 showed a higher possibility of being in the "low in all domain" group (*IL-6*: RRR [95% CI] = 1.42 [1.07 – 1.88]; p=0.016; *TNFR-1*: RRR = 1.46 [1.09 – 1.96]; p=0.011; *GDF-15*: RRR = 1.99 [1.45 – 2.73]; p<0.001) than in the "robust with high vitality" group. In addition, increased IL-6 and GDF-15 levels were associated with a higher risk of belonging to the "low locomotion" group (*IL-6*: RRR = 1.37 [1.10 – 1.71]; p=0.005; *GDF-15:* RRR = 1.48 [1.17 – 1.89]; p=0.001). We found a weaker association between GDF-15 and the "low psychological domain" group (RRR = 1.29 [1.01 – 1.64]; p=0.044).

Despite not reaching statistical significance, people with increased IL-6 and GDF-15 seem to be more likely to be in the robust group rather than in the "robust with high vitality" group (*IL-6*: RRR [95% CI] = 1.20 [0.97 – 1.48]; p=0.094; *GDF-15*: RRR = 1.23 [0.98 – 1.54]; p=0.070) (**Table 5.3**).

Sensitivity analysis including biomarker outliers provided similar results for TNFR-1 and GDF-15. IL-6 was associated with all IC multi-trajectory groups after considering biomarker outliers, with a trend of increasing RRRs from the robust group to the "low in all domains" group.

Table 5.3 Association between plasma biomarkers and IC multi-trajectory groups

	Low in all domains vs. robust with high vitality		Low locomotion vs. robust with high vitality		Low psychological domain vs. robust with high vitality		Robust vs. robust with high vitality	
	RRR (95% CI)	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**
CRP	1.22 (0.93, 1.60)	0.157	1.20 (0.98, 1.47)	0.084	1.02 (0.82, 1.26)	0.891	0.97 (0.80, 1.18)	0.762
IL-6	1.42 (1.07, 1.88)	0.016	1.37 (1.10, 1.71)	0.005	1.25 (0.99, 1.56)	0.056	1.20 (0.97, 1.48)	0.094
TNFR-1	1.46 (1.09, 1.96)	0.011	1.21 (0.98, 1.51)	0.083	1.06 (0.85, 1.33)	0.579	0.99 (0.81, 1.21)	0.913
MCP-1	0.98 (0.74, 1.29)	0.860	0.91 (0.73, 1.13)	0.381	1.11 (0.90, 1.38)	0.334	0.99 (0.81, 1.21)	0.905
GDF-15	1.99 (1.45, 2.73)	<0.001	1.48 (1.17, 1.89)	0.001	1.29 (1.01, 1.64)	0.044	1.23 (0.98, 1.54)	0.070
ATPase IF1	0.89 (0.66, 1.18)	0.413	0.90 (0.73, 1.11)	0.321	1.05 (0.85, 1.30)	0.664	0.92 (0.76, 1.11)	0.377

All biomarker values were log-transformed and then standardized; all models were adjusted for age, sex, educational levels, MAPT group allocation, and the number of chronic diseases; CI, confidence interval; CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IC, intrinsic capacity; IF1, inhibitory factor 1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; RRR, relative risk ratio; TNFR-1, tumor necrosis factor receptor-1.

Identification of global IC trajectories

GBTM began with 1 group, fixing at cubic function, then increased the number of trajectory groups to 5 (**Table 5.4**). The 5-group model gave rise to one group with <5% of total participants. After removing insignificant cubic and quadratic terms, the 4-group model with linear function showed improved BIC value (higher than models in quadratic and cubic functions) and had APPA >0.7 and OCC >5 for all groups. Thus the 4-group model with all linear trajectories was chosen (values shown in bold in **Table 5.4**). Four global IC trajectories were labelled as *low baseline IC with steep decline* (5.3%), *intermediate baseline IC with moderate decline* (18.9%), *intermediate baseline IC with mild decline* (35.7%), and *high baseline IC with very mild decline* (40.1%) (**Figure 5.4**). All trajectories followed a similar pattern, with lower initial composite IC scores tending to have steeper IC declining slopes over time. The sensitivity analysis on females and males obtained similar trajectory group membership (**Figure 5.5**).

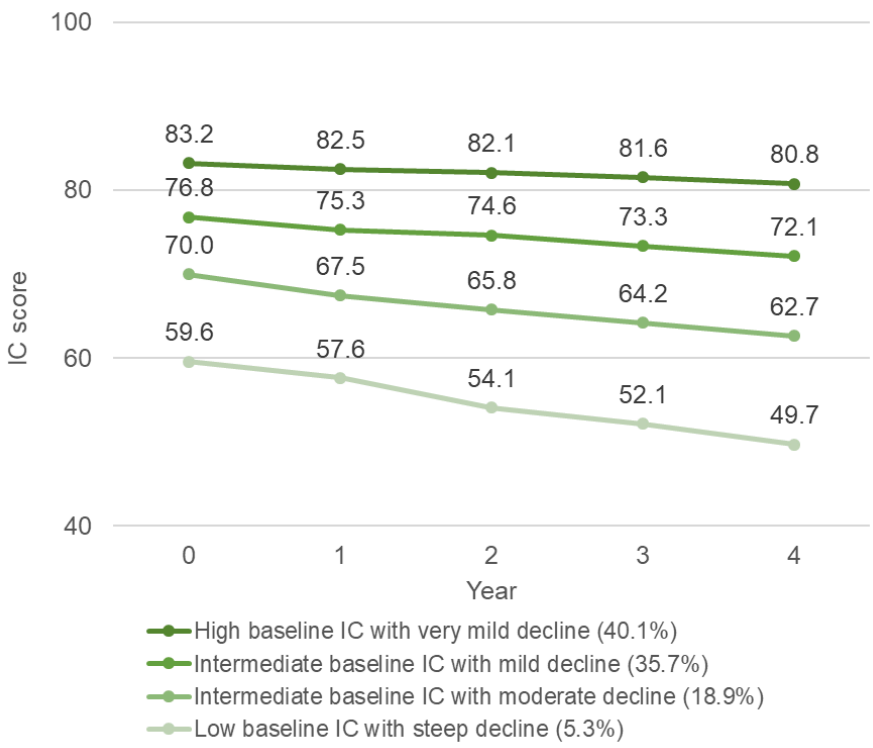

Figure 5.4 Trajectories of global IC score

Table 5.4 Fit indices for group-based trajectory modeling of composite IC score

Number of trajectory groups	Polynomial function order[1]	BIC	APPA		OCC		Proportions in each trajectory group (%)				
			Mean	The lowest value across group(s)	Mean	The lowest value across group(s)	1	2	3	4	5
1	3	-18170.07	1	1	NA	NA	100	-	-	-	-
2	3, 3	-17014.39	0.953	0.936	23.37	16.40	34.1	65.9	-	-	-
3	3, 3, 3	-16576.04	0.933	0.913	44.47	17.61	12.7	37.3	50.0	-	-
4	3, 3, 3, 3	-16400.95	0.910	0.870	93.03	11.98	5.4	19.2	35.8	39.6	-
4	2, 2, 2, 2	-16388.41	0.909	0.872	85.39	12.30	5.4	19.2	35.6	39.7	-
4	**1, 1, 1, 1**	**-16375.90**	**0.911**	**0.871**	**96.98**	**12.18**	**5.3**	**18.9**	**35.7**	**40.1**	**-**
5	3, 3, 3, 3, 3	-16306.71	0.876	0.837	85.91	8.99	4.2	13.5	25.4	36.4	20.5

The model that best fit our data is shown in bold. [1] Polynomial function order: 1 = linear, 2 = quadratic, 3 = cubic.

APPA, average posterior probability of assignment; BIC, Bayesian information criterion; IC, intrinsic capacity; NA, not applicable; OCC, odds of correct classification.

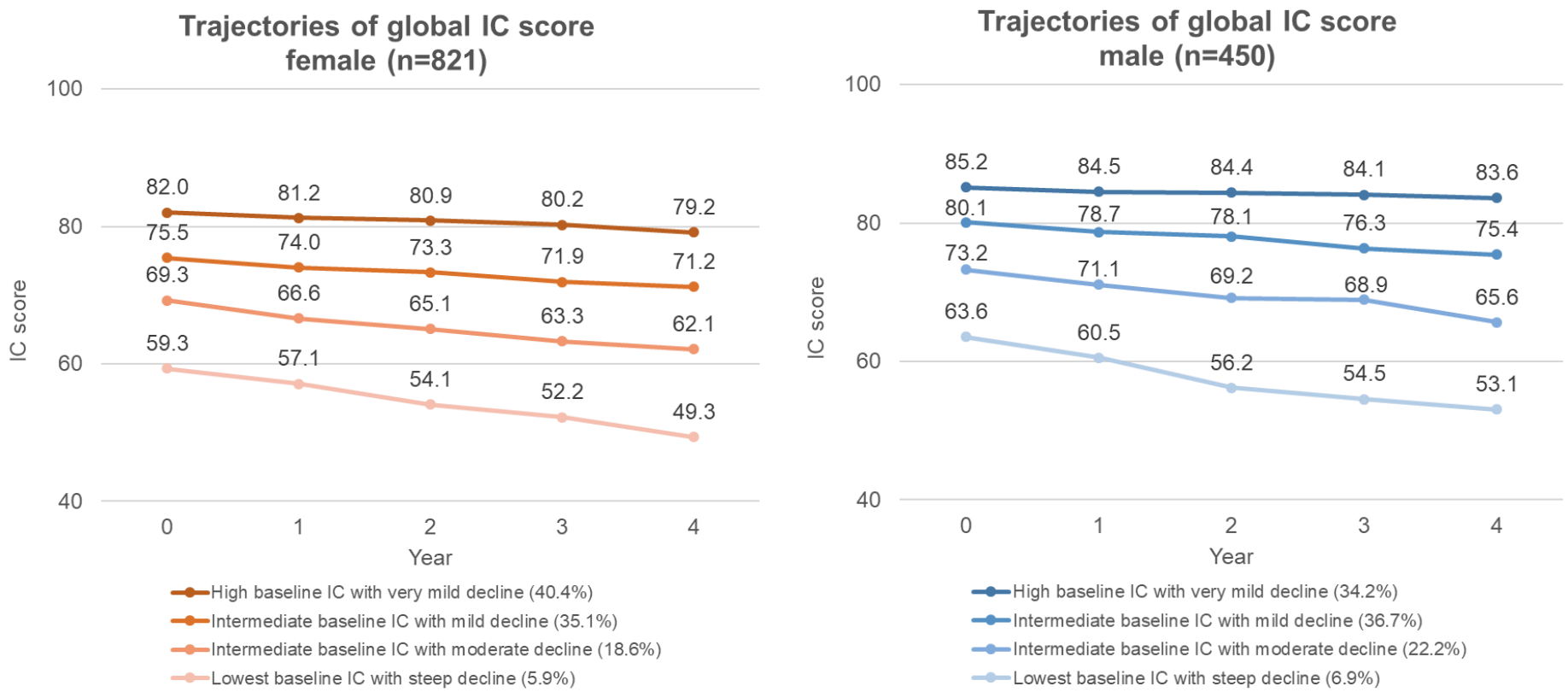

Figure 5.5 Trajectories of global IC score in the female and male population

Table 5.5 displays how participants were distributed in the four global IC trajectories and the five IC multi-trajectory groups. For people with steep IC decline, 91% were in the "low in all IC domains" group. Individuals with a high baseline IC tended to be "robust (48.2%)" or "robust with high vitality (51.4%)". Furthermore, participants with moderate declining and mild declining were distributed across three and more IC multi-trajectories, suggesting that individuals may have different declining patterns in IC components even if their global IC trajectories were similar.

Table 5.5 Participant distribution across two trajectory group memberships

	Global IC trajectory groups			
	Low baseline IC with steep decline	**Intermediate baseline IC with moderate decline**	**Intermediate baseline IC with mild decline**	**High baseline IC with very mild decline**
IC multi-trajectory groups				
Low in all domains	61 (91.0%)	46 (19.2%)	0	0
Low locomotion	6 (9.0%)	114 (47.5%)	191 (42.1%)	2 (0.4%)
Low psychological domain	0	80 (33.3%)	132 (29.1%)	0
Robust	0	0	114 (25.1%)	246 (48.2%)
Robust with high vitality	0	0	17 (3.7%)	262 (51.4%)
Total	67 (100%)	240 (100%)	454 (100%)	510 (100%)

Values were presented in number (%); IC, intrinsic capacity.

Participants' baseline characteristics according to their global IC trajectories are presented in **Table 5.6**. Compared to the high baseline IC group, the steep declining group was significantly older and had more females, lower education levels, and a higher number of chronic diseases.

Table 5.6 Comparison of baseline characteristics between global IC trajectory groups

	Low baseline IC with steep decline (n=67 [5.3%])	Intermediate baseline IC with moderate decline (n=240 [18.9%])	Intermediate baseline IC with mild decline (n=454 [35.7%])	High baseline IC with very mild decline (n=510 [40.1%])	p
Age (years)	78.7 (4.8)[a,b]	78.4 (4.8)[c,d]	76.2 (4.2)[a,c,e]	74.4 (3.2)[b,d,e]	<0.001
Female	50 (74.6%)[a]	181 (75.4%)[b]	313 (68.9%)[c]	277 (54.3%)[a,b,c]	<0.001
MAPT group					
Multidomain intervention + omega-3	15 (22.4%)	58 (24.2%)	113 (24.9%)	133 (26.1%)	
Omega-3	23 (34.3%)	61 (25.4%)	111 (24.5%)	118 (23.1%)	0.794
Multidomain intervention	16 (23.9%)	56 (23.3%)	113 (24.9%)	133 (26.1%)	
Placebo	13 (19.4%)	65 (27.1%)	117 (25.8%)	126 (24.7%)	
Education					
No diploma	6 (9.1%)[a,b]	19 (8.0%)[c]	20 (4.4%)[a,d]	15 (3.0%)[b,c,d]	
Primary school certificate	24 (36.4%)	54 (22.8%)	86 (19.0%)	57 (11.4%)	
Secondary education	23 (34.9%)	74 (31.2%)	155 (34.3%)	170 (34.0%)	<0.001
High school diploma	3 (4.6%)	31 (13.1%)	71 (15.7%)	73 (14.6%)	
University level	10 (15.2%)	59 (24.9%)	120 (26.6%)	185 (37.0%)	
Number of chronic diseases	1.4 (1.1)[a]	1.2 (1.0)	1.1 (1.0)	1.1 (0.9)[a]	0.019
Global IC score, 0-100	59.2 (6.6)[a,b,c]	69.8 (5.4)[a,d,e]	76.7 (4.6)[b,d,f]	83.3 (3.9)[c,e,f]	<0.001
IC indicators					
Cognition: MMSE, 0-30	26.2 (2.5)[a,b,c]	27.7 (1.8)[a,d,e]	28.1 (1.8)[b,d,f]	28.7 (1.3)[c,e,f]	<0.001

Locomotion: SPPB, 0-12	7.5 (2.3)[a,b,c]	9.8 (1.8)[a,d,e]	10.8 (1.5)[b,d,f]	11.5 (0.8)[c,e,f]	<0.001
Psychological: GDS, 0-15	6.8 (2.9)[a,b,c]	4.9 (2.7)[a,d,e]	3.0 (2.1)[b,d,f]	1.5 (1.4)[c,e,f]	<0.001
Vitality: Handgrip strength, kg	19.4 (7.8)[a,b]	21.5 (8.0)[c,d]	25.2 (8.0)[a,c,e]	31.5 (9.9)[b,d,e]	<0.001
Plasma biomarkers					
CRP, mg/L	4.9 (8.8)	3.5 (5.1)	3.2 (5.5)	3.1 (4.6)	0.124
IL-6, pg/mL	4.0 (3.0)	3.7 (3.2)[a]	3.6 (3.6)[b]	2.9 (2.5)[a,b]	0.002
TNFR-1, pg/mL	1372.2 (549.3)[a,b]	1328.8 (434.3)[c,d]	1192.1 (354.5)[a,c]	1150.9 (375.8)[b,d]	<0.001
MCP-1, pg/mL	227.2 (82.1)	229.9 (82.3)	221.4 (76.3)	215.6 (87.0)	0.256
GDF-15, pg/mL	1337.3 (565.0)[a,b]	1251.5 (521.1)[c,d]	1092.3 (460.5)[a,c]	1025.6 (427.3)[b,d]	<0.001
ATPase IF1, ng/mL	637.9 (287.1)	601.1 (288.4)	570.7 (261.3)	576.3 (283.2)	0.311

Values are presented in mean (standard deviation) for continuous variables or number (%) for categorical variables. p-value based on ANOVA or Chi-square test across groups. Bonferroni-corrected threshold for significance were applied for pairwise comparisons in Chi-square test. Post-hoc pairwise comparisons were performed using the Tukey–Kramer test for continuous variables. Same letters were assigned to the groups showing significant difference based on the Tukey–Kramer test or Chi-square test. Data were missing for some participants for education (n=16), CRP (n=288), IL-6 (n=290), TNFR-1 (n=287), MCP-1 (n=289), GDF-15 (n=287), and IF1 (n=286). Values for global IC score and each IC indicator are based on data at baseline, which were missing for some participants for MMSE (n=18), SPPB (n=35), GDS (n=22), and handgrip strength (n=116). CRP=C-reactive protein. GDF-15=growth differentiation factor-15. GDS=Geriatric Depression Scale. IC=intrinsic capacity. IF1=inhibitory factor 1. IL-6=interleukin-6. MAPT=Multidomain Alzheimer Preventive Trial. MCP-1=monocyte chemoattractant protein-1. MMSE=Mini-Mental State Examination. SPPB=Short Physical Performance Battery. TNFR-1=tumor necrosis factor receptor-1.

Association of global IC trajectories with plasma biomarkers

After adjusting age, sex, education, MAPT groups, and chronic diseases number, plasma IL-6, TNFR-1, and GDF-15 were significantly associated with global IC trajectories (**Table 5.7**). Again, GDF-15 levels had the strongest association with IC trajectory groups. For one SD increase in logGDF-15 levels, the RRR compared to the high baseline IC trajectory was 1.92 (95% CI = 1.34 – 2.74; p<0.001) for the steep declining group and 1.58 (95% CI = 1.27 – 1.98; p<0.001) for the moderate declining group. Higher IL-6 levels were associated with an increased risk of steep IC decline, moderate IC decline, and mild IC decline, compared to the high baseline IC trajectory. Participants with higher TNFR-1 levels were more likely to be in the steep declining group and the moderate declining group rather than in the high baseline IC group (**Table 5.7**). Associations between plasma biomarkers and global IC trajectories remained unchanged after including biomarker outliers.

Adjusting for baseline IC levels in multinomial logistic regression

We performed multinomial logistic regression with additional adjustment for initial IC levels. The results regarding IC multi-trajectories are shown in **Table 5.8**, and global IC trajectories are displayed in **Table 5.9**. After controlling the baseline IC values, IL-6 was no longer associated with any trajectory groups, indicating that the prior associations with trajectories were mainly driven by the correlation with baseline IC. On the other hand, people with higher GDF-15 tended to be in the low locomotion group (RRR = 1.50; 95% CI = 1.05 – 2.14; p=0.028), and those with higher MCP-1 were more likely to be in the low psychological group (RRR = 1.40; 95% CI = 1.02 – 1.93; p=0.040), after adjusting for the baseline levels of each IC domain (**Table 5.8**). TNFR-1, MCP-1 and GDF-15 levels remained associated with moderate IC decline (**Table 5.9**). GDF-15 still showed the highest RRR when compared to other biomarkers.

Table 5.7 Association between plasma biomarkers and IC trajectory groups examining by multinomial logistic regression

	Low baseline IC with steep decline vs. high baseline IC with very mild decline		Intermediate baseline IC with moderate decline vs. high baseline IC with very mild decline		Intermediate baseline IC with mild decline vs. high baseline IC with very mild decline	
	RRR (95% CI)	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**
CRP	1.18 (0.87, 1.61)	0.283	1.13 (0.93, 1.36)	0.229	1.00 (0.86, 1.17)	0.974
IL-6	1.54 (1.13, 2.10)	0.006	1.32 (1.08, 1.62)	0.007	1.26 (1.07, 1.48)	0.005
TNFR-1	1.57 (1.12, 2.18)	0.008	1.48 (1.20, 1.82)	<0.001	1.13 (0.96, 1.32)	0.138
MCP-1	1.07 (0.78, 1.47)	0.665	1.12 (0.92, 1.35)	0.264	1.07 (0.92, 1.25)	0.391
GDF-15	1.92 (1.34, 2.74)	<0.001	1.58 (1.27, 1.98)	<0.001	1.16 (0.98, 1.38)	0.090
ATPase IF1	1.12 (0.80, 1.57)	0.498	1.01 (0.83, 1.23)	0.939	0.95 (0.82, 1.11)	0.542

All biomarker values were log-transformed and then standardized. All models were adjusted for age, sex, educational levels, MAPT group allocation, and the number of chronic diseases. CRP=C-reactive protein. GDF-15=growth differentiation factor-15. IC=intrinsic capacity. IF1=inhibitory factor 1. IL-6=interleukin-6. MCP-1=monocyte chemoattractant protein-1. RRR=relative risk ratio. TNFR-1=tumor necrosis factor receptor-1.

Table 5.8 Association between plasma biomarkers and IC multi-trajectory groups examining by multinomial logistic regression with additional adjustment for baseline IC levels

	Low in all domains vs. robust with high vitality		Low locomotion vs. robust with high vitality		Low psychological domain vs. robust with high vitality		Robust vs. robust with high vitality	
	RRR (95% CI)	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**
CRP	1.28 (0.83, 1.99)	0.265	1.13 (0.83, 1.55)	0.438	1.18 (0.84, 1.64)	0.339	1.03 (0.78, 1.36)	0.831
IL-6	1.06 (0.67, 1.67)	0.816	1.17 (0.84, 1.64)	0.352	1.27 (0.89, 1.80)	0.185	1.21 (0.89, 1.64)	0.222
TNFR-1	1.04 (0.65, 1.66)	0.872	1.17 (0.83, 1.64)	0.365	1.07 (0.75, 1.52)	0.719	0.98 (0.73, 1.32)	0.893
MCP-1	0.97 (0.63, 1.51)	0.906	1.00 (0.73, 1.37)	0.988	1.40 (1.02, 1.93)	0.040	1.06 (0.80, 1.40)	0.683
GDF-15	1.56 (0.94, 2.57)	0.082	1.50 (1.05, 2.14)	0.028	1.30 (0.90, 1.89)	0.159	1.21 (0.88, 1.68)	0.245
ATPase IF1	0.90 (0.56, 1.44)	0.661	0.95 (0.68, 1.33)	0.764	0.99 (0.71, 1.39)	0.963	0.84 (0.63, 1.11)	0.216

All biomarker values were log-transformed and then standardized. All models were adjusted for age, sex, educational levels, MAPT group allocation, the number of chronic diseases, baseline levels of cognition, locomotion, psychological domain, and vitality.

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IC, intrinsic capacity; IF1, inhibitory factor 1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; RRR, relative risk ratio; TNFR-1, tumor necrosis factor receptor-1.

Table 5.9 Association between plasma biomarkers and IC trajectory groups examining by multinomial logistic regression with additional adjustment for baseline IC levels

	Low baseline IC with steep decline vs. high baseline IC with very mild decline		Intermediate baseline IC with moderate decline vs. high baseline IC with very mild decline		Intermediate baseline IC with mild decline vs. high baseline IC with very mild decline	
	RRR (95% CI)	**p**	**RRR (95% CI)**	**p**	**RRR (95% CI)**	**p**
CRP	0.79 (0.47, 1.34)	0.383	0.97 (0.72, 1.30)	0.818	0.97 (0.79, 1.19)	0.768
IL-6	0.88 (0.52, 1.51)	0.647	0.97 (0.72, 1.32)	0.859	1.20 (0.96, 1.49)	0.103
TNFR-1	1.13 (0.64, 1.97)	0.675	1.42 (1.03, 1.94)	0.030	1.19 (0.96, 1.48)	0.119
MCP-1	1.09 (0.64, 1.84)	0.761	1.36 (1.02, 1.82)	0.039	1.19 (0.98, 1.46)	0.083
GDF-15	1.38 (0.73, 2.61)	0.323	1.54 (1.10, 2.14)	0.011	1.18 (0.94, 1.50)	0.160
ATPase IF1	1.29 (0.77, 2.18)	0.330	1.20 (0.87, 1.65)	0.268	0.97 (0.79, 1.20)	0.789

All biomarker values were log-transformed and then standardized. All models were adjusted for age, sex, educational levels, MAPT group allocation, the number of chronic diseases, and global IC scores at baseline.

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IC, intrinsic capacity; IF1, inhibitory factor 1; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; RRR, relative risk ratio; TNFR-1, tumor necrosis factor receptor-1.

5.4 Interpretation of main findings

This work supported the heterogeneity of functional capacity during aging between individuals by identifying five IC multi-trajectories over four years among older adults aged ≥70. About half of the study population exhibited high and stable trajectories in cognition, locomotion, and psychology (i.e., "robust" and "robust with high vitality" groups), and around 8% of people showed impairments in all IC domains. We further observed that higher plasma IL-6, TNFR-1 and GDF-15 increased the likelihood of having the "low in all IC domains" trajectory. Older people with elevated IL-6 and GDF-15 levels also tended to suffer from lower and faster declining locomotion. Exploratory analysis showed that higher IL-6, TNFR-1, and GDF-15 remained associations with lower and faster declining IC trajectories when integrating IC domains as a single score.

- ***Comparing IC trajectories identified in our study and previous works***

Our findings are consistent with the literature, showing heterogeneity in IC trajectories among older individuals. The prior Taiwanese study (investigated 9448 individuals aged ≥50 under long-term care; **Figure 1.12**)[220] and our multi-trajectory analysis both found three patterns of IC domain impairment, including a group with impairments in almost all domains, a stable group with the highest proportion, and a group with relatively high IC than remaining subjects. Apart from these three basic patterns, other types of IC trajectories with impairments in specific domains were identified based on the investigated sample. For example, the Taiwanese study also recognized a trajectory group characterized by vision and locomotion impairments, probably because their work focused on individuals requiring long-term care services, which selected more people with sensory and physical dysfunctions that need care assistance. Conversely, our study found two trajectory groups with descending psychological function, indicating that psychological capacity is a crucial domain for individuals at risk of cognitive

decline like MAPT, whose psychological capacity can decline dramatically within years and requires early prevention. Given that neither the previous study nor ours used samples representing the general older population, future population-based research can provide further information on domain-specific IC trajectories.

We did not identify IC trajectories exhibited increased IC over time as the work of Salinas-Rodríguez et al.[208] (**Figure 1.11**). Several differences between Salinas-Rodríguez et al.'s study and ours are worth highlighting. First, the study sample in Salinas-Rodríguez et al.'s work was younger (aged ≥50 years) and was followed over a longer period (eight years). Second, unlike our study that enrolled clinical trial subjects at risk for cognitive decline, they used data from a nationally representative sample (SAGE cohort). Thus, their population may be more resilient to functional impairment, enabling the detection of increased IC. Finally, they applied self-reported difficulties to the sensory domain, which had the potential to improve after adaptation.

Interestingly, all IC trajectories in our study showed descending trends when integrating IC domains into a composite score (**Figure 5.4**). It may reveal the declining nature of IC in older adults, especially without appropriate IC monitoring and enhancing interventions. In addition, lower initial IC levels seem to correlate with faster IC declining rates in the future.

- ***Association of IC trajectories with plasma biomarkers***

GDF-15 outperformed other biomarkers by showing the strongest associations with IC trajectory groups. This may be due to and pleiotropic profile of GDF-15 and its connection with several aging hallmarks (Section 1.6). High GDF-15 had been suggested to mirror the underlying process of physiological decline, as it was strongly associated with acute events and shorter survival through the life course[258]. In this sense, increased GDF-15 levels may reflect the reduction of the physiological reserve, resulting in lower levels and a faster decline in IC domains. Furthermore, although not reaching statistical significance, our findings suggest that

GDF-15 levels might be able to differentiate robust people with high or low vitality levels. Of note, vitality has been considered a core domain representing the underlying physiological determinant of IC and shares similar concept with physiological reserve (Section 1.5.1).

As previously mentioned, IC trajectories determined by GBTM included both characteristics of baseline IC and pattern of decline. We could not exclude the possibility that our trajectory membership depends highly on baseline IC; people might be assigned to different trajectory groups mainly due to their differences in initial IC levels (of certain domains or the global levels) rather than longitudinal trends. In our exploratory analysis, associations with IC decline were attenuated for some biomarkers, especially IL-6, after controlling baseline IC. It means that the investigated biomarkers may correlate more with baseline IC levels (cross-sectional relationship) rather than longitudinal trends of trajectories.

GBTM has an advantage in detecting the latent groups of participants shared with non-a-prior characteristics. However, it is not the best way to evaluate how biomarkers affect the slope of IC over time. Future studies focusing specifically on the potential effect of biomarkers on IC change over time and using complementary statistical techniques, such as multi-level models, are needed.

5.5 Strengths and limitations

As one of the first works to investigate the biological risk factors of longitudinal IC trajectories, we highlight the use of GBTM to explore global and domain-specific trajectories and the comparisons between multiple aging biomarkers in a large population of older adults. There are some limitations to this research. First, the sensory domain was not considered in our IC measure. It is plausible that more IC trajectories may exist in older adults when the sensory domain is considered. Second, although we have measured biomarkers of inflammation and mitochondrial function, biomarkers of other biological aging mechanisms

were not included due to data availability. Third, this is a secondary analysis of a RCT composed of participants ≥70 years, with either subjective memory complaint or IADL limitation or slow gait speed. More studies are required to ascertain the generalizability of our findings to other populations. Finally, three-quarters of our population received MAPT interventions until the 36-month visit. However, our previous work did not observe any intervention effect on IC in MAPT[236]. We also adjusted the MAPT group allocation as a covariate in the regression analysis to minimize this bias.

CHAPTER 6. STUDY III: Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults.

The elements of this chapter came from a published paper: *Lu WH, Gonzalez-Bautista E, Guyonnet S, Lucas A, Parini A, Walston JD, Vellas B, de Souto Barreto P. Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults. J Cachexia Sarcopenia Muscle. 2023;14(2):930-39.* This original article is provided in the Appendix of this thesis.

6.1 Rationale for this study

In Study II, we recognized several limitations of GBTM in investigating IC and biomarkers. First, GBTM was not the best way to evaluate how biomarkers affect IC change over time because the trajectories contained both information on initial IC levels (intercept) and the slope of IC decline. It was necessary to confirm the longitudinal association of IC with plasma biomarkers used in Study II by a statistical technique retaining the continuous IC profile (without grouping participants by trajectory) and controlling the between-individual difference in initial IC levels. Therefore, we planned to use linear mixed-effect regression because it nests repeated measures within individuals and with a random effect for individual intercept and individual slope.

Focusing only on four IC domains was another major limitation in Study II. In Study II, we did not include the sensory domain because sensory data was only available for two time points in the thesis. Although sensory data was only available in the partial MAPT population with a relatively short follow-up (one year), we think it was important to examine the association of biomarkers with a complete 5-domain IC construct.

Finally, as aforementioned, there is no consensus on IC scaling methods in the literature (Section 1.4.3). To our knowledge, no study has examined different IC scaling methods within

the same population to ascertain whether they might result in distinct IC distributions and associations with specific variables.

Thus, this study used linear mixed-effect regression to evaluate the cross-sectional and longitudinal associations between plasma biomarkers and IC. IC was operationalized as 4-domain and 5-domain scores, respectively, using a modified POMP method with final IC scores ranging from 0 to 100 points. The association of plasma biomarkers with individual domains was also examined. We further conducted an exploratory analysis using z-standardization to operationalize IC scores to see if the main results remained.

6.2 Methods

Study source and population

This secondary analysis of MAPT recruited participants with available data on both plasma biomarkers and IC over four years (from the 12- to 60-month visits), leading to a sample of 1238 participants. Among them, 535 participants in the MAPT multidomain intervention groups (either combined omega-3 supplementation or not) underwent vision and hearing assessments at the 12-month and 24-month visits and were included to estimate the five-domain IC.

Plasma biomarkers

Plasma CRP, IL-6, TNFR-1, MCP-1, and GDF-15 were examined in this study. We did not investigate the association of IC with IF1 in this study because it showed weak associations with IC (Study II) and other aging biomarkers (Study I) in prior studies.

Intrinsic capacity (IC)

This study explored both 4-domain and 5-domain scores since they had different strengths. The

data availability of MAPT allowed us to obtain 4-domain IC scores, composed of MMSE (cognition), SPPB (locomotion), GDS (psychology), and handgrip strength (vitality), for the entire MAPT cohort over four years. The 5-domain IC construct consisted of the prior four domains plus the sensory is recommended by the literature (Section 1.5.1); however, we could only calculate the 5-domain IC scores among the MAPT subsample (n=535) for the one-year follow-up. Thus, in this study, we defined the primary outcome as the 4-domain IC score (available for a larger sample size with a longer length of follow-up) and the 5-domain IC score as a secondary outcome. The sensory capacity was defined as the average performance of the near-vision acuity (measured by a Monoyer chart) and the hearing ability (indicated by the HHIE-S).

To obtain the global IC score, we first rescaled the measurements of each domain into the 100-scale system according to the modified POMP method (Equation 5.1). As detailed in Section 5.2, we adapted the POMP method by defining the 0-100 IC scores as the range between the lowest and highest values on the measurement tools. The original scores of GDS (psychological capacity) and HHIE-S (hearing capacity) were weighted as -1 before rescaling to make the rescaled values have the same direction as other domains (i.e., higher indicates better function). The score of the sensory domain was calculated by averaging the rescaled values of the HHIE-S and visual acuity. The 4-domain IC score was determined as the arithmetic mean value of the four IC domains (cognition, locomotion, psychological, and vitality). The five-domain IC score was determined as the mean value of the five IC domains (plus sensory).

Statistical analysis

- ***Main analysis***

Linear mixed-effect regression creates an individual intercept (known as a random effect at the participant level) and an individual slope of outcome evolution (known as a random slope

on time) to consider the repeated outcome measurements of an individual (i.e., IC scores in the current study). A random effect at the participant level and a random slope on time were included in the mixed-effect models when examining the 4-domain IC score (i.e., four repeated measurements over four years). For the 5-domain IC score (i.e., two repeated measures over one year), only a random effect at the participant level was considered because the random effect on time was insignificant.

We first used a linear mixed-effect model to identify the longitudinal trend of IC in our study population. This model introduced only time and demographic covariates (age, sex, education, and MAPT group allocation) as independent variables. The coefficient of the time variable represented annual IC changes.

The association of IC scores with plasma biomarkers was examined by linear mixed-effect regression introducing plasma biomarkers and the interaction terms between plasma biomarkers and time as independent variables; a separate model was performed per biomarker. IC was evaluated as a global score and as separate IC domains. The biomarker coefficients in the models indicated the cross-sectional association with baseline IC, and the coefficients for biomarker-time interaction indicated the longitudinal association with IC over time (i.e., the degree of IC change varied as one unit of plasma biomarker increased). We kept all biomarker values in our main analysis because there are no established cut-offs for extreme values of plasma biomarkers in the literature. We further conducted a sensitivity analysis with biomarker outliers (defined as above or below four SDs from the population mean) removed to test whether such values have altered the associations.

- ***Exploratory analysis: using composite IC z-score***

As stated earlier, global IC scores were created by the modified POMP method. Since we were the first team to adapt this method to build a 100-scale IC index, we thought it was

necessary to validate our findings using the composite IC z-score, another IC scaling method that did not limit the scores from 0 to 100, as an exploratory analysis. To create composite IC z-scores, each domain's values were standardized by subtracting the baseline population mean and dividing by the baseline standard deviation. We combined individual z-scores from domains and divided the sum by the respective number of domains (four or five). The composite IC z-scores indicated the number of SDs by which an individual's IC levels deviated from the population average. A positive z-score indicated that the individual's value was above the mean, whereas a negative value showed the opposite. Person correlation was performed to evaluate the correlation between composite IC z-score and 100-scale IC scores derived from the modified POMP method. The association between composite IC z-score and plasma biomarkers was examined by linear mixed-effect models adjusted with demographic covariates.

6.3 Results

Characteristics of the study population

Baseline characteristics of study population is presented in **Table 6.1**. Of 1238 participants, the mean ± SD age was 76.2 ± 4.3 years, and 63.7% were female. The mean ± SD value of the baseline 4-domain IC score were 78.9 ± 9.3 of 100 points possible. The linear mixed-effect model showed that the annual IC decline rate was 1.17 (95% CI = -1.30 to -1.05) points per year on average, when measuring IC as a 4-domain construct (**Table 6.2**). Similar baseline IC score (78.9 ± 8.3) and annual IC decline rate (-0.93 points/year) were found using the five-domain measure (**Table 6.1 and 6.2**).

Being older age, female, and lower educational levels were significantly associated with faster IC decline. Conversely, receiving MAPT interventions were not associated with IC change over time (**Table 6.2**).

Table 6.1 Baseline characteristics of study population

	N	Median (IQR) or n (%)
Age (year), *mean (SD)*	1238	76.2 (4.3)
Female	1238	789 (63.7%)
MAPT group		
Multidomain intervention + omega-3	1238	309 (25.0%)
Omega-3		303 (24.5%)
Multidomain intervention		317 (25.6%)
Placebo		309 (25.0%)
Education		
No diploma	1238	62 (5.0%)
Primary school certificate		210 (17.0%)
Secondary education		408 (33.0%)
High school diploma		185 (14.9%)
University level		373 (30.1%)
Measurement for IC domain		
Cognitive: MMSE, 0-30	1238	29 (27, 29)
Locomotion: SPPB, 0-12	1217	11 (10, 12)
Psychological: GDS, 0-15	1233	3 (1, 4)
Vitality: Handgrip strength (kg)	1135	25 (20, 34)
Sensory:		
Visual acuity – Monoyer scale, 0-10	510	7 (5.5, 9)
HHIE-S, 0-40	536	4 (0, 12)
Global IC score, 0-100, *mean (SD)*		
4 domains (without sensory)	1115	78.9 (9.3)
5 domains (with sensory)	462	78.9 (8.3)
Plasma biomarker		
CRP, mg/L	1060	1.8 (1, 3.7)
IL-6, pg/mL	1062	2.57 (1.81, 3.8)
TNFR-1, pg/mL	1063	1142 (957, 1386)
MCP-1, pg/mL	1063	204 (170, 252)
GDF-15, pg/mL	1062	1003.5 (804, 1309)

Value presented in median (IQR) for continuous variables or number (%) for categorical variables except where indicated other. CRP, C-reactive protein; GDF-15, Growth differentiation factor-15; GDS, Geriatric Depression Scale; HHIE-S, Hearing Handicap Inventory for the Elderly – the Screening version; IC, intrinsic capacity; IL-6, interleukin-6; MAPT, Multidomain Alzheimer Preventive Trial; MCP-1, Monocyte chemoattractant protein-1; MMSE, Mini-Mental State Examination; SPPB, Short Physical Performance Battery; TNFR-1, Tumor necrosis factor receptor-1.

Table 6.2 Longitudinal changes of IC examining by linear mixed-effects regression

	4-domain IC score		5-domain IC score	
	β (95% CI)	**p**	**β (95% CI)**	**p**
Age at baseline (year)	-0.70 (-0.80, -0.60)	<0.001	-0.58 (-0.72, -0.45)	<0.001
Female	-7.82 (-8.69, -6.96)	<0.001	-6.40 (-7.59, -5.22)	<0.001
MAPT group				
Multidomain intervention + omega-3	0.11 (-1.05, 1.27)	0.853	0.11 (-1.02, 1.25)	0.844
Omega-3	-0.80 (-1.97, 0.38)	0.183	-	-
Multidomain intervention	-0.06 (-1.21, 1.09)	0.916	Ref	-
Placebo	Ref	-	-	-
Education				
No diploma	-4.56 (-6.55, -2.58)	<0.001	-5.35 (-8.17, -2.53)	<0.001
Primary school certificate	-2.63 (-3.89, -1.38)	<0.001	-4.30 (-6.10, -2.49)	<0.001
Secondary education	-1.15 (-2.19, -0.11)	0.030	-1.75 (-3.15, -0.35)	0.014
High school diploma	-0.40 (-1.71, 0.90)	0.545	-1.54 (-3.33, 0.25)	0.092
University level	Ref	-	Ref	-
Time (year)	-1.17 (-1.30, -1.05)	<0.001	-0.93 (-1.46, -0.39)	0.001

All models were adjusted for age, sex, MAPT group allocation and educational level.

Association between plasma biomarkers and IC

Table 6.3 displays the cross-sectional and longitudinal associations between plasma biomarkers and IC. Regarding the 4-domain IC, higher CRP, IL-6, TNFR-1, and GDF-15 were associated with lower IC scores at baseline. In addition, participants with higher TNFR-1, MCP-1, and GDF-15 had faster IC declines over time. Regarding the 5-domain IC, significant associations of IL-6, TNFR-1, and GDF-15 with IC were observed at the cross-sectional level. However, none of these biomarkers was associated with IC change over time (**Table 6.3**).

Sensitivity analysis removing the extreme plasma biomarkers values provided similar results, except for plasma TNFR-1, which was not associated with baseline 5-domain IC score after removing one outlier (adjusted β= -3.20; 95% CI: -8.38 to 1.98; p=0.225).

Table 6.3 Associations between plasma biomarkers and IC

	4-domain IC score		5-domain IC score	
	β (95% CI)	**p**	**β (95% CI)**	**p**
Cross-sectional				
CRP	-1.56 (-2.64, -0.48)	0.005	-1.13 (-2.70, 0.43)	0.156
IL-6	-3.16 (-4.82, -1.50)	<0.001	-2.76 (-5.19, -0.32)	0.026
TNFR-1	-6.86 (-10.25, -3.47)	<0.001	-5.01 (-9.99, -0.02)	0.049
MCP-1	1.08 (-1.99, 4.16)	0.490	0.01 (-4.28, 4.29)	0.998
GDF-15	-7.07 (-10.02, -4.12)	<0.001	-5.20 (-9.48, -0.92)	0.017
Longitudinal				
CRP	-0.13 (-0.45, 0.19)	0.429	0.40 (-0.96, 1.76)	0.566
IL-6	-0.36 (-0.85, 0.13)	0.150	1.59 (-0.52, 3.70)	0.139
TNFR-1	-1.28 (-2.29, -0.27)	0.013	-2.03 (-6.20, 2.14)	0.341
MCP-1	-1.33 (-2.24, -0.42)	0.004	0.25 (-3.52, 4.03)	0.895
GDF-15	-1.42 (-2.26, -0.58)	0.001	-1.24 (-4.66, 2.19)	0.479

All values of biomarkers were log-transformed; all models were adjusted for age, sex, educational level, and MAPT group allocation; CI, confidence interval; CRP, C-reactive protein; GDF-15, Growth differentiation factor-15; IC, intrinsic capacity; IL-6, interleukin-6; MCP-1, Monocyte chemoattractant protein-1; TNFR-1, Tumor necrosis factor receptor-1.

Association between plasma biomarkers and individual IC domains

The cross-sectional and longitudinal associations between plasma biomarkers and individual IC domains are presented in **Figure 6.1**. All plasma biomarkers showed associations with locomotion change after adjusting for demographic covariates (*CRP:* adjusted β [95% CI] = -0.77 [-1.44 to -0.09]; *IL-6:* adjusted β = -1.33 [-2.36 to -0.29]; *TNFR-1:* adjusted β = -2.49 [-4.61 to -0.36]; *MCP-1:* adjusted β = -2.10 [-4.04 to -0.16]; *GDF-15:* adjusted β = -2.69 [-4.45 to -0.93]). Elevated MCP-1 and GDF-15 were associated with worsening function in the psychological domain (*MCP-1:* adjusted β = -1.96 [-3.70 to -0.22]; *GDF-15:* adjusted β = -2.13 [-3.72 to -0.54]). Increasing MCP-1 levels were also associated with worse vitality over time (*MCP-1:* adjusted β = -1.48 [-2.94 to -0.03]). None of the five plasma biomarkers predicted the

change in the cognitive domain.

It is worth noting that we observed the marginal association between increasing IL-6 levels and improved sensory capacity (adjusted β = 4.50 [0.45 to 8.55]). However, we did not find individual associations of IL-6 levels with vision (adjusted β = 4.38 [-1.69 to 10.45]) and hearing (adjusted β = 3.54 [-1.32 to 8.41]).

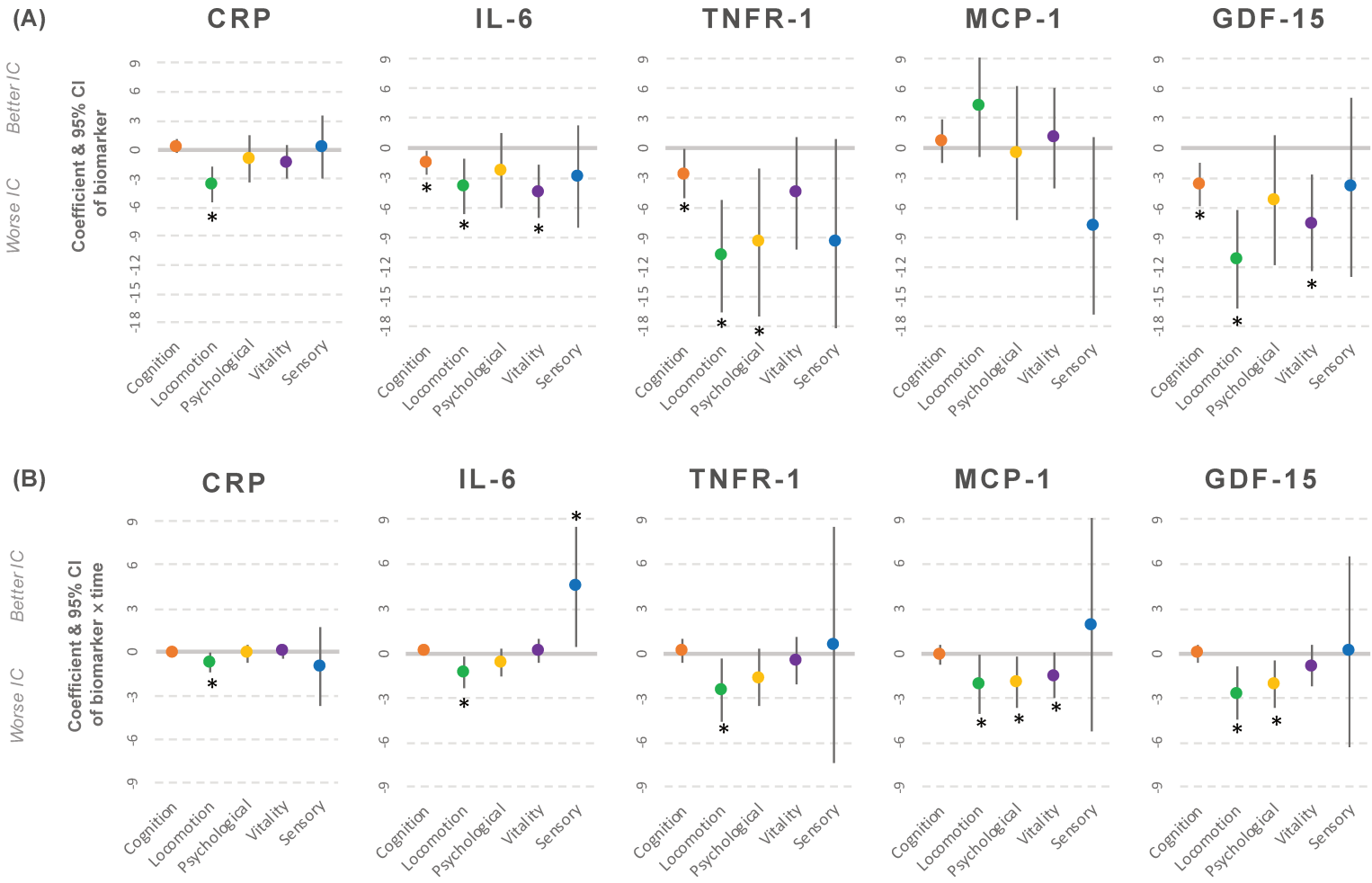

Figure 6.1 (A) Cross-sectional; (B) longitudinal associations between plasma inflammation-related biomarkers and IC domains

The sensory domain is composed of vision and hearing capacities. *p<0.05

Correlation between the 100-scale IC score and composite IC z-score

There is a high correlation between IC scores from the two scaling approaches. The Pearson correlation coefficient (r) between the 100-scale IC score and IC composite z-score over the observation period was 0.97 ($p<0.001$) for the 4-domain IC construct and 0.86 ($p<0.001$) for the 5-domain IC construct.

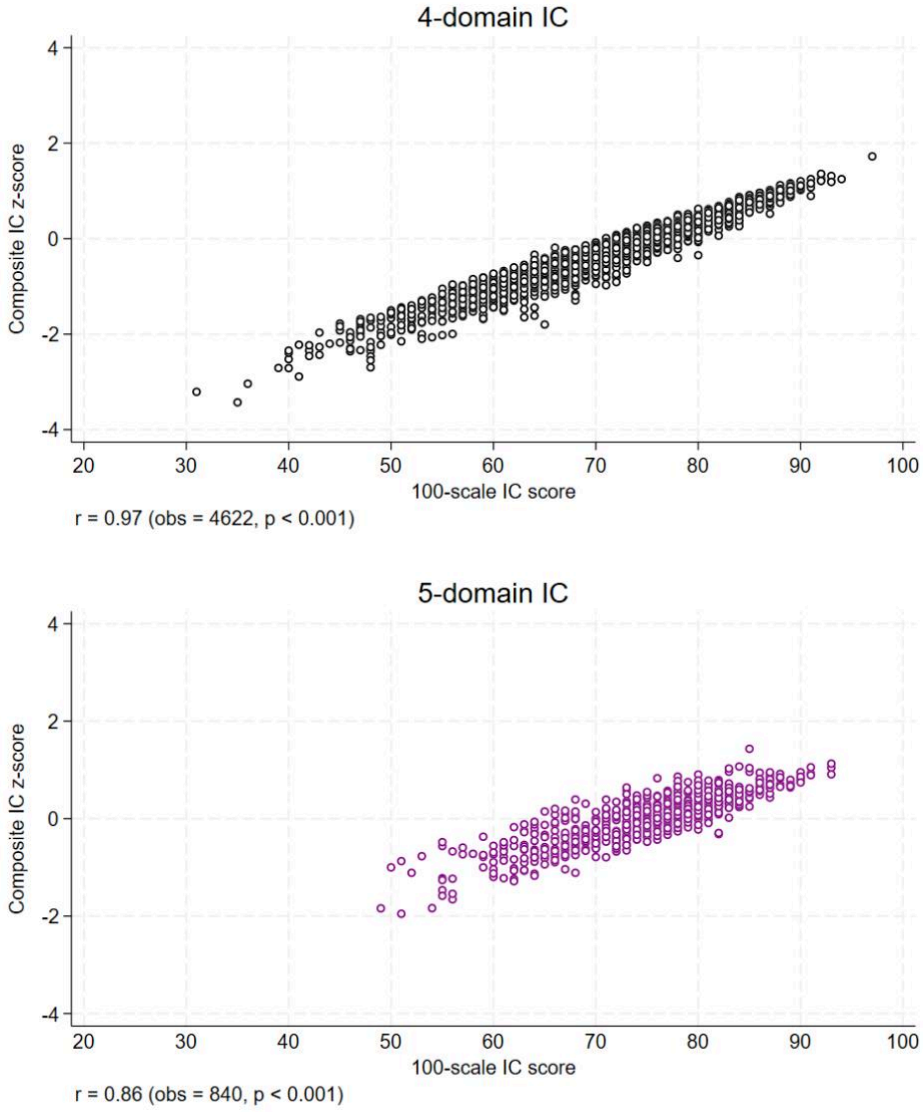

Figure 6.2 Scatterplots of the 4-domain IC scores (up) and the 5-domain IC scores (bottom) using two scaling approaches

The Pearson correlation coefficients (r), number of observations, and p-values are presented in the lower-left corner.

Association between plasma biomarkers and IC using composite IC z-score

Sensitivity analysis using composite IC z-score provided similar results, except for TNFR-1, which remained an inverse relationship with 4-domain IC at the longitudinal level but did not reach statistical significance (adjusted β= -0.06; 95% CI: -0.13 to 0.01; p-0.096).

Table 6.4 Associations between plasma biomarkers and IC composite z-score

	4-domain IC score		5-domain IC score	
	β (95% CI)	**p**	**β (95% CI)**	**p**
Cross-sectional				
CRP	-0.09 (-0.17, -0.02)	0.014	-0.11 (-0.21, -0.01)	0.024
IL-6	-0.24 (-0.35, -0.12)	<0.001	-0.21 (-0.36, -0.05)	0.008
TNFR-1	-0.51 (-0.75, -0.27)	<0.001	-0.27 (-0.58, 0.05)	0.094
MCP-1	0.08 (-0.14, 0.29)	0.480	0.14 (-0.13, 0.41)	0.302
GDF-15	-0.54 (-0.74, -0.34)	<0.001	-0.38 (-0.64, -0.11)	0.006
Longitudinal				
CRP	-0.01 (-0.03, 0.01)	0.511	0.04 (-0.05, 0.13)	0.355
IL-6	-0.02 (-0.05, 0.02)	0.375	0.13 (-0.01, 0.28)	0.066
TNFR-1	-0.06 (-0.13, 0.01)	0.096	-0.04 (-0.32, 0.25)	0.807
MCP-1	-0.07 (-0.14, -0.01)	0.023	-0.01 (-0.27, 0.25)	0.926
GDF-15	-0.08 (-0.14, -0.02)	0.009	-0.05 (-0.28, 0.19)	0.681

All values of biomarkers were log-transformed; all models were adjusted for age, sex, educational level, and MAPT group allocation; CI, confidence interval; CRP, C-reactive protein; GDF-15, Growth differentiation factor-15; IC, intrinsic capacity; IL-6, interleukin-6; MCP-1, Monocyte chemoattractant protein-1; TNFR-1, Tumor necrosis factor receptor-1.

6.4 Interpretation of main findings

In summary, Study III demonstrated that higher TNFR-1 and GDF-15 were consistently associated with 4-domain IC, consisting of cognition, locomotion, psychological and vitality capacities, at both cross-sectional and longitudinal levels. However, none of the five plasma biomarkers was longitudinally associated with IC after considering the sensory domain (the 5-domain construct). Domain-specific analysis revealed that all biomarker concentrations

predicted the locomotion change over time. Taken together, our results supported that inflammation, measured via plasma TNFR-1, MCP-1 and GDF-15, may be involved in the faster IC decline of older individuals.

Despite the slight differences in the measurement for each IC domain, our study population presented similar baseline IC levels (78.9 ± 9.3 points) from Stolz et al.'s work in USA (77 ± 11 points; mean age 78.4 years)[192] and was relatively higher compared with the Mexican population in the SAGE study (range from 36.9 to 61.2 points in a cohort aged ≥50 years)[208]. We demonstrated that IC decreased 1.2 points per year in older adults. Furthermore, a 10-fold increase in plasma levels of TNFR-1, MCP-1 and GDF-15 was associated with an additional 1.3–1.4 points of IC decline per year. Although there is no established cut-off for clinically meaningful IC decline, Stolz et al.[192] found that a 1-point decrease in IC was associated with a 7% increased risk of ADL disability and a 5% increased risk of mortality in older adults. Taken together, the inflammation-related IC decline in older individuals may indicate a doubling or even higher risk of adverse health outcomes in the future.

- ***Association between plasma inflammation-related biomarkers and IC***

The current study showed significant longitudinal associations with IC in plasma TNFR-1, MCP-1, and GDF-15. It is worth noting that the exploratory analysis in Study II, which additionally adjusted for the initial IC levels as covariates to examine the associations with impaired IC trajectories, also observed the same markers remaining significant associations with IC trajectory groups (**Table 5.8 and 5.9**). The consistency between these two analyses confirmed that high TNFR-1, MCP-1, and GDF-15 were more correlated with the rate of IC decline, while increased CRP and IL-6 were more correlated with the current IC levels.

Concerning the domain-specific analysis, all plasma biomarkers were associated with locomotion decline. These five biomarkers were all related to chronic inflammation; hence, the

findings potentially suggest that locomotion could be the initial or most vulnerable domain of IC affected by chronic inflammation. Conversely, none of the plasma biomarker levels predicted cognitive capacity changes. This result was compatible with previous research on inflammatory markers and cognitive function, which showed associations between circulating IL-6 and cognitive declines in the studies with longer follow-up (7–9 years)[112,116] but not in the one with short observation (median 2.7-year follow-up)[120].

Circulating CRP, IL-6 and TNFR-1 have been commonly regarded as markers of systemic inflammatory response in literature (Section 1.4.3). In the current study, only plasma TNFR-1 was associated with longitudinal IC change, suggesting that TNFR-1 may be a more reliable marker of inflammation-related functional decline than CRP and IL-6. Indeed, TNFR-1 levels are relatively stable in circulation[259]. Similar results were observed in the prior study, which found significant associations of cognitive decline with TNFR-1 but not with IL-6[117].

Despite lack of association with baseline IC, higher plasma MCP-1 levels were associated with longitudinal IC declines, particularly in the locomotion, psychological and vitality domains. MCP-1 has been known to enhance neuroinflammation and leads to cognitive impairment[260]; however, studies on plasma MCP-1 with other functional outcomes are poorly investigated. To the best of our knowledge, this is the first study that found that higher MCP-1 was prospectively associated with physical function and handgrip strength (as vitality). MCP-1 had been recognized as a marker of aging through cellular senescence[246]. However, compared with other inflammation-related biomarkers in this study, the evidence on MCP-1, cellular senescence and age-related outcomes is scarce and remains to be clarified by more updated research.

- ***Composite IC z-scores***

As detailed in Section 5.2, we adapted the POMP method by defining the 0-100 IC scores as the range between the lowest and highest values on the measurement tools. Since we were the

first team to apply the modified POMP method to create an IC index, we thought it was necessary to validate our findings using the composite IC z-score, another IC scaling method that did not limit the scores from 0 to 100. Our sensitivity analysis confirmed that IC scores exhibited a very similar distribution across the study sample regardless of the scaling methods used (**Figure 6.2**). Additionally, using different scaling methods had a weak impact on the evaluation of the association with plasma biomarkers, with only the association of TNFR-1 becoming statistically insignificant but remaining a trend with IC.

6.5 Strengths and limitations

This is one of the first works to investigate the associations between plasma inflammation-related biomarkers and IC using a longitudinal approach and appropriate statistical technique. Both the 4-domain and 5-domain construct of IC were tested using two different scaling methods proposed in previous studies. However, significant IC-biomarker associations discovered in this study required careful interpretation due to some limitations. First, the MAPT Study enrolled subjects at risk of cognitive decline, which might affect the generalizability of our results to other populations. Second, we measured plasma biomarkers in a subset of MAPT participants one year after the study enrolment. Because three out of four subjects in the current study had received interventions, it is not excluded that their biomarker levels may be affected by MAPT intervention. Although our analyses added MAPT group allocation as a covariate to minimize this bias, the intervention effects cannot be omitted, particularly in the analyses for five-domain IC and sensory domains. In other words, the lack of association in our study may be related to the potential beneficial effect from interventions and require cautious interpretation. Finally, the full-domain IC including sensory was only explored in half of our population for 1-year follow-up. Future longitudinal studies operationalizing the complete IC domains with several years of follow-up and a large sample are required.

CHAPTER 7. INTEGRATIVE DISCUSSION

7.1 Summary of main findings

This doctoral thesis suggested that inflammation was associated with older individuals' IC as an operational definition of vitality capacity and as a correlate (in cross-sectional associations) and a predictor of longitudinal IC evolution.

In Study I, our results indicated that low vitality capacity contributed to functional disability primarily by mediating other expressed IC domains. A composite index of plasma inflammation-related biomarkers could serve as a vitality measure, which predicted the evolution of locomotion and IADL difficulties over time.

In Study II, we identified multiple longitudinal IC trajectories in the older population. About half of the total population exhibited high and stable trajectories in cognition, locomotion, and psychology, and around 8% of people showed impairments in all IC domains. Higher plasma IL-6, TNFR-1 and GDF-15 distinguished older people with multi-impaired IC trajectories from those with high-stable IC. GDF-15 outperformed other biomarkers by showing the strongest associations with IC trajectory groups.

In Study III, increased levels of TNFR-1 and GDF-15 in blood were consistently associated with IC at both cross-sectional and longitudinal levels. However, the longitudinal association between inflammatory biomarkers and IC did not retain after including the sensory domain (a 5-domain IC construct).

7.2 Methodological considerations for the studies in this thesis

Despite the studies in this thesis applied sound methodology, there is still room for improvement in the following aspects: (1) a relatively healthy study sample, (2) potential selective attrition during the longitudinal follow-up, and (3) outcome variables that may not be randomly missed

in the datasets.

- **A relatively healthy study sample**

All studies in this thesis were derived from the MAPT Study cohort. Participants eligible to take part in the MAPT Study might be healthier than the general older population, as individuals with diagnosed dementia, MMSE <24, and any ADL limitations were excluded (Section 3.1.1). In addition, the volunteer bias occurs when recruiting older participants for intervention studies since people who accepted to participate are more likely to be younger, functionally and physically more active, and have a slower rate of decline in physical and psychological functioning than those who declined or withdrew[261]. The issue of a relatively homogeneous and healthy older cohort should be aware of when interpreting IC trajectories since we may underestimate the diversity of IC patterns and the rate of IC decline.

- **Potential selective attrition during the longitudinal follow-up**

In longitudinal studies of older adults like the MAPT Study, participants may experience declining health or severe adverse events (i.e., death and institutionalization) that prohibit them from continuing to participate in the studies[262]. Early withdrawal of participants from the study may introduce selection bias, as we may exclude participants most likely to experience accelerated IC decline and onset of disability, our outcomes of interest, and thus underestimate the association between inflammation and outcomes. Adjusting for baseline functional levels as a predictor of both study drop-out and functional outcome is not recommended, as it could introduce another bias into estimates of the effect of an exposure on the outcome[263,264].

- **Outcome variables that may not be randomly missed in the datasets**

Building upon the earlier statement, the selective attrition during the longitudinal follow-up implies that our outcome data may not be missing at random (MAR). This is against the

assumption of GBTM and linear mixed-effect regression in our studies and may lead to biased estimates. Innovative methods such as inverse probability weighting (IPW) may reduce this bias, in which participants are weighted according to the probability of non-response or censoring[265]. However, the validity of IPW relies on the logistic model for estimating weight, including all prognostic variables associated with non-response or censoring. Thus, the results can be more controversial if a correctly specified logistic model cannot be assured[266].

7.3 Implication of plasma biomarkers for quantifying vitality capacity

Study I demonstrated that a composite measure of plasma biomarkers related to inflammation and energy metabolism might be operationalized as vitality capacity. Still, it was not superior to nutritional and neuromuscular indicators. Given that vitality capacity covers a broad range of biomolecular systems (illustrated in **Figure 1.9**), combined panels of plasma biomarkers from multiple attributes will be recommended in future research.

The question of which biomarkers should be used to quantify vitality remains difficult. The meeting for the WHO working definition of vitality capacity highlighted that ideal biomarkers of vitality are clinically implementable, informative for monitoring (i.e., change over time and are responsive to interventions), and feasible to collect in low-resource settings[194]. Indeed, IC was conceptualized as a metric that could be easily assessed and monitored in the healthcare system; thus, laboratory biomarkers already used to diagnose or exclude certain diseases (e.g., albumin and hemoglobin A1c [HbA1c]) should be a priority choice. These biomarkers are unavailable in MAPT but are well-collected in several population-based cohorts and some claims databases. Future studies applying routine laboratory biomarkers, together with other common clinical markers (i.e., blood pressure and respiratory muscle strength), into our path models in Study I could contribute knowledge on their utility for quantifying vitality.

As combining different attributes of vitality measures is an inevitable trend, how to integrate

these biomarkers technically needs to be solved. Techniques like factor analysis can efficiently condense multiple different-scale markers into one or few variables (i.e., dimension reduction); however, the complicated statistical process might be a barrier to clinical application. Clinical practice requires measurements that are easy to process. A balance between clinically feasible and maintaining individual distinctiveness will be a challenge for future vitality studies.

7.4 Implication of plasma biomarkers for predicting functional decline in older adults

The strength of this thesis are the longitudinal design and inclusion of novel markers like GDF-15, which contribute knowledge regarding IC and inflammation. Study II and III results align with the literature showing that TNFR-1 and IL-6 are more prominent markers of inflammation in age-related functional decline. In addition, GDF-15 shows potential in predicting IC decline, even better than the traditional inflammatory markers like TNFR-1 and IL-6, probably because multiple types of stressors other than chronic inflammation, such as oxidative stress, mitochondrial dysfunction, impaired autophagy, and endoplasmic reticulum stress can stimulate its upregulation[64]. The nature of the observational design did not allow us to distinguish a causal relationship between increased levels of inflammatory mediators and decreased IC. Still, these findings provide perspective in clinical and geroscience research aspects.

7.4.1 Clinical aspect

Functional decline in old age is considered a complex mechanism caused by the dysregulation of multiple physiological systems, and the process involves changes in numerous molecules and signaling pathways. The clinical practice seeks low-cost, easy-to-implement, and highly predictive biomarkers in major clinical events to help physicians risk-stratify patients for better care planning. The fact that a biomarker is a bystander or consequence rather than a true instigator of damage has less impact on its utility in risk stratification.

The innovative biomarkers used in the thesis, like GDF-15 and IF1, have great potential in aging research, but they still require more investigation before applying to clinical practice. With respect to plasma IL-6 and TNFR-1, which were often used for measuring inflammation in clinical trials and epidemiologic cohorts, we are still uncertain about how to define the "abnormal" values of these biomarkers in a way that correlates with clinical significance (detailed in Section 5.5).

Another unsolved question before utilizing these biomarkers in clinical practice is how we should "react to" these abnormal values. The information is lacking because the majority of evidence comes from the individual relationship between anti-inflammation interventions and the change of inflammatory markers, and between the high inflammatory markers and poor health prognosis (**Figure 7.1**). The study design of the ENRGISe Study[267] is a good example for future research in this field, which attempted to answer if decreasing inflammatory marker levels by interventions could have a beneficial impact on preserving function in older adults (**Figure 7.2**).

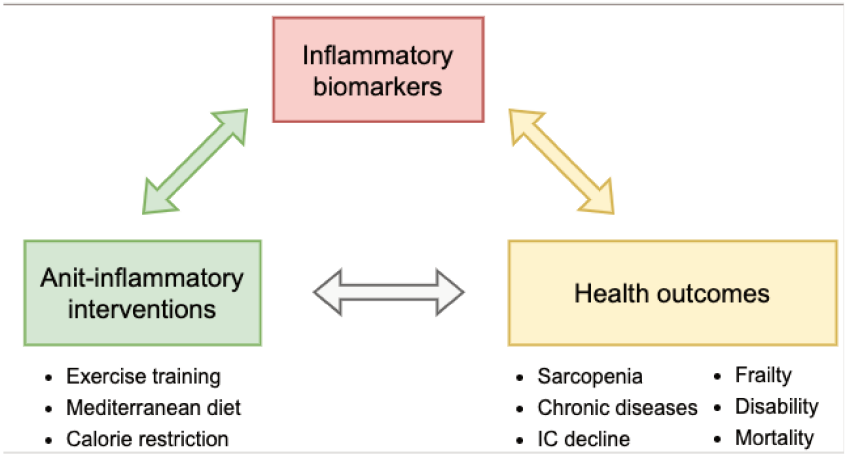

Figure 7.1 Diagram of the relationship between anti-inflammatory interventions, inflammatory biomarkers, and functional outcomes

Exercise training, Mediterranean diet, and calorie restriction are potential interventions to decrease the levels of inflammatory markers, with prior RCTs observing their anti-inflammatory effects in adults[268–271]. The majority of the previous studies only focused on associations between each two elements.

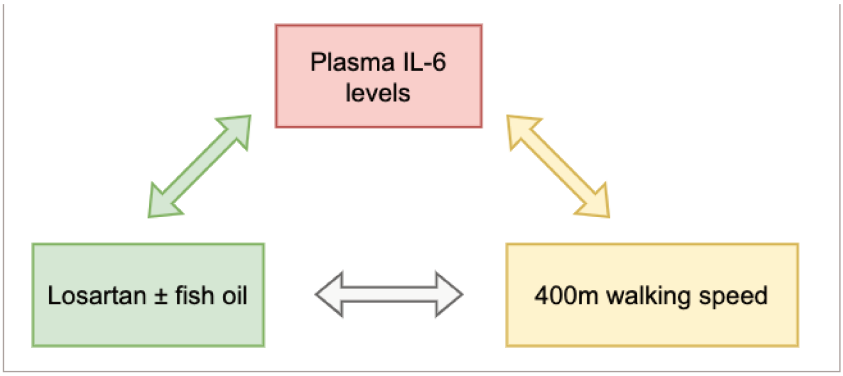

Figure 7.2 Example of ENRGISe Study

The ENRGISe Study enrolled older participants with elevated IL-6 levels at baseline and investigated the effects of losartan and fish oil on walking speed and change in IL-6 levels. The pilot study, however, did not demonstrate a beneficial effect on walking speed or IL-6 levels after 1-year intervention[267].

7.4.2 Geroscience research aspect

Upregulated levels of GDF-15 are one of the most promising markers of aging and age-related diseases, and our thesis supports its potential in predicting functioning outcomes. One of the remaining challenges about GDF-15 is related to the unclear pathways involved in age-related changes and the correspondent receptors[64]. Currently GFRAL is the only known receptor for GDF-15, whose expression is limited on the cell surface of neurons within the brainstem area and functions to reduce food intake and subsequent weight loss[272]. It remains unknown about the role of the GDF15-GFRAL pathway in age-related conditions or whether GDF-15 acts on other peripheral tissues through other GDF15-specific receptors. As the stressors of GDF-15 upregulation seem to be linked to different hallmarks of aging, clarifying the GDF-15 targeted cells and receptors and the following signal pathways may help understand how aging hallmarks interrelate.

7.5 Perspective for measuring chronic inflammation

The current literature lacks a standard measure to quantify a chronic LGI, including established

biomarker levels and exposure time frames to exclude confounding by acute events. Plasma CRP, IL-6, and TNFR-1 have been used to measure an inflammatory state in humans for decades. Still, very few studies have been dedicated to investigating an appropriate way to measure LGI using these markers. To our knowledge, the threshold of defining LGI had been proposed for CRP (3-10 mg/L)[211] and IL-6 (2.5-30 pg/mL)[273]. Of note, only the cut-off of IL-6 >2.5 pg/mL was selected based on an existed evidence showing that people with IL-6 >2.5 pg/mL were at higher risk of mobility disability[274]. Other threshold values, however, were determined by clinical experience[59] or findings on cardiovascular risk[61]. The definition of "chronic" inflammation was also unknown. The time interval for repeated measuring biomarkers ranged from 6 months to 5 years in previous studies, and most studies defined chronic inflammation based on two measurements of biomarkers[119,211,275].

In order to define a standard definition of chronic LGI, future studies are encouraged to have two or even more measurements of the investigated plasma biomarkers to consider the evolution of biomarker concentrations. The information at the moment of assessing blood sample potential factors that may influence inflammatory marker evolution should be collected in order to consider their confounding effects, particularly the factors that can help us exclude acute inflammation (such as recent hospitalization, trauma or infectious diseases) and the anti-inflammatory medications that might directly influence inflammatory mediators (such as the cytokine receptor antagonists). Retrospective studies investigating the inflammatory marker patterns in people with functional impairment known to be related to age-related chronic inflammation (e.g., sarcopenia and cognitive impairment) can provide insights into determining appropriate monitoring intervals.

Furthermore, previous studies showed that inflammatory marker levels increase gradually with age[52] and, depending on markers, may exist sex differences[276] (**Figure 7.3**). Whether the

thresholds of LGI should be established separately for young and middle-aged individuals (under 60 years) and older populations (above 60) remains investigation. Potential sex differences on LGI cut-offs should also be explored. One will need to use cohorts with large enough sample sizes to answer these questions to ensure sufficient statistical power. Last but not least, the proposed LGI definitions should be validated in different population-based cohorts.

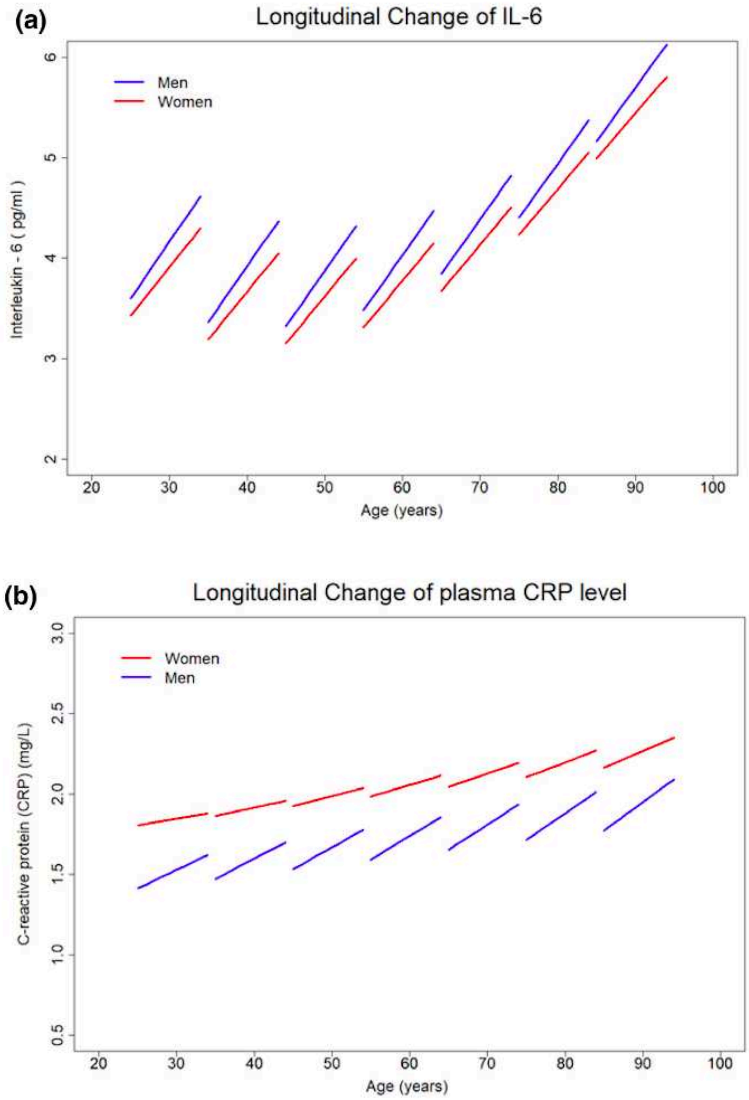

Figure 7.3 Longitudinal changes in IL-6(a) and CRP(b) levels by age groups and sex observed in the Baltimore Longitudinal Study of Aging

Data source: *Kuo PL, et al. J Intern Med. 2020;287(4):373-394.*[276]

7.6 The role of anti-inflammatory activity in healthy aging

In 2007, Franceschi et al. proposed that extended longevity is a consequence of balancing pro-inflammatory and anti-inflammatory network activities[36,277]. This idea came from the paradox of centenarians; that is, these long-living people experienced increased pro-inflammatory molecules but escaped from major age-related diseases with a strong inflammatory pathogenetic component. Franceschi et al. further hypothesized that, in centenarians, the development of inflammaging was compensated by the concomitant development of robust and efficient anti-inflammatory responses and by having gene polymorphisms related to reduced inflammatory responses[277]. When young, these people might be more susceptible to infectious diseases, but they survive longer in late life (**Figure 7.4**).

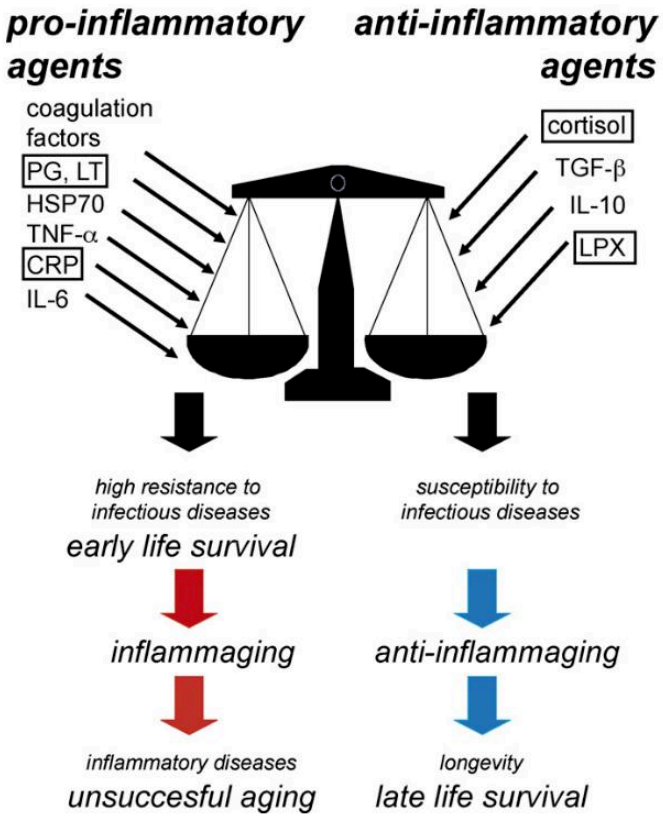

Figure 7.4 The balancing between pro- and anti-inflammatory agents and their relation with longevity

Data source: *Franceschi C, et al. Mech Ageing Dev. 2007;128(1):92-105.*

As Franceschi et al. noted in their paper published in 2007, the role of anti-inflammatory activity in successful aging is still under debate due to insufficient data. After so many years, research investigating how the anti-inflammatory activity changes during normal aging process is still lacking, and most studies on inflammaging ignore the role of anti-inflammatory activity[278] . Following Franceschi et al.'s hypothesis, we believe that age-related dysregulation in the pro-inflammation/anti-inflammation network should be characterized by a measure that considers both pro-inflammatory and anti-inflammatory activities. The iAge® created by the Stanford team in 2021 is a good example of integrating both pro-inflammatory and anti-inflammatory markers in serum to represent "the biological age of the immune system" (**Figure 7.5**).

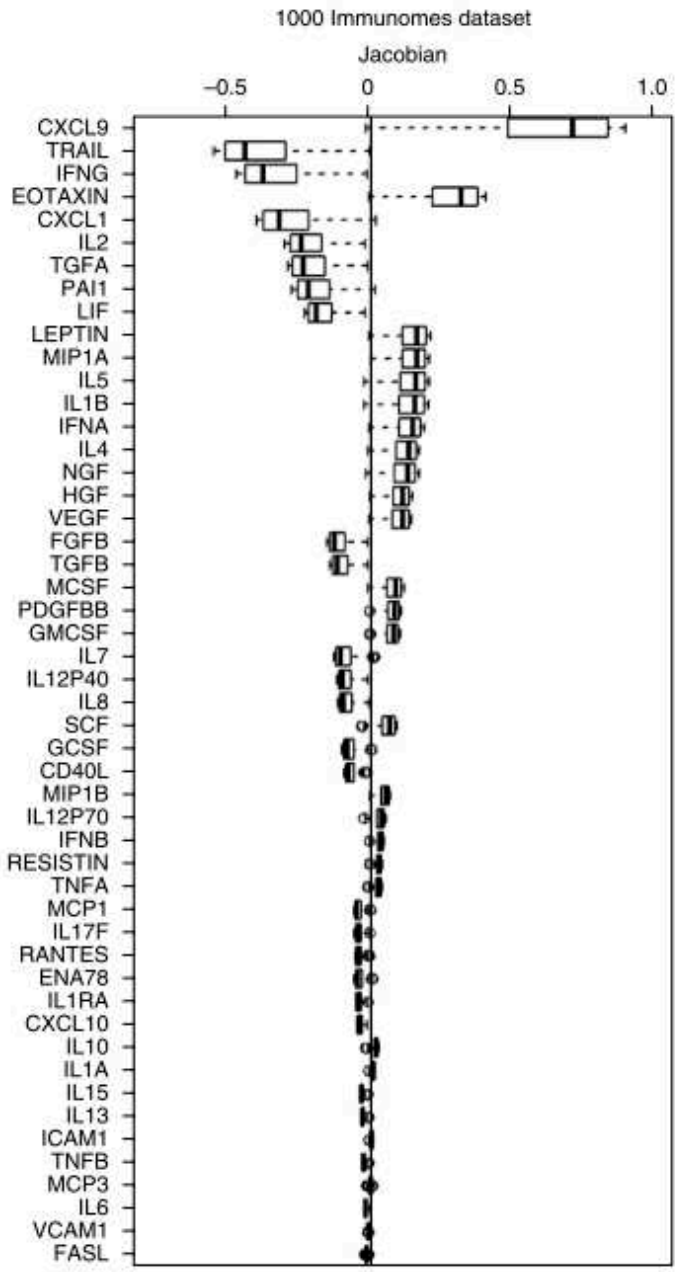

Figure 7.5 The iAge contained both pro- and anti-inflammatory immunomes identified by artificial intelligence

Data source: *Sayed N, et al. Nat Aging. 2021;1:598-615.*

As the anti-inflammatory network involves processes outside the immune system, such as neuroendocrine pathways and anti-inflammatory hormone production[279], whether these molecules change with aging and collectively determine healthspan and should be included in the future measure of inflammaging awaits more investigation.

7.7 Physical resilience and chronic inflammation

IC, frailty, and physical resilience are three entities showing distinct health attributes[6,280,281]. IC focuses on a person's biological capacity to cope with daily activities and achieve personal accomplishment. Conversely, frailty describes a state of vulnerability to internal or external stressors due to accumulated deficits in physiological systems. Like IC, resilience is a positive attribute and generally refers to the capacity to bounce or spring back after a stressful encounter or adversity[282]; it has been applied to a wide range of systems ranging from cells to individuals to communities, and to psychological, physiologic, clinical, and social outcomes[283]. Physical resilience at the individual level is defined as a characteristic that determines one's ability to resist or recover from functional decline after an acute or chronic health stressor[284]. The WHO healthy aging model also encompasses the concept of physical resilience, which describes it as the capability to maintain or improve functional ability in the face of adversity in life[4]. Jean Woo initially described IC as one of the determinants of physical resilience[285] and later supported by Chhetri et al.[280], as both IC and physical resilience are linked by the same underlying physiological changes. Since IC can be reversible with interventions, appropriate actions that enhance IC may also improve physical resilience[281]. Currently, the connection between IC and physical resilience remains a conceptualized idea, lacking support from animal or human data. Therefore, it would be intriguing to further validate whether individuals with low and rapidly declining IC (as discovered in this thesis) also exhibit reduced resilience to acute health stressors. Additionally, investigating whether interventions to reverse or maintain IC can enhance resilience would provide valuable insights[286].

Given that both IC and physical resilience are connected to physiological reserve, biological mechanisms that reduce or impair physiological reserve, including chronic inflammation, might impact not only IC but also physical resilience. Indeed, the immune system has long been known to modulate our response to stress[287]. There is emerging evidence on the role of

dysregulated immune responses on resilience[288] or how it might mediate the adverse outcomes in older adults from acute illness[289]. Although Ryan and Ryznar proposed inflammatory mediators may be one of the promising biomarkers of resilience[287], only a preliminary study has explored the association between plasma inflammatory markers and physical resilience among older patients with cancer[290]. Establishing the same association of inflammatory biomarkers with physical resilience in general older adults is needed to confirm their utility as resilience biomarkers.

7.8 Connecting chronic inflammation with other hallmarks of aging and phenotypic aging measures

All biological aging pathways may eventually lead to a common state of low physiological reserve and resilience and an increased susceptibility to age-related disease and functional impairment[291]. Ideally, multiple biomarkers representing different aging mechanisms can be combined to predict aging-related conditions, with markers complementing each other, thereby improving the predictive power[292]. Belsky et al. used different methods to quantify biological aging (**Figure 7.6**) and found low agreement between different measurements, implying that they measure different aspects and levels of the aging process[293]. López-Otín et al. also highlighted in their latest paper that hallmarks of aging are connected to the eight strata of organismal organization, from the molecular to the meta-organismal levels (**Figure 7.7**)[20]. Events that cause health perturbations start from a single level and propagate up or down to other strata[294]. Collectively, no matter where it starts, the aging process does not rest at a single level/marker but spreads out in multiple dimensions and interacts with multiple hallmarks, leading to complex aging features.

		Measurement Design			
		Cross-Sectional	Longitudinal		
Variety of Assays	Single Assay	Telomere Length, Epigenetic Clocks	Telomere Erosion, Epigenetic Ticking	Cellular-Level Measures Assayed in Blood	Biological Level of Measurement
	Multiple-Assay Composite	KDM Biological Age, Age-Related Homeostatic Dysregulation	Pace of Aging	Patient-Level Measures Derived From Assays of Multiple Organ Systems	

Figure 7.6 The biological aging measures in Belsky et al.'s work can be divided into cellular and patient (organismal) levels

Data source: *Belsky DW, et al. Am J Epidemiol. 2018;187(6):1220-1230.*

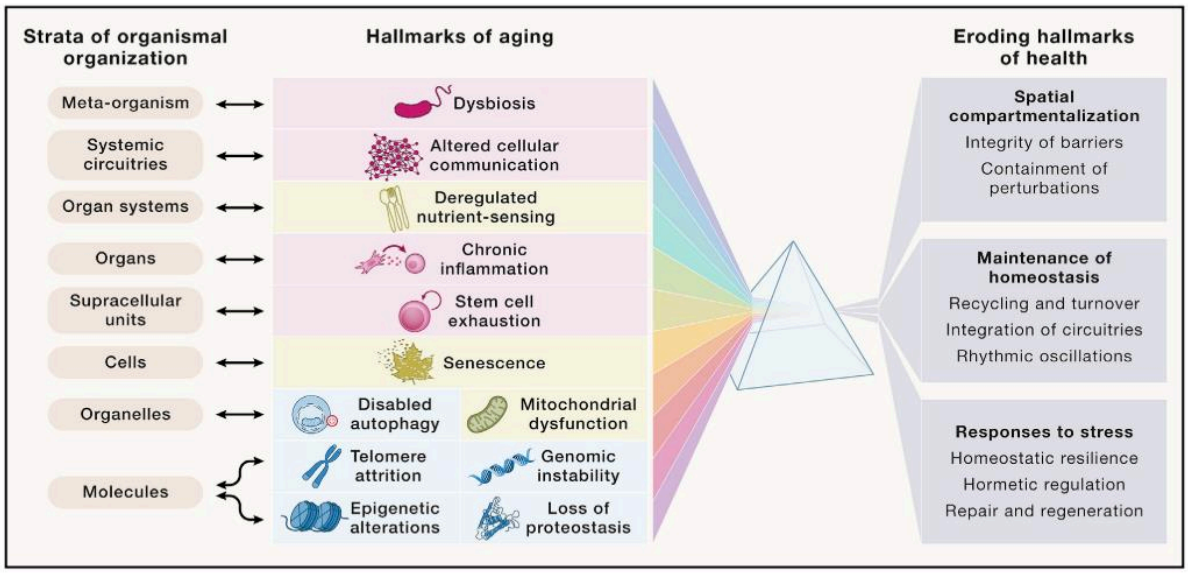

Figure 7.7 Eight strata of organismal organization and twelve hallmarks of aging

Data source: *López-Otín C, et al. Cell. 2023;186(2):243-278.*

Indeed, growing evidence in cellular and animal studies has shown that chronic inflammation is closely related to other aging hallmarks. From an epidemiological perspective, the next question is whether chronic inflammation is accompanied by impairments in other hallmarks,

and whether they together determine the onset of phenotypic aging outcomes. For instance, how many times of the frailty and disability risks increase in individuals with chronic inflammation when other hallmarks are impaired? Do older adults with epigenetic age acceleration or shorter telomere lengths but with normal levels of inflammatory markers are more likely to stay functionally intact? Do people under a chronic inflammatory state and accompanied by mitochondrial dysfunction, cellular senescence, or impaired proteostasis pathways (i.e., various origins of inflammatory sources) have different rates and patterns of functional decline?

Interestingly, Belsky et al. showed that the clinical-biomarker composite was superior in predicting age-related outcomes than single, cellular-level markers[293]. Similar results were observed in their other work using another nationally-representative cohort[295]. We may say that phenotypic and functional measures (which are outcome measures themselves) are important predictors for immediate / short-term risk of dependency, whereas biomarkers can be used as an earlier, preventative marker of risk stratification. Phenotypic and functional measures are still potent markers of unhealthy aging, which can thus supplement the biological measure to identify individuals who will derive the greatest benefit from geroprotective agents[291]. As accelerated IC decline is one of the earliest markers of phenotypic aging, we envision that IC would help the discovery of future biomarkers of aging and collectively serve as targets for geroscience intervention trials.

7.9 Future works on IC and biomarkers of aging in the INSPIRE program

The ongoing INSPIRE program in Toulouse could potentially provide insights into understanding the interplay between IC and biomarkers from aging hallmarks. The INSPIRE program aims to foster research in geroscience and healthy aging[296]. Both cohort of animal (the INSPIRE Animal Cohort) and human (the INSPIRE Human Translational Cohort;

named as the INSPIRE-T cohort) were built to explore potential biomarkers of aging, age-related diseases/disabilities (**Figure 7.8**). The INSPIRE Animal Cohort was established with the intention of "mirroring" the INSPIRE-T cohort of humans, thus facilitating the translation of findings from basic research to clinical research[297]. The animal cohort includes models of Swiss mice and African Killifish designed to mimic the human accelerated aging phenotypes (such as frailty and functional decline)[296]. In the INSPIRE Animal Cohort, we can more comprehensively investigate alterations within inflammatory and anti-inflammatory networks throughout life course as mice undergo both normal aging and an accelerated aging phenotype. Such investigations could potentially lead to the identification of crucial biomarkers associated with accelerated aging within anti-inflammatory networks, which could further translate to the INSPIRE-T human cohort. Furthermore, within the animal model, it becomes feasible to systematically assess physical resilience through appropriate stressors, thereby allowing for the observation of the trajectory of physical resilience and its intricate interplay with dysregulated immune function and cytokine production.

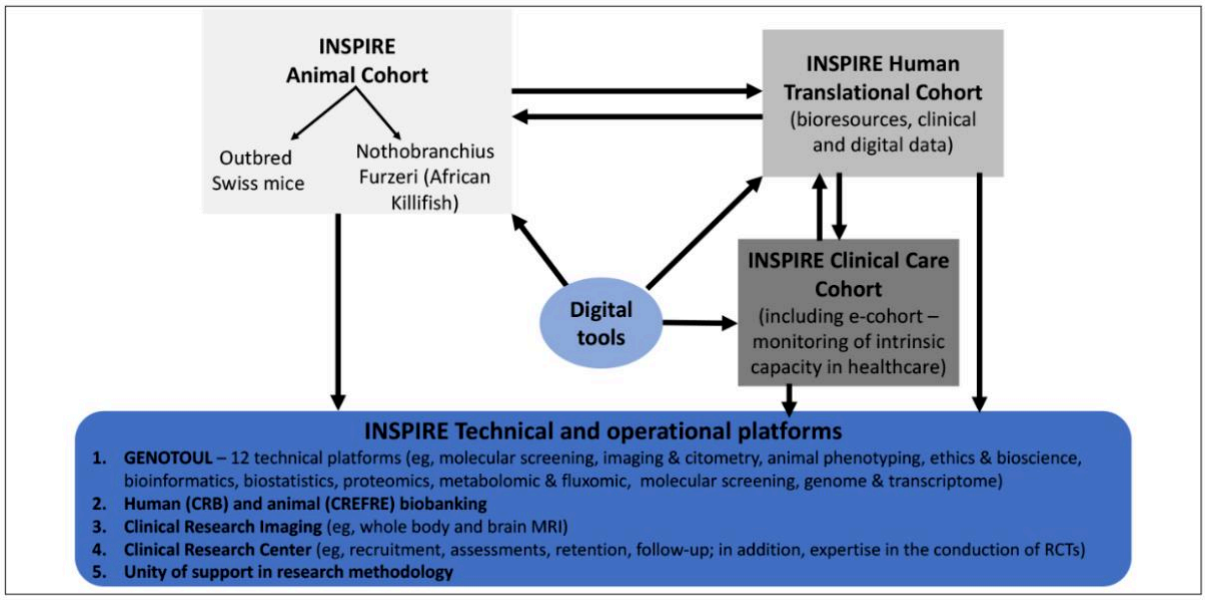

Figure 7.8 The structure of INSPIRE program

Data source: *de Souto Barreto P, et al. J Frailty Aging. 2020:1-8.*

The ongoing INSPIRE-T human cohort recruited community-dwelling adults aged ≥20 (no upper limit for age), dwelling in the Toulouse area (southwest France), and collected their biological (biobank composed of fluids and cells), clinical, digital, and imaging data. Participants could be at any level of functional capacity (robust, frail, or disabled). The cohort oversampled individuals aged ≥60 in order to capture major clinical events. The study recruitment began in 2019 with a preplanned 10-year follow-up period[298]. Until 2023, the INSPIRE-T cohort has included 1,014 adults (age ranging from 20 to 102 years old).

One of the limitations in the thesis is that we explored IC in an older cohort with relatively homogeneous characteristics. Evidence on IC distribution over a large age range and per sex is still lacking. One of the WHO's goal in implementing IC assessments into routine care is to establish a reference curve for IC in adults. This IC reference curve can track individuals' IC trajectories and identify those who deviate from normality, which allow better risk stratification and monitoring of adults' functional capacity during aging in a similar way that weight and height growth charts are valuable in tracking children's growth over childhood[299–301].

We are currently investigating how IC is distributed across adulthood using the baseline data from the INSPIRE-T cohort. We demonstrated a method to establish age- and sex-specific IC reference centiles to be used in a community-based cohort, which can identify an individual's IC level relative to their peers. Our findings, which have just been published in *Nature Aging*[302], show that IC levels were relatively high at young-to-middle age and markedly lower after age 65; inter-individual variation in IC values became larger in old age than in youth (**Figure 7.9**). We further validate the clinical significance of IC centiles by examining their associations with other clinical outcomes. We observed that participants at low IC percentiles (≤P10) showed a higher likelihood of having comorbidity, frailty, functional limitations in ADL and IADL, and falling, independent of age, sex, and educational levels. This suggests that assessing deviations

from the IC norm can provide valuable information about potential adverse events to encourage appropriate interventions.

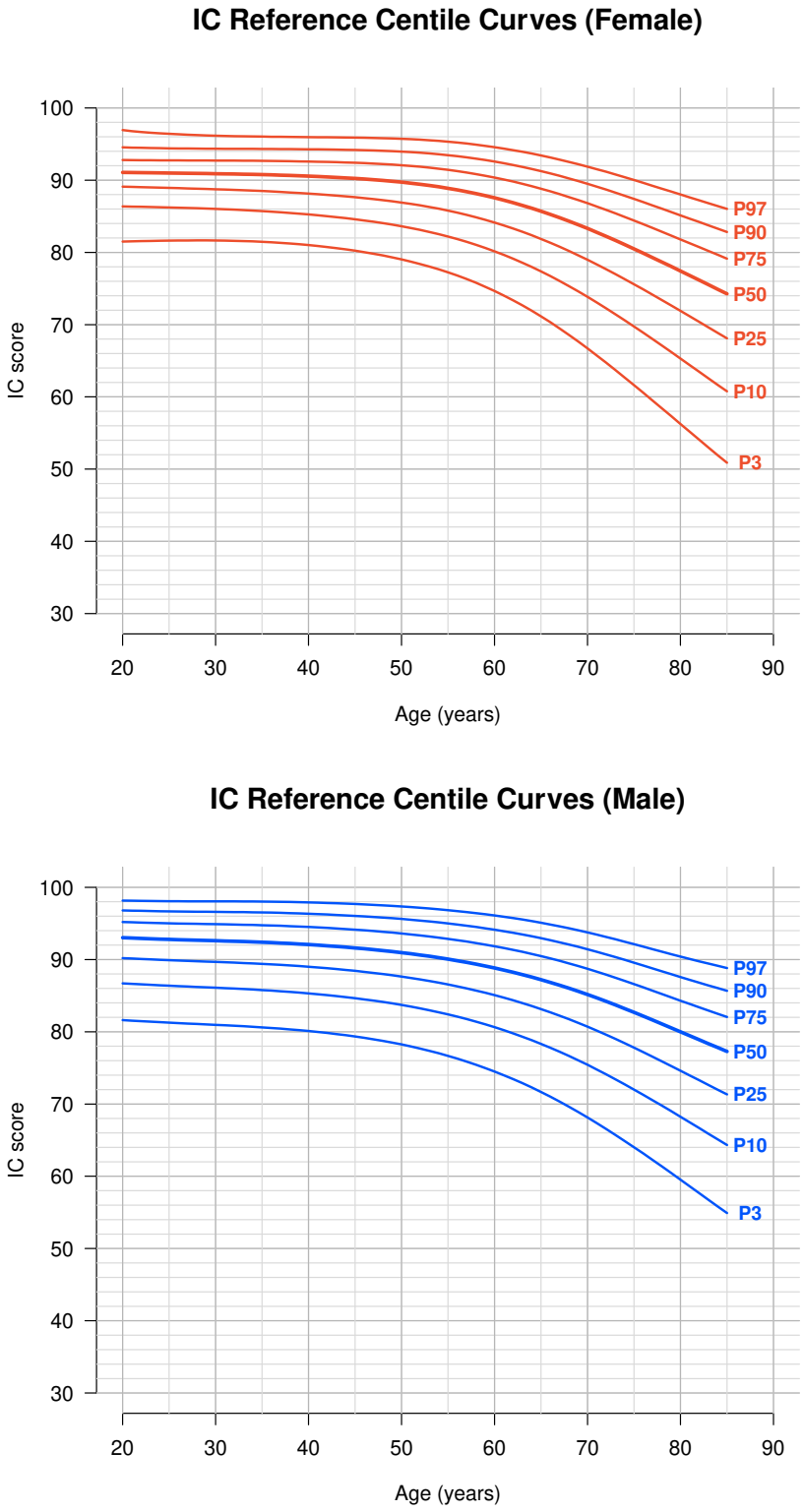

Figure 7.9 Smoothed reference centile curves for IC based on the INSPIRE-T cohort

Data source: *Lu WH, et al. Nat Aging. Nat Aging. 2023;3(12):1521-28.*

We are curious to know whether people in the lowest IC percentile had accelerated biological aging, potentially explaining why they had a higher likelihood of experiencing clinical events compared to their peers of the same chronological age. Therefore, our next step is to investigate IC with the biological clocks from two hallmarks of aging – DNA methylation age (also known as epigenetic age) and inflammatory age (iAge®) in the INSPIRE-T cohort.

DNA methylation age is an estimated age based on the DNA methylation pattern across specific cytosine guanine dinucleotide (CpG) sites[303]. Higher DNAmAge relative to chronological age is considered epigenetic age acceleration (EAA) suggesting that a person is biologically older than their chronological age. EAA has been recently validated in the Women's Health Initiative study published in JAMA to predict longevity among women and discriminate between total and health longevity[304]. The iAge® in the INSPIRE-T cohort was established with the cooperation with Stanford team. It is a metric of systematic chronic inflammation clock derived from a deep learning algorithm applied to serum immune markers[71]. Compared to individual biomarkers of inflammation, iAge better represents the chronic inflammatory load that the body currently experiences[305]. To our knowledge, no study has examined the association of IC with EAA, iAge or other biological clocks. Understanding how these biological clocks are collectively associated with IC, particularly with low and accelerated IC decline can put insight on the crosstalk between aging hallmarks.

CONCLUSION

The findings of this thesis suggested that inflammation is implicated in IC decline in community-dwelling older adults.

The vitality framework proposed in Beard et al.'s study is supported by our results, which showed that other IC domains partly mediated the association between vitality and functional disabilities. We demonstrated the feasibility and predictive ability to operationalize the vitality domain by multiple plasma biomarkers.

Our study supported the heterogeneity of functional aging between individuals by identifying multiple IC trajectories with different domain impairments. Furthermore, plasma biomarkers reflecting inflammation distinguished older people with multi-impaired IC trajectories from those with high-stable IC. We further demonstrated that higher plasma TNFR-1 and GDF-15 were consistently associated with IC evolution at both cross-sectional and longitudinal levels using the linear mixed-effect regression.

Although our studies focused on plasma inflammation-related biomarkers, the overall findings can facilitate the utility of plasma biomarkers as a measure of vitality capacity and as predictors of future functional decline in older adults. How to construct a standard measure of chronic inflammation in humans that better reflects age-related dysregulation in the inflammation network and whether impairments in other hallmarks of aging accompany chronic inflammation to determine the onset of phenotypic aging outcomes (including IC decline) will be the following questions in this field.

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APPENDIXES

I. **Published paper of Study I:** Investigating three ways of measuring the intrinsic capacity domain of vitality: nutritional status, handgrip strength, and aging biomarkers. *Age Ageing. 2023;52(7):afad133.*

II. **Published paper of Study II:** Association between aging-related biomarkers and longitudinal trajectories of intrinsic capacity in older adults. *Geroscience. 2023;45(6):3409-18.*

III. **Published paper of Study III:** Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults. *J Cachexia Sarcopenia Muscle. 2023;14(2):930-39.*

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RESEARCH PAPER

Investigating three ways of measuring the intrinsic capacity domain of vitality: nutritional status, handgrip strength and ageing biomarkers

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Abstract

Background: Vitality is conceptually considered as the underlying capacity influencing other intrinsic capacity (IC) domains and being related to nutrition, physiological reserve and biological ageing. However, there is no consensus on its operationalisation.
Objective: To investigate the structure and magnitude of the association of vitality with other IC domains and functional difficulties using three operational definitions of vitality.
Methods: We included 1,389 older adults from the Multidomain Alzheimer Preventive Trial with data on Mini Nutritional Assessment (MNA), handgrip strength and plasma biomarkers (comprising inflammatory and mitochondrial markers). Using path analysis, we examined the effects of vitality on difficulties in basic and instrumental activities of daily living (ADL and IADL) exerted directly and indirectly through the mediation of other IC domains: cognition, locomotion, psychological, vision and hearing. We further explored the longitudinal association of vitality with IC domains, ADL and IADL over 4 years using linear mixed-effect regression.
Results: We observed significant indirect effects of vitality on IADL, mainly through cognitive, locomotor and psychological domains, regardless of the vitality measurement. Participants with higher vitality had fewer IADL difficulties at follow-up (MNA score: β [95% CI] = −0.020 [−0.037, −0.003]; handgrip strength: −0.011 [−0.023, 0.000]; plasma biomarker-based index: −0.015 [−0.028, −0.002]). Vitality assessed with the plasma biomarker-based index predicted improved locomotion over time.
Conclusion: Vitality was associated with disability primarily through the mediation of other IC domains. The three indicators examined are acceptable measurements of vitality; biomarkers might be more suitable for the early detection of locomotion decline.

Keywords: disability, healthy ageing, biological ageing, inflammation, structural equation modelling, older people

Key Points

- Vitality was associated with disability primarily through the mediation of other intrinsic capacity domains.

- Nutritional status, handgrip strength and plasma biomarker levels are acceptable measurements of vitality.
- Plasma biomarkers might serve to early detect locomotion decline.

Introduction

According to the World Health Organization (WHO), an individual's functional ability is determined by intrinsic capacity (IC), the environment and the interactions between the two [1, 2]. IC is defined as 'the composite of all physical and mental capacities' [1] and is often operationalised by five constructs: cognition, locomotion, psychological, sensory (vision and hearing) and vitality [2]. This IC construct has been validated using population-based data from different countries [3–6]. Beard and colleagues further suggested that IC domains are not at parallel levels; instead, vitality serves as an underlying capacity and influences the manifestations of the other more overtly expressed IC domains [3, 4]. In other words, how vitality contributes to overall functional capacity is partially by modulating other IC domains.

There is a lack of consensus on measuring the IC domain of vitality in the literature [7]. The concept of vitality, a capacity to maintain homeostasis to function properly, is derived from the evidence showing that imbalanced energy intake and expenditure (e.g. weight loss, abnormal body mass index) was a strong predictor of disability in older people [2]. Following this definition, the WHO programme Integrated Care for Older People suggests using nutritional status assessments, such as the Mini Nutritional Assessment (MNA), to evaluate older individuals' vitality [8]. On the other hand, the concept of vitality is similar to 'physiological reserve' and 'biological age' [9]. That is, vitality can be understood as the sum of all functions of physiological and biomolecular systems that resist the challenges and determine lifespan and healthspan. In this sense, handgrip strength, a vital sign of physiological reserve [10] and biological age [11], was often used to measure vitality [3–6]. Biomarkers related to ageing mechanisms at the biomolecular level can be promising indicators of vitality, considering they reflect homeostatic dysregulation since the subclinical stage. To our knowledge, no study has used a combination of plasma biomarkers to operationalise vitality. Furthermore, the most appropriate operational definition of vitality is unknown since no investigation compared the performance of different vitality operationalisations as a structural component of the IC model and an element associated with the evolution of the other IC domains and functional ability over time.

This study aimed to investigate the pathways that connect vitality with functional difficulties (i.e. basic and instrumental activities of daily living—ADL and IADL), directly and indirectly through the overt IC domains (cognition, locomotion, psychological, sensory [vision and hearing]) in older adults. Three vitality measurements reflecting energy balance, physiological reserve and biology of ageing were examined: nutritional status, handgrip strength and a combination of plasma ageing-related biomarkers. Moreover, we evaluated the association of vitality with longitudinal changes in other IC domains, ADL and IADL.

Methods

Study design and participants

This study is a secondary analysis of the Multidomain Alzheimer Preventive Trial (MAPT), whose detailed information is provided in Supplementary Material and published elsewhere [12, 13]. In brief, the MAPT Study is a randomised controlled trial investigating whether omega-3 polyunsaturated fatty acid supplementation and multidomain lifestyle interventions (exercise advice, cognitive training and nutritional counselling) prevented cognitive decline in community-dwelling older adults. Compared with controls, the three-year MAPT interventions did not show a beneficial effect on cognitive function [13], IC [14] and functional difficulties (Supplementary Material). The MAPT Study recruited 1,679 older adults aged ≥ 70 who presented either spontaneous memory complaints, difficulties in one IADL or gait speed ≤ 0.8 m/s. In the current study, we chose the 12-month visit, the moment when plasma biomarkers were measured, as the starting point of follow-up. Participants with data on at least one vitality measurement at the 12-month visit were included, leading to a final sample of 1,389 subjects. Participants with complete information on three vitality measurements, other IC indicators and functional outcomes were further included in the path analysis ($n = 220$ for exploring ADL and $n = 280$ for IADL; Figure S1).

Measurement of vitality

We investigated three vitality measurements: nutritional status (evaluated by MNA [15]), handgrip strength and the composite of plasma biomarkers. Multiple plasma biomarkers were selected based on data availability and the following criteria: (i) being related to the hallmarks of ageing [16, 17] and (ii) having been used to measure vitality in prior studies [3, 4]. Seven plasma biomarkers were included in our analysis: C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor receptor-1 (TNFR-1), monocyte chemoattractant protein-1 (MCP-1), growth differentiation factor-15 (GDF-15), ATPase inhibitory factor 1 (IF1) and haemoglobin. CRP, IL-6, TNFR-1 and MCP-1 are markers of chronic inflammation [18, 19]. GDF-15 and IF1 are closely related to energy metabolism and mitochondrial dysfunction [20, 21]. Haemoglobin has been applied previously as a vitality indicator of the IC

construct [3, 4]. More details about vitality measurements are described in Supplementary Material.

Measurement of other IC domains

The cognitive domain was evaluated by the 30-item Mini-Mental State Examination (MMSE; scores varying from 0 to 30, with higher means better) [22]. Locomotion was based on the Short Physical Performance Battery (SPPB), involving the tests for usual gait speed, repeated chair stand and standing balance with a summary score ranging from 0 to 12 (higher is better) [23]. The psychological domain was measured by the 15-item Geriatric Depression Scale (GDS), with scores varying from 0 to 15 (higher is worse) [24]. Visual acuity of the left and right eyes was examined by a Monoyer chart (distance vision with a score ranging from 0 to 10, with higher being better). The average score of both eyes was applied for the analysis. Hearing capacity was assessed by the Hearing Handicap Inventory for the Elderly—Screening version (HHIE-S), a 10-item questionnaire evaluating the degree of hearing difficulty in daily life with scores varying from 0 to 40 (higher is worse) [25, 26]. MMSE, SPPB and GDS were measured annually from 12 to 60 months for the whole population, whereas visual acuity and HHIE-S assessments were only available at 12 and 24 months for the subjects who received the multidomain intervention.

Measurement of functional difficulties

The outcomes of interest were the number of difficulties in ADL [27] and IADL [28]. Participants were considered functional difficulties if they reported difficulties or were fully dependent on these activities. In the original MAPT Study, ADL was evaluated at the study enrolment, 48 and 60 months, whereas IADL was assessed at the study enrolment, 36, 48 and 60 months. Because of the lack of ADL and IADL data at 12 months (the start point of the follow-up in this study), we assumed that our participants maintained the same ADL and IADL performances between the study enrolment and the 12-month visit. It is worth highlighting that all study participants, as per inclusion/exclusion criteria, were fully independent in ADL (i.e. no difficulty in ADL) at study enrolment.

Other variables

Covariates used for the analyses were age, sex, MAPT group allocation and education. We also collected information for eight major chronic diseases. Frailty was assessed based on the Fried frailty criteria [29].

Statistical analysis

We performed path analysis from a structural equation modelling (SEM) framework to test the hierarchical IC structure, in which vitality directly determined the change in ADL/IADL and indirectly through impairments in overtly expressed IC domains. Three path models using different vitality measurements were built, and the model fit was evaluated. Models were estimated using maximum likelihood estimation with robust standard errors (SE) to account for the non-normality of the variables. We defined ΔADL as the change in ADL from 12 to 48 months, and ΔIADL as the change in IADL from 12 to 36 months; a positive value of ΔADL and ΔIADL indicates an increased number of functional difficulties.

Regarding plasma biomarkers, we first conducted confirmatory factor analysis (CFA) and created a latent factor called 'biological substrate', which included all investigated biomarkers as indicators. Only the biomarker indicators with significant factor loadings were retained for subsequent investigations using the path analysis. The values of plasma biomarkers were log-transformed, and the outliers (defined as values above or below four standard deviations [SD] from the population mean) were excluded. Based on the SEM results for ADL, we created a bio-vitality index with weights derived from the standardised factor loadings of 'biological substrate' (latent variable). We referred to the results from ADL rather than IADL since the former is part of the functional ability according to the WHO's definition [30]; moreover, ADL is a better indicator of care dependency than IADL, even though both are related to coping in daily life. We summed up the weighted biomarker levels and rescaled the value into a score ranging from 0 to 1 (hereafter called the 'bio-vitality index') using the proportion of maximum scaling method [31]. The largest observed value was defined as 1 (the maximum possible vitality), and the smallest observed value was defined as 0 (the minimum possible vitality). Other values were transformed into the scores by subtracting the smallest value and then dividing by the range. A higher bio-vitality index represented lower overall levels of plasma biomarkers and better vitality capacity. To test the construct validity of the bio-vitality index, we examined the association between the bio-vitality scores and clinical characteristics using multivariate linear regression.

Finally, we evaluated the variation in overtly expressed IC domains (i.e. cognition, locomotion, psychology, vision and hearing) and the variation of ADL and IADL over time according to different vitality measurements using linear mixed-effect regression. The mixed models for cognitive, locomotor and physiological capacities include a random effect at the participant level and a random slope on time. The mixed models for vision, hearing, ADL and IADL include a random effect at the participant level because of the insignificant random effect on time.

Two sensitivity analyses were performed. First, our path models assumed no covariance between four expressed IC domains. Thus, we evaluated the standardised covariance residual of each path model to see if covariance residuals for the pair of IC domains (mediators) were >2.58 [32] (which indicated the correlations between these two domains should not be omitted), and then reran the path analysis included significant mediator covariance. Second, we created a bio-vitality index based on the SEM results for IADL and examined if the IADL-based bio-vitality index provided similar construct validity and longitudinal association with outcomes as the ADL-based index. All

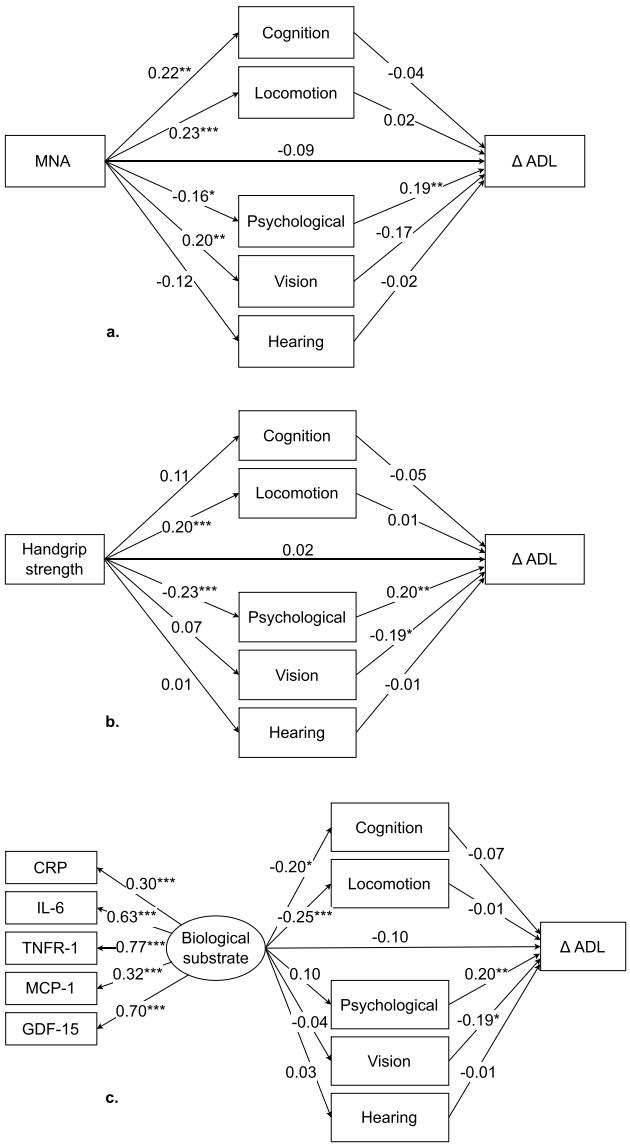

Figure 1. Direct and indirect effects of vitality on change in ADL difficulties. **a**) MNA score as vitality; **b**) Handgrip strength as vitality; **c**) Plasma biomarker levels as vitality. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

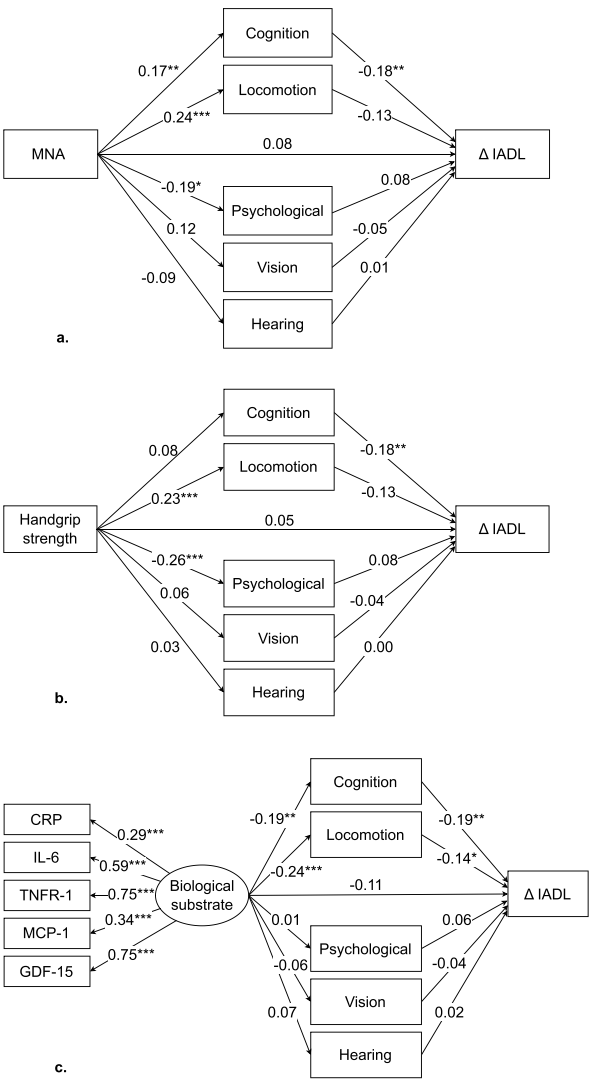

Figure 2. Direct and indirect effects of vitality on change in IADL difficulties. **a**) MNA score as vitality; **b**) Handgrip strength as vitality; **c**) Plasma biomarker levels as vitality. $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$.

statistical analyses were performed using the SAS software version 9.4 (SAS Institute, Inc., Cary, NC) with a significance level of 0.05.

Results

Characteristics of the study population

Tables S2 and S3 present the characteristics of study participants included in the path and longitudinal analyses. Amongst the overall population ($n = 1{,}389$), the mean (SD) age was 76.2 (4.4) years, with 63.8% being women (Table S2). Differences in baseline characteristics between MAPT participants included in the present study and those not included are shown in Tables S4–S6.

CFA for plasma biomarkers

The CFA showed that CRP, IL-6, TNFR-1, MCP-1 and GDF-15 were statistically correlated with the latent variable 'biological substrate', whose standardised factor loadings ranged from 0.27 to 0.78. On the other hand, the standardised factor loadings of IF1 and haemoglobin were insignificant and lower than 0.10, suggesting these two biomarkers contributed little information to the latent variable (Table S7). Therefore, we did not include IF1 and haemoglobin in the following path analysis. The model fit indices before and after excluding IF1 and haemoglobin did not change significantly (Table S7).

Path analysis for vitality, other IC domains and functional difficulties

Figures 1 and 2 illustrate the path diagrams from vitality to ADL/IADL difficulties; vitality was measured by MNA score

Table 1. Total, direct and indirect standardised effects from vitality to change in ADL and IADL.

	Vitality measurement					
	MNA score		Handgrip strength		Plasma biomarkers	
	Standardised coefficient (SE)	*P*	Standardised coefficient (SE)	*P*	Standardised coefficient (SE)	*P*
Outcome: ΔADL difficulties						
Total effect	−0.152 (0.074)	0.040	−0.042 (0.049)	0.392	−0.058 (0.057)	0.303
Total indirect effect	−0.067 (0.044)	0.133	−0.064 (0.035)	0.069	0.043 (0.041)	0.300
Direct effect	−0.085 (0.074)	0.250	0.021 (0.050)	0.670	−0.101 (0.068)	0.136
Outcome: ΔIADL difficulties						
Total effect	−0.004 (0.059)	0.941	−0.014 (0.058)	0.814	−0.032 (0.095)	0.737
Total indirect effect	−0.085 (0.025)	0.001	−0.068 (0.027)	0.011	0.077 (0.033)	0.021
Direct effect	0.081 (0.071)	0.255	0.054 (0.050)	0.278	−0.108 (0.093)	0.242

(Figures 1a and 2a), handgrip strength (Figures 1b and 2b) and plasma biomarkers ('biological substrate'; Figures 1c and 2c), respectively. Table S8 displays the model fit statistics. All path models demonstrated similar and adequate fits with standardized root mean squared residual (SRMR) around 0.08, implying that none of the three vitality measurements outperformed the others. Only MNA showed a marginally significant total effect on ΔADL; however, the direct and total indirect effects were insignificant (Table 1). On the other hand, all three vitality measurements showed significant indirect effects on ΔIADL (Table 1), mainly through cognitive, psychological and locomotor domains (Figure 2). The results of the path models considered significant covariances between other IC domains are displayed in Tables S9 and S10; the overall model fit was improved after considering important mediator covariance, but the indirect effects of vitality on ΔIADL remain unchanged.

Construct validity of the bio-vitality index

We calculated the bio-vitality index for the study sample of longitudinal analysis based on the weights in Table S11. Amongst these participants, a lower bio-vitality index was associated with increasing age, having ≥3 chronic diseases, being prefrail and frail. Females tended to have a higher bio-vitality index than males (Figure 3 and Table S12). The IADL-based bio-vitality index showed similar construct validity (Table S13).

Variation in overtly expressed IC domains, ADL and IADL according to vitality measurements

After adjusting for covariates, a higher bio-vitality index (better vitality capacity) was associated with improved locomotor capacity over time (increased SPPB score). Neither MNA nor handgrip strength was longitudinally associated with other IC domains (Table 2). Significant associations existed between better vitality and decreased IADL difficulties across all vitality measurements (MNA score: β [95% CI] = −0.020 [−0.037, −0.003]; handgrip strength: −0.011 [−0.023, 0.000]; bio-vitality index: −0.015 [−0.028, −0.002]; Table 2). Significant associations with ADL change over time were observed if vitality was measured as MNA or handgrip strength (Table 2). The association of the bio-vitality index with the changes in locomotion and IADL remain significant by using the IADL-based index (Table S14).

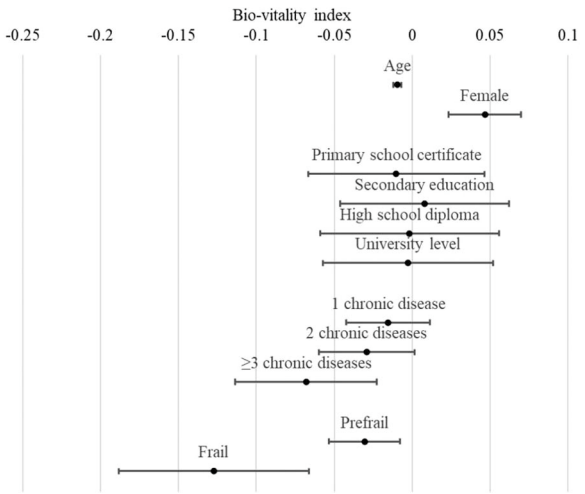

Figure 3. Construct validity of the bio-vitality index. Results from the multivariate linear regression with clinical characteristics as the independent variables and bio-vitality index as the dependent variable. Black dots and horizontal bars represent the coefficients and their confidence intervals.

Discussion

This work showed that the vitality domain of IC was indirectly associated with IADL when using all three operational definitions: MNA, handgrip strength and a combination of plasma biomarkers (bio-vitality index). For longitudinal analysis, participants with better vitality demonstrated fewer

Table 2. Linear mixed-effect regression examining longitudinal association of vitality with other IC domains, ADL and IADL.

Outcome	Vitality measurement								
	MNA score ($N = 599$)			Handgrip strength ($N = 1,283$)			Bio-vitality index ($N = 874$)		
	n	*β* (95% CI)	*P*	*n*	*β* (95% CI)	*P*	*n*	*β* (95% CI)	*P*
Cognition: MMSE	590	−0.015 (−0.061, 0.032)	0.535	1,258	0.004 (−0.025, 0.033)	0.799	855	−0.023 (−0.058, 0.010)	0.173
Locomotion: SPPB	591	0.037 (−0.013, 0.086)	0.143	1,264	0.018 (−0.013, 0.049)	0.262	855	0.050 (0.010, 0.090)	0.015
Psychological: GDS	590	−0.009 (−0.066, 0.049)	0.771	1,257	0.027 (−0.010, 0.064)	0.154	855	−0.030 (−0.075, 0.015)	0.190
Vision: Monoyer score	584	0.035 (−0.132, 0.202)	0.680	570	−0.091 (−0.266, 0.084)	0.307	381	0.035 (−0.143, 0.214)	0.698
Hearing: HHIE-S	593	−0.100 (−0.544, 0.345)	0.660	579	0.189 (−0.284, 0.663)	0.433	390	0.138 (−0.412, 0.689)	0.622
ADL difficulties	595	−0.017 (−0.026, −0.008)	<0.001	1,267	−0.008 (−0.014, −0.002)	0.009	859	−0.004 (−0.011, 0.003)	0.271
IADL difficulties	595	−0.020 (−0.037, −0.003)	0.018	1,267	−0.011 (−0.023, 0.000)	0.047	859	−0.015 (−0.028, −0.002)	0.027

All models were adjusted for age, sex, education and MAPT group allocation. The coefficient (β) indicates the annual change from 12 months in each outcome (IC domain, ADL or IADL) per SD increment in vitality measurement. Higher MMSE, SPPB and Monoyer values indicate better function, whereas higher GDS, HHIE-S, ADL difficulties and IADL difficulties indicate worse function.

IADL difficulties over the follow-up across all vitality definitions. Higher vitality was associated with a lower decline in ADL when operationalising vitality as MNA and handgrip strength. Finally, higher vitality assessed with plasma biomarkers was associated with increased locomotor capacity over time.

The vitality framework proposed in Beard *et al.*'s study [3, 4] is supported by our path analysis, which showed that other IC domains mediated the association between vitality and change in IADL. The reasons we did not obtain the same results for ADL could be related to the smaller sample size and relatively fit population, with unchanged ADL performance for most participants between 12 and 48 months. Regardless of the measurement, vitality showed no direct effect on ADL and IADL difficulties. Taken together, our data suggest that vitality is associated with disability through its potential influences on other IC domains. According to Nagi's disablement model [33], the active pathology and impairments in body systems lead to limitations in functional performances and, consequently, inabilities in activities required in a social context. Our work further explains the disablement process in older adults by showing that limitations in different functional performances are not happening simultaneously, and low vitality capacity seems to be the starting point of this process.

In addition, we found that vitality measurements were associated with the overtly expressed capacities in the path models. MNA equally contributed to all capacities except hearing (Figure 1a). Handgrip strength had significant associations with locomotor and psychological domains (Figures 1b and 2b). Plasma biomarkers were correlated with cognition and locomotion (Figures 1c and 2c). Indeed, several nutrients play essential roles in maintaining the health of skeletal muscle [34], visual system [35] and brain [36], whereas a healthy dietary pattern was associated with a lower risk of depression [37]. The underlying mechanisms of low handgrip strength—loss of muscle mass and impaired neuromuscular coordination [38]—can have apparent impacts on mobility and are sometimes manifested as psychological symptoms, such as fatigue and reduced social activities. Our result of plasma biomarkers may be driven by the relatively high weighting of plasma IL-6, TNFR-1 and GDF-15, since these inflammatory markers have more evidence of the association with cognitive [39–41] and physical function [41–44].

Our study found that none of the vitality measurements fitted the MAPT data better than the other. This result suggests that each vitality operationalisation only partially reflected the aggregate of biological mechanisms involved in homeostasis regulation. The advantage of using plasma biomarkers over the nutritional assessment or handgrip strength for vitality is that the latter still relies on clinical manifestation. In other words, abnormal plasma biomarker levels might indicate subclinical dysregulation in the body even before symptoms of malnutrition (e.g. loss of appetite, involuntary weight loss) and muscle weakness appear. Indeed, in this study, only the bio-vitality index predicted the variation in expressed IC domain (i.e. locomotion), indicating that biomarkers can identify individuals at risk of locomotion decline several years in advance. Interestingly, all plasma biomarkers retained in the bio-vitality index are related to inflammation, which is also an underlying mechanism of malnutrition [45] and low muscle strength [38]. Thus, despite all these measurements being closely related to homeostasis, we might say that plasma biomarkers describe the biomolecular (intracellular/intercellular) changes, whereas nutritional status and handgrip strength reflect variations at the physiologic level (organs/systems).

The three vitality measurements explored in our study may have different clinical applications. Simplicity and low cost make MNA more suitable for routine monitoring of older individuals in clinical settings, even in low- and medium-income countries. In addition, MNA and handgrip strength provide clear targets for subsequent intervention to enhance IC. On the other hand, plasma biomarkers

are ideal indicators of vitality in middle-aged and young-old adults without any phenotypic manifestation. Using biomarkers for early risk identification and prevention in this population is a cost-effective strategy considering social productivity and long-term healthcare utilisation. The urgent need is for a more robust and predictive bio-vitality index, and combining markers from different biological ageing hallmarks may help improve our current approach.

All IC domains are important determinants of functional disability. However, in our path analysis, significant associations with ADL/IADL change were found mainly in cognitive and psychological capacities. It is noteworthy that our study participants were at risk of cognitive decline (as per MAPT inclusion criteria) and showed a high rate of depressive symptoms (23.3% of the study cohort with GDS ≥ 5 at the 12-month visit). In contrast, our study sample was relatively robust in locomotor, vision and hearing domains, with only 6.4% with SPPB < 8, 4.0% of people had visual impairment (equivalent to visual acuity ≤ 0.25), and only 3.8% of subjects had a severe hearing handicap (HHIE-S = 26–40) at the 12-month visit. Indeed, according to a previous study investigating the association between depression and functional difficulties in older people, GDS ≥ 5 could significantly predict disability in IADL [46]. On the other hand, in another paper, older individuals with ADL disability tended to be SPPB < 8 [23].

This is the first study to test the hierarchical IC structure that vitality underlies all other capacities and contributes to disability through multiple pathways. We compared three vitality approaches in a sample of community-dwelling older people and provided evidence for both structure and magnitude of the cross-sectional and longitudinal association. However, some limitations should be noted. First, this is a secondary analysis of a randomised controlled study that enrolled older individuals with either spontaneous memory complaints or functional/physical limitations. Furthermore, because of the data available in the MAPT Study, we used different sample sizes to explore vitality definitions. It is worth mentioning that, in some analyses, study subjects had slightly different characteristics compared with the rest of the MAPT population. Finally, this study only included plasma biomarkers related to inflammation and energy metabolism. Further investigations using various biomarkers are needed to evaluate the impact of other mechanisms involved in maintaining homeostasis, such as hormonal function.

Conclusion

In conclusion, our study observed that vitality led to IADL difficulties primarily through the mediation of other IC domains. Nutritional status, handgrip strength and plasma inflammation-related biomarkers are acceptable measurements of vitality. Biomarkers might be more suitable for the early detection of individuals at risk of locomotion decline. Studies enrolling different biomarkers of ageing to create a complete vitality index might be encouraged.

Supplementary Data: Supplementary data mentioned in the text are available to subscribers in *Age and Ageing* online.

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Data Availability Statement: Request for de-identified data related to the results reported in this article (ie, text, tables, figures, and the appendix) and a data dictionary will be evaluated and may be made available under request to the MAPT scientific committee: nicola.coley@inserm.fr & guyonnet.s@chu-toulouse.fr.

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ORIGINAL ARTICLE

Association between aging-related biomarkers and longitudinal trajectories of intrinsic capacity in older adults

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Abstract Intrinsic capacity (IC), the composite of physical and mental capacities, declines with age at different rates and patterns between individuals. We aimed to investigate the association between longitudinal IC trajectories and plasma biomarkers of two hallmarks of aging—chronic inflammation and mitochondrial dysfunction—in older adults. From the Multidomain Alzheimer Preventive Trial (MAPT), we included 1271 community-dwelling older people (mean [SD] age = 76.0 [4.3] years) with IC data over four years. Group-based multi-trajectory modeling was performed to identify clusters of the participants with similar longitudinal patterns across four IC domains: cognition, locomotion, psychology, and vitality. Five IC multi-trajectory groups were determined: low in all domains (8.4%), low locomotion (24.6%), low psychological domain (16.7%), robust (i.e., high in all domains except vitality; 28.3%), and robust with high vitality (22.0%). Compared to the best trajectory group (i.e., robust with high vitality), elevated levels of plasma interleukin-6 (IL-6), tumor necrosis factor receptor-1 (TNFR-1), and growth differentiation factor-15 (GDF-15) were associated with a higher risk of belonging to the "low in all domains" group (IL-6: relative risk ratio (RRR) [95% CI] = 1.42 [1.07 – 1.88]; TNFR-1: RRR = 1.46 [1.09 – 1.96]; GDF-15: RRR = 1.99 [1.45 – 2.73]). Higher IL-6 and GDF-15 also increased the risk of being in the "low locomotion" group. GDF-15 outperformed other biomarkers by showing the strongest associations with IC trajectory groups. Our findings found that plasma biomarkers reflecting inflammation and mitochondrial impairment distinguished older people with multi-impaired IC trajectories from those with high-stable IC.

Keywords Healthy aging · Functioning · Biological aging · Inflammaging

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Introduction

Since 2015, the World Health Organization (WHO) has committed to promoting healthy aging, which

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aims to support individuals' functional ability to achieve well-being in older age [1]. Functional ability is defined as the interplay between environmental factors and intrinsic capacity (IC) [1]. IC, the composite of a person's total physical and mental capacities that they can draw on, is represented as a combination of five essential body functions: cognition, locomotion, psychology, sensory (vision and hearing), and vitality [2, 3]. The composite score of five IC domains had been associated with adverse health outcomes in older adults, including frailty, disability, hospital admission, institutionalization, and mortality [4–6]. Previous literature suggests that IC is a dynamic construct that varies across the lifespan [7], becoming lower and fast declining at an advanced age [8]. IC is recommended to be monitored regularly in primary care [9] and can be enhanced through interventions at the early decline phase to prevent or delay future care dependence [10].

Biomarkers linked to cellular and molecular mechanisms of aging may serve as measurable parameters that predict IC variation. Growing evidence showed that blood-derived biomarkers, particularly those related to inflammation, were associated with IC impairments in older adults [11, 12]. Indeed, a chronic, low-grade, sterile inflammatory state has been found in old age, often termed inflammaging, which resulted from genomic instability, the accumulation of damaged cells and debris, and senescent cells with senescence-associated secretory phenotype (SASP) [13]. Another hallmark of aging – mitochondrial dysfunction, characterized by reduced ATP production and increased reactive oxygen species (ROS) generation [14], may also cause IC decline through inflammation, cellular senescence and apoptosis, and dysregulation of energy metabolism [15].

To the best of our knowledge, whether aging biomarkers are associated with lower and faster declining IC trajectories has not been investigated. The close correspondence between biomarkers and IC trajectories can help understand the underlying mechanisms of IC decline and serve as targets for risk identification and early interventions. Therefore, this study is aimed at examining the association of longitudinal IC trajectories with plasma biomarkers representing key biological aging mechanisms, including inflammation and mitochondrial dysfunction. The multi-trajectories across four IC domains — cognition, locomotion, psychology, and vitality — were investigated. The trajectories of global IC, a summarized score of four IC domains, were also examined as an exploratory outcome. We hypothesized that abnormal levels of plasma biomarkers could identify older adults with impaired IC trajectories.

Methods

Data source and study population

This study used data from the Multidomain Alzheimer Preventive Trial (MAPT; http://clinicaltrials.gov [NCT00672685]), which has been detailed elsewhere [16]. In brief, the MAPT Study was a 3-year randomized, multicenter, placebo-controlled trial investigating the preventive effects of two interventions (omega-3 polyunsaturated fatty acid supplementation and a multidomain intervention composed of cognitive training, physical activity, and nutritional counseling) on cognitive function among community-dwelling older adults. The combined or alone interventions did not show significant effects on cognitive change over three years [16]. After the 3-year interventional phase, an additional 2-year observation was conducted. The MAPT Study was approved by the French Ethical Committee in Toulouse (CPP SOOM II) and authorized by the French Health Authority. Informed consent was obtained from all MAPT participants.

The MAPT Study recruited older adults aged ≥70 who met at least one criterion: spontaneous memory complaints or limitation in one instrumental activity of daily living or slow walking speed (≤0.8 m/s). The current study's population was composed of 1,271 subjects with repeated IC measurements during the follow-up period (Figure S1). Comparisons of baseline characteristics between MAPT participants included and excluded from the current study are shown in Table S1.

Plasma biomarkers

Multiple plasma biomarkers were selected if they showed direct links with the hallmarks of aging proposed by López-Otín et al. [17]. Plasma C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor receptor 1 (TNFR-1), and monocyte chemoattractant protein-1 (MCP-1) act as markers of chronic

inflammation [18, 19]. In addition, IL-6, TNFR-1, and MCP-1 were identified as the SASP components [20–22]. Growth differentiation factor-15 (GDF-15) is a stress-response cytokine upregulated during inflammation, mitochondrial dysfunction, and cellular senescence [23, 24]. ATPase Inhibitory Factor 1 (IF1) is an endogenous inhibitor of ATP synthase that regulates mitochondrial bioenergetics. A lower circulating IF1 level has been proposed to indicate higher intracellular concentrations of IF1, which may serve as a marker of mitochondrial impairment [25]. All biomarkers were measured from the blood samples collected at the 12-month visit, with detailed procedures described in Supplemental Text 1. To compare the effect of biomarkers with different units and distributions, we log-transformed and standardized biomarker values before statistical analysis. The outlier values, defined as above or below 4 standard deviations (SD) from the population mean, were removed from the analysis.

Intrinsic capacity (IC)

Four IC domains were evaluated in the present study: cognition, locomotion, psychology, and vitality. We did not investigate the sensory domain because visual and hearing data were available for only half of the MAPT population at the 12- and 24-month visits. In this study, the same visit where plasma biomarkers were measured (i.e., the 12-month visit in MAPT) was selected as the starting point of follow-up; data collected before this starting point was not used in the present study. Four IC domains were annually evaluated from the 12-month visit to the 60-month visit (a total of four years of follow-up).

Cognition was evaluated using the 30-item Mini-Mental State Examination (MMSE; higher indicates better function [26]). Locomotion was assessed by the Short Physical Performance Battery (SPPB), an instrument consisting of the walk, chair-rise, and standing balance tests with overall scores ranging from 0 to 12 (higher is better) [27]. The psychological domain was measured using the 15-item Geriatric Depression Scale (GDS); a lower score means better capacity (less likely to have depression) [28]. Vitality was represented by handgrip strength, measured with a hydraulic dynamometer (Jamar, Bolingbrook, IL) using standardized procedures [29]. Participants were asked to stand up with the arm straight down, hold the dynamometer with their dominant hands, and squeeze it as hard as possible; the maximum value of three attempts (in kilograms [kg]) was recorded.

Variables for each IC domain were transformed to a 0-to-100 scale; higher is better. Values representing the best performance in the original instruments (30 for MMSE, 12 for SPPB, and 0 for GDS) were rescaled as 100 points, while values indicating the worst performance (0 for MMSE and SPPB, 15 for GDS) were rescaled as 0 point. Regarding the vitality domain, we defined 100 points based on the maximum value of handgrip strength among all the observations during the follow-up period (i.e., the maximum possible value achieved by our study cohort); a different maximum value was applied for each sex (i.e., 51 kg for women and 72 kg for men).

Covariates

Additional demographic covariates were collected: age, sex, MAPT group allocation, educational level, and the number of chronic diseases at the MAPT study enrollment.

Statistical analysis

We conducted group-based multi-trajectory modeling [30], a generalization of group-based trajectory modeling (GBTM), to identify latent clusters of older adults with similar joint evolution of the four IC domains over four years. Compared to univariate GBTM, multi-trajectory modeling determined separate trajectories for each IC domain. Individuals from the same multi-trajectory groups were represented with similar patterns across the four domains. IC scores for each domain were modeled as following the censored normal distribution. The optimal number and shape of trajectory groups were determined by the model best fitting our data (detailed in Supplemental Text 2). We performed group-based multi-trajectory modeling in each sex as a sensitivity analysis to see if we could obtain trajectory group membership similar to the primary analysis.

To evaluate the association between plasma biomarkers and IC multi-trajectory groups, we used multinomial logistic regression to derive relative risk ratios (RRRs) for each trajectory group according to plasma biomarker levels. Regression models were adjusted for demographic covariates mentioned above. Sensitivity analysis with biomarker outliers included was performed.

We were also interested if associations between the investigated plasma biomarkers and IC trajectories remained when composing IC domains into a single value. This analysis was exploratory since only four components were included in the IC construct instead of five (with sensory). Following the prior studies [31, 32], the global IC was calculated as a composite score that averaged the performance of the four domains. The trajectories of the global IC were identified by using the univariate GBTM [33]. We further evaluated the association of global IC trajectories with six plasma biomarkers using multinomial logistic regression. All analyses were performed using SAS version 9.4 (SAS Institute, Inc, Cary, NC) and STATA version 17 (College Station, TX), with a significance level of 0.05.

Results

Multi-trajectories of four IC domains

Among 1,271 participants, we identified five multi-trajectory groups across four IC domains, labeled as low in all domains (8.4%), low locomotion (24.6%), low psychological domain (16.7%), robust (i.e., high in all domains except vitality; 28.3%), and robust with high vitality (22.0%) (Fig. 1). The model selection process regarding IC multi-trajectories is detailed in Supplemental Text 2 and Table S2. Five trajectories were replicated in the sensitivity analysis on females and males, with slightly different percentages in each trajectory group (Figure S2 and S3). Table 1 displays the participants' baseline characteristics according to their IC multi-trajectories. The oldest age, the highest percentages of low educational levels, and the highest number of chronic diseases were observed in the "low in all domain" group.

We observed the associations of IL-6, TNFR-1, and GDF-15 levels with IC multi-trajectories, independently of age, sex, educational levels, MAPT group allocation, and chronic disease number (Table 2). GDF-15 levels showed the strongest (the highest RRRs) and multiple associations with IC multi-trajectory groups. Compared to the "robust with high vitality" group, older individuals with higher plasma IL-6, TNFR-1, and GDF-15 were more likely to be the "low in all domain" group (IL-6: RRR [95% CI] = 1.42 [1.07 – 1.88]; p = 0.016; TNFR-1: RRR = 1.46 [1.09 – 1.96]; p = 0.011; GDF-15: RRR = 1.99 [1.45 – 2.73]; p <

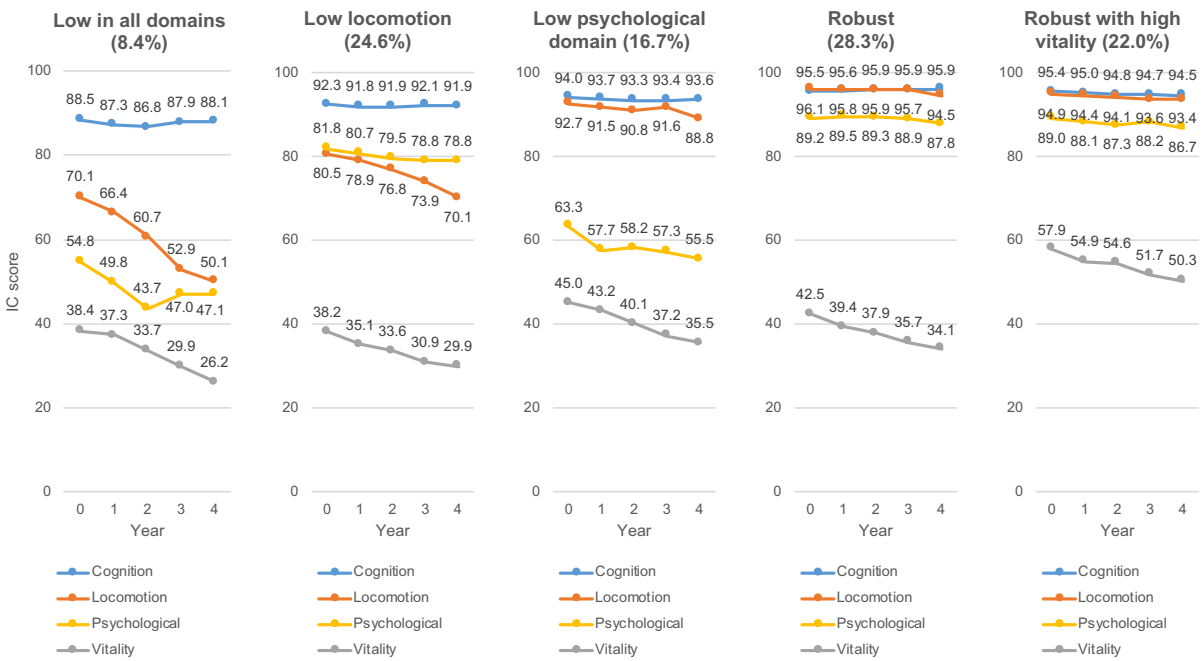

Fig. 1 Multi-trajectories across four IC domains

Table 1 Characteristics of the study population at the 12-month visit

	Total (n = 1271)	Multi-trajectory group membership					
		Low in all domains (n = 107 [8.4%])	Low locomotion (n = 313 [24.6%])	Low psychological domain (n = 212 [16.7%])	Robust (n = 360 [28.3%])	Robust with high vitality (n = 279 [22.0%])	p value
Age (years)	76.0 (4.3)	78.6 (4.6)[a,b,c]	78.0 (4.7)[d,e,f]	76.1 (4.1)[a,d,g,h]	74.8 (3.6)[b,e,g]	74.3 (3.1)[c,f,h]	<0.001
Female	821 (64.6%)	76 (71.0%)[a]	233 (74.4%)[b]	135 (63.7%)[c,d]	281 (78.1%)[c,e]	96 (34.4%)[a,b,d,e]	<0.001
MAPT group							
Multidomain intervention + omega-3	319 (25.1%)	27 (25.2%)	75 (24.0%)	49 (23.1%)	99 (27.5%)	69 (24.7%)	0.835
Omega-3	313 (24.6%)	34 (31.8%)	72 (23.0%)	54 (25.5%)	81 (22.5%)	72 (25.8%)	
Multidomain intervention	318 (25.0%)	25 (23.4%)	82 (26.2%)	54 (25.5%)	92 (25.6%)	65 (23.3%)	
Placebo	321 (25.3%)	21 (19.6%)	84 (26.8%)	55 (25.9%)	88 (24.4%)	73 (26.2%)	
Education							
No diploma	60 (4.8%)	10 (9.4%)[a,b,c,d]	23 (7.4%)[a,e]	7 (3.3%)[b]	9 (2.5%)[c,e]	11 (4.0%)[d]	<0.001
Primary school certificate	221 (17.6%)	39 (36.8%)	66 (21.4%)	38 (17.9%)	39 (11.0%)	39 (14.3%)	
Secondary education	422 (33.6%)	36 (34.0%)	101 (32.7%)	74 (34.9%)	128 (36.0%)	83 (30.5%)	
High school diploma	178 (14.2%)	6 (5.7%)	46 (14.9%)	27 (12.7%)	58 (16.3%)	41 (15.1%)	
University level	374 (29.8%)	15 (14.2%)	73 (23.6%)	66 (31.1%)	122 (34.3%)	98 (36.0%)	
Number of chronic diseases	1.1 (1.0)	1.4 (1.1)[a,b]	1.2 (1.0)	1.1 (1.0)	1.0 (0.9)[a]	1.1 (1.0)[b]	0.003
Global IC score, 0–100	77.2 (8.0)	62.9 (7.8)[a,b,c,d]	73.1 (5.7)[a,e,f]	74.3 (5.5)[b,g,h]	80.9 (3.7)[c,e,g,i]	84.4 (4.1)[d,f,h,i]	<0.001
IC indicators							
Cognitive: MMSE, 0–30	28.2 (1.7)	26.5 (2.3)[a,b,c,d]	27.7 (1.9)[a,e,f,g]	28.2 (1.6)[b,e,h,i]	28.6 (1.4)[c,f,h]	28.6 (1.3)[d,g,i]	<0.001
Locomotion: SPPB, 0–12	10.7 (1.7)	8.4 (2.2)[a,b,c,d]	9.6 (1.7)[a,e,f,g]	11.1 (1.1)[b,e,h]	11.5 (1.0)[c,f,h]	11.4 (0.9) [d,g]	<0.001
Psychological: GDS, 0–15	3.0 (2.5)	6.8 (2.7)[a,b,c,d]	2.7 (1.7)[a,e,f,g]	5.5 (2.4)[b,e,h,i]	1.6 (1.5)[c,f,h]	1.6 (1.4)[d,g,i]	<0.001
Vitality: handgrip strength, kg	26.8 (9.8)	22.1 (8.0)[a,b]	22.0 (7.8)[c,d,e]	26.8 (8.1)[a,c,f,g]	23.7 (6.4)[d,f,h]	37.9 (8.5)[b,e,g,h]	<0.001
Plasma biomarkers							
CRP, mg/L	3.3 (5.3)	4.9 (8.9)[a]	3.6 (5.1)	3.0 (5.0)	3.2 (5.4)	2.8 (3.5)[a]	0.032
IL-6, pg/mL	3.3 (3.1)	3.9 (3.4)	3.6 (2.9)	3.5 (3.9)	3.1 (2.7)	3.0 (3.1)	0.045
TNFR-1, pg/mL	1207.9 (395.1)	1372.6 (497.5)[a,b,c]	1276.8 (420.1)[d,e]	1192.5 (365.6)[a]	1141.9 (371.1)[b,d]	1176.1 (354.7)[c,e]	<0.001
MCP-1, pg/mL	220.9 (82.2)	224.3 (75.0)	219.2 (77.6)	229.5 (80.6)	217.9 (86.6)	218.4 (84.9)	0.610
GDF-15, pg/mL	1104.9 (473.1)	1330.4 (507.6)[a,b,c]	1200.5 (545.0)[d,e]	1106.8 (465.6)[a]	1011.3 (378.2)[b,d]	1045.9 (456.0)[c,e]	<0.001

Table 1 (continued)

	Total (n = 1271)	Multi-trajectory group membership					
		Low in all domains (n = 107 [8.4%])	Low locomotion (n = 313 [24.6%])	Low psychological domain (n = 212 [16.7%])	Robust (n = 360 [28.3%])	Robust with high vitality (n = 279 [22.0%])	p value
ATPase IF1, ng/mL	581.7 (276.6)	586.5 (279.1)	579.3 (274.5)	606.5 (292.6)	581.0 (281.7)	563.9 (258.9)	0.674

Values were presented in mean (standard deviation) for continuous variables or number (%) for categorical variables. p value based on ANOVA or Chi-square test across groups. Bonferroni-corrected threshold for significance was applied for pairwise comparisons in Chi-square test. Post hoc pairwise comparisons were performed using the Tukey–Kramer test for continuous variables. Same letters were assigned to the groups showing significant difference based on the Tukey–Kramer test or Chi-square test. Data were missing for some participants for education (n = 16), CRP (n = 288), IL-6 (n = 290), TNFR-1 (n = 287), MCP-1 (n = 289), GDF-15 (n = 287), and IF1 (n = 286). Values for global IC score and each IC indicator are based on data at the 12-month visit, which were missing for some participants for MMSE (n = 18), SPPB (n = 35), GDS (n = 22), and handgrip strength (n = 116). *CRP*, C-reactive protein; *GDF-15*, growth differentiation factor-15; *GDS*, Geriatric Depression Scale; *IC*, intrinsic capacity; *IF1*, inhibitory factor 1; *IL-6*, interleukin-6; *MAPT*, Multidomain Alzheimer Preventive Trial; *MCP-1*, monocyte chemoattractant protein-1; *MMSE*, Mini-Mental State Examination; *SPPB*, Short Physical Performance Battery; *TNFR-1*, tumor necrosis factor receptor-1

0.001). In addition, increased IL-6 and GDF-15 levels were associated with a higher risk of belonging to the "low locomotion" group (IL-6: RRR = 1.37 [1.10 – 1.71]; p = 0.005; GDF-15: RRR = 1.48 [1.17 – 1.89]; p = 0.001). There is a weaker association between GDF-15 and "low psychological domain" group (RRR = 1.29 [1.01 – 1.64]; p = 0.044). Despite not reaching significance, we observed that people with increased IL-6 and GDF-15 tended to be in the robust group rather than in the "robust with high vitality" group (IL-6: RRR [95% CI] = 1.20 [0.97 – 1.48]; p = 0.094; GDF-15: RRR = 1.23 [0.98 – 1.54]; p = 0.070) (Table 2). Sensitivity analysis including biomarker outliers provided similar results for TNFR-1 and GDF-15. IL-6 was associated with all IC multi-trajectory groups after considering biomarker outliers, with a trend of increasing RRRs from the robust group to the "low in all domains" group (Table S4).

Trajectories of the composite IC score

As detailed in Supplemental Text 2 and Table S3, four trajectory groups of global IC were identified: low baseline IC with steep decline (5.3%; called hereafter "steep declining"), intermediate baseline IC with moderate decline (18.9%; called hereafter "moderate declining"), intermediate baseline IC with mild decline (35.7%; called hereafter "mild declining"), and high baseline IC with very mild decline (40.1%)

Table 2 Association between plasma biomarkers and IC multi-trajectory groups examining by multinomial logistic regression

	Low in all domains vs. robust with high vitality		Low locomotion vs. robust with high vitality		Low psychological domain vs. robust with high vitality		Robust vs. robust with high vitality	
	RRR (95% CI)	p value	RRR (95% CI)	p value	RRR (95% CI)	p value	RRR (95% CI)	p value
CRP	1.22 (0.93, 1.60)	0.157	1.20 (0.98, 1.47)	0.084	1.02 (0.82, 1.26)	0.891	0.97 (0.80, 1.18)	0.762
IL-6	1.42 (1.07, 1.88)	0.016	1.37 (1.10, 1.71)	0.005	1.25 (0.99, 1.56)	0.056	1.20 (0.97, 1.48)	0.094
TNFR-1	1.46 (1.09, 1.96)	0.011	1.21 (0.98, 1.51)	0.083	1.06 (0.85, 1.33)	0.579	0.99 (0.81, 1.21)	0.913
MCP-1	0.98 (0.74, 1.29)	0.860	0.91 (0.73, 1.13)	0.381	1.11 (0.90, 1.38)	0.334	0.99 (0.81, 1.21)	0.905
GDF-15	1.99 (1.45, 2.73)	<0.001	1.48 (1.17, 1.89)	0.001	1.29 (1.01, 1.64)	0.044	1.23 (0.98, 1.54)	0.070
ATPase IF1	0.89 (0.66, 1.18)	0.413	0.90 (0.73, 1.11)	0.321	1.05 (0.85, 1.30)	0.664	0.92 (0.76, 1.11)	0.377

All biomarker values were log-transformed and then standardized. All models were adjusted for age, sex, educational levels, MAPT group allocation, and the number of chronic diseases. *CRP*, C-reactive protein; *GDF-15*, growth differentiation factor-15; *IC*, intrinsic capacity; *IF1*, inhibitory factor 1; *IL-6*, interleukin-6; *MCP-1*, monocyte chemoattractant protein-1; *RRR*, relative risk ratio; *TNFR-1*, tumor necrosis factor receptor-1

(Figure S4). Four trajectories followed a similar pattern, with lower initial composite IC scores tending to have steeper IC declining slopes over time. Sensitivity analysis on females and males obtained similar trajectory group membership (Figure S5).

Table S5 displays how participants were distributed in the four global IC trajectories and the five IC multi-trajectory groups. For people with steep IC decline, 91.0% were in the "low in all domains" group. Individuals with the high baseline IC tended to be "robust (48.2%)" or "robust with high vitality (51.4%)". Furthermore, participants with moderate declining and mild declining were distributed across three and more IC multi-trajectories, suggesting that individuals may have different declining patterns in IC components even if their global IC trajectories were similar.

Significant differences in baseline characteristics exist between four global IC trajectory groups (Table S6). Participants with higher plasma IL-6, TNFR-1, and GDF-15 were more likely to have lower and accelerated global IC trajectories (Table S7). Again, GDF-15 had the strongest association with IC trajectory groups. For one SD increase in logGDF-15 levels, the RRR compared to the high baseline IC trajectory was 1.92 (95% CI = 1.34 – 2.74; $p < 0.001$) for the steep declining group and 1.58 (95% CI = 1.27 – 1.98; $p < 0.001$) for the moderate declining group. Associations between plasma biomarkers and global IC trajectories remained unchanged after including biomarker outliers (Table S8).

Discussion

This study identified five IC multi-trajectories over four years among older adults aged ≥70. About half of the total population exhibited high and stable trajectories in cognition, locomotion, and psychology, and around 8% of people showed impairments in all IC domains. We observed that higher plasma IL-6, TNFR-1, and GDF-15 increased the likelihood of having the "low in all IC domains" trajectory. Older people with elevated IL-6 and GDF-15 levels also tended to suffer from lower and faster declining locomotion. Exploratory analysis showed that higher IL-6, TNFR-1, and GDF-15 remained associations with lower and faster declining IC trajectories when integrating IC domains as a single score.

Our study demonstrated that higher plasma IL-6, TNFR-1, and GDF-15 were independent risk factors for older adults with multi-impaired IC trajectories. Indeed, low IC had been significantly associated with the markers reflecting systematic inflammatory states, including IL-6 [11] and TNFR-1 [12], compared to other biochemical markers. In the present study, GDF-15 outperformed other plasma biomarkers by showing the highest RRRs and associations with several trajectory groups. Various tissues or organs express and secrete GDF-15, such as the adipose tissue, the skeletal muscle, and the liver, and its expression is upregulated in various age-related pathological conditions [23, 24]. GDF-15 is also involved in regulating energy intake and body weight change via the GDNF α-like receptor (GFRAL) [24]. Secretion of small amount of GDF-15 during specific periods of life is adaptive as it participates to restore/maintain homeostasis through regulating inflammatory and metabolic signals. However, homeostatic mechanisms become impaired with age and GDF-15 elevation prolongs, leading to an overall detrimental effect on body function [23]. High GDF-15 had been suggested to mirror the underlying process of physiological decline, as it was strongly associated with acute events and shorter survival through the life course [34]. In this sense, increased GDF-15 levels may reflect the reduction of the physiological reserve, resulting in lower levels and a faster decline in IC domains. Taken together, the close link between GDF-15, energy metabolism, and physiological homeostasis may explain the strong relationship of high GDF-15 on multiple impaired trajectories, from domain-specific to global IC, and from moderate to steep declines. Furthermore, although not reaching statistical significance, our findings suggest that GDF-15 levels might be an indicator of vitality able to differentiate robust people with high or low vitality levels. Of note, vitality has been considered a core domain representing the underlying physiological determinant of IC, and early detection of vitality deterioration could allow interventions to preserve or increase IC [35]. Whether GDF-15 may be an indicator of vitality and whether its levels may help to better stratify high functioning individuals according to their future risk of IC declining and onset of clinical events deserve further investigation.

The mechanisms linking plasma IL-6 to IC trajectories are not fully understood. It had been suggested

that elevated IL-6 contributes to functional disability through its detrimental effect on muscle [36] or mediated by medical conditions, such as cardiovascular diseases and diabetes [18]. Recently, IL-6 has been proposed to help energy allocation by promoting catabolic states, including in the muscle tissue, to support the high energy demand from the immune system during chronic inflammation [37]. Perhaps this explains why increasing IL-6 was notably correlated to declining locomotion trajectory in our study, possibly resulting from IL-6-induced loss of muscle mass and functioning. In contrast, plasma TNFR-1 was not associated with any domain-specific IC trajectory. Prior investigations found that TNFR-1 predicted future cognitive decline at the preclinical stage [38], and its signaling was involved in the early pathogenesis of dementia [39]. Thus, it is possible that TNFR-1 is more sensitive to cognitive capacity, and its association would be more likely to be observed in IC trajectories driven by impaired cognitive domain or at least trajectories influenced/composed by cognitive function, such as the "low in all domains" group or the global IC score (which considers the overall effects of all IC domains).

Plasma biomarkers showing significant associations with IC trajectories (i.e., GDF-15, IL-6, and TNFR-1) tended to be those that had demonstrated associations with chronological age in younger and older adults [40, 41] and a myriad of age-related clinical conditions (such as chronic diseases [23, 39, 42] and functional capacity [43, 44]), suggesting these markers are involved in cascades leading to different phenotypic expressions of aging. In contrast, evidence on MCP-1 upregulation and IF1 reduction in blood was commonly discovered in people who suffered from age-related pathologies (e.g., neurodegeneration for MCP-1 [45] and insulin resistance for IF1 [46]); thus, perhaps the expressions of these biomarkers correlate more with specific pathologies rather than aging itself. Future studies, especially those with a more comprehensive selection of plasma biomarkers, are needed to validate this assumption.

As one of the first works to investigate the biological risk factors of longitudinal IC trajectories, we highlight the use of GBTM to explore domain-specific trajectories and the comparisons between multiple aging biomarkers in a large population of older adults. Nevertheless, some limitations should be noted. First, although we have measured biomarkers of inflammation and mitochondrial function, biomarkers of other biological aging mechanisms were not included due to data availability. Second, the sensory domain was not considered in our IC measure. It is plausible that more IC trajectories may exist in older adults when the sensory domain is considered. Third, this is a secondary analysis of a randomized controlled trial composed of participants ≥70 years, with either subjective memory complaint or IADL limitation or slow gait speed. More studies are required to ascertain the generalizability of our findings to other populations. Fourth, three-quarters of our population received MAPT interventions until the 36-month visit. However, our previous work did not observe any intervention effect on IC in MAPT [31]. We also adjusted the MAPT group allocation as a covariate in the regression analysis to minimize this bias.

In conclusion, our study supported the heterogeneity of functional aging between individuals by identifying IC trajectories with different domain impairments. Plasma biomarkers reflecting inflammation and mitochondrial impairment distinguished older people with multi-impaired IC trajectories from those with high-stable IC. Future research should focus on how aging biomarkers may facilitate the IC-based risk stratification of community-dwelling older people for individualized medicine and serve as potential targets/surrogate markers for future geroscience intervention. More population-based studies using the full five-domain IC measure are encouraged to confirm biomarkers' utility in clinical care.

Author contributions WHL designed and conceptualized the research, performed the analyses, interpreted the data, and drafted the manuscript. SG interpreted the data and revised the draft critically for important intellectual content. LOM managed data of plasma IF1, interpreted the data, and revised the draft critically for important intellectual content. AL and AP managed data of plasma IL-6, TNFR-1, MCP-1, and GDF-15; interpreted the data; and revised the draft critically for important intellectual content. BV conceived the MAPT study, interpreted the data, and revised the draft critically for important intellectual content. PdSB designed and conceptualized the research, interpreted the data, and revised the draft critically for important intellectual content. All authors have read and agreed with the final version to be submitted.

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Declarations

Competing interests The authors declare no competing interests.

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Plasma inflammation-related biomarkers are associated with intrinsic capacity in community-dwelling older adults

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Abstract

Background How inflammation relates to intrinsic capacity (IC), the composite of physical and mental capacities, remains undefined. Our study aimed to investigate the cross-sectional and longitudinal associations between plasma inflammation-related biomarkers and IC in older adults.

Methods This secondary analysis of the Multidomain Alzheimer Preventive Trial (MAPT) included 1238 community-dwelling older individuals with IC assessments from 12 to 60 months. Plasma C-reactive protein (CRP), interleukin-6 (IL-6), tumour necrosis factor receptor-1 (TNFR-1), monocyte chemoattractant protein-1 (MCP-1) and growth differentiation factor-15 (GDF-15) were measured at 12 months. IC was operationalized as a score ranging from 0 to 100, derived from four domains: cognition, *Mini-Mental State Examination*; locomotion, *Short Physical Performance Battery*; psychological, *Geriatric Depression Scale*; and vitality, *handgrip strength*. A five-domain IC score (plus sensory) was investigated in a subsample (n = 535) with a 1-year follow-up as an exploratory outcome.

Results The mean age of the 1238 participants was 76.2 years (SD = 4.3); 63.7% were female. Their initial four-domain IC scores averaged 78.9 points (SD = 9.3), with a yearly decline of 1.17 points (95% CI = −1.30 to −1.05; $P < 0.001$). We observed significant associations of lower baseline IC with higher CRP, IL-6, TNFR-1 and GDF-15, after controlling age, sex, MAPT group allocation and educational level [*CRP:* adjusted β (95% CI) = −1.56 (−2.64 to −0.48); $P = 0.005$; *IL-6:* adjusted β = −3.16 (−4.82 to −1.50); $P < 0.001$; *TNFR-1:* adjusted β = −6.86 (−10.25 to −3.47); $P < 0.001$; *GDF-15:* adjusted β = −7.07 (−10.02 to −4.12); $P < 0.001$]. Higher TNFR-1, MCP-1 and GDF-15 were associated with faster decline in four-domain IC over 4 years [*TNFR-1:* adjusted β (95% CI) = −1.28 (−2.29 to −0.27); $P = 0.013$; *MCP-1:* adjusted β = −1.33 (−2.24 to −0.42); $P = 0.004$; *GDF-15:* adjusted β = −1.42 (−2.26 to −0.58); $P = 0.001$]. None of the biomarkers was significantly associated with the five-domain IC decline.

Conclusions Inflammation was associated with lower IC in older adults. Among all plasma biomarkers, TNFR-1 and GDF-15 were consistently associated with IC at the cross-sectional and longitudinal levels.

Keywords Biological ageing; Geroscience; Functional decline; TNFR-1; MCP-1; GDF-15

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Introduction

Intrinsic capacity (IC) is the composite of physical and mental capacities that individuals can draw upon as they age, encompassing the domains of cognition, locomotion, psychological, vitality and sensory.[1,2] Overall, an individual's IC reaches a peak in early adulthood and then declines gradually with age.[1] Thus, monitoring and enhancing IC is useful to prevent functional impairment and promote healthy ageing.[S1] Indeed, a lower IC predicts the onset of disability,[3,S2] institutionalization[3] and mortality[4] in older adults. Despite the fact that IC shows an overall declining trend, there is a wide variation in IC levels and decline rates among older individuals.[3,5] Heterogeneity in IC trajectories is possibly explained by individuals' variability in biological ageing. Yet, the link between biological ageing and IC is not fully understood.

Inflammation is one of the hallmarks of ageing[S3,S4] and has been implicated in several age-related diseases, including dementia[S5] and depression.[S6,S7] In the skeletal muscle, inflammation can directly trigger muscle catabolism or inhibit growth factors,[6,7] resulting in declined muscle strength[8] and mobility disability.[9,10] In addition, inflammation has been associated with the incidence of age-related sensory impairments.[S8,S9] Previous epidemiologic studies have measured circulating pro-inflammatory factors—including acute-phase proteins, cytokines and chemokines—to quantify the inflammatory status in older adults.[11] Plasma markers like growth differentiation factor-15 (GDF-15), which is induced by age-related inflammation[12] and strongly predicts adverse health outcomes,[13,S10] are also promising candidates in recent research. Although the link between inflammation-related biomarkers and individual IC domains has been extensively explored,[8,14-17,S8,S11–S13] the association with the IC composite remains poorly investigated. To the best of our knowledge, only three observational studies investigated the association between inflammation-related biomarkers with IC.[4,18,19] However, their findings were inconsistent, probably due to their cross-sectional approach,[4,19] categorical measures of IC,[4,19] small sample size[19] and a focus on pro-inflammatory markers.[4,18,19]

Considering the limitations in the literature, we aimed to investigate the cross-sectional and longitudinal associations between IC and five plasma inflammation-related biomarkers: C-reactive protein (CRP, acute-phase protein), interleukin-6 (IL-6, cytokine), tumour necrosis factor receptor-1 (TNFR-1, soluble cytokine receptor), monocyte chemoattractant protein-1 (MCP-1, chemokine) and GDF-15. Moreover, we compared the inflammatory biological profiles of individuals according to their longitudinal IC trajectories. We hypothesized that older adults with higher levels of inflammation-related biomarkers would have lower IC at baseline and faster IC decline over time.

Methods

Data source

This is a secondary analysis of the Multidomain Alzheimer Preventive Trial (MAPT). MAPT Study is a multicentre, randomized controlled trial investigating the preventive effects of omega-3 polyunsaturated fatty acid (PUFA) supplementation and multidomain lifestyle interventions on cognitive decline in community-dwelling older adults.[S14] Participants were randomized into four groups: receiving both omega-3 PUFA supplementation and multidomain intervention (including exercise advice, cognitive training and nutritional counselling), receiving omega-3 PUFA supplementation only, receiving placebo and multidomain intervention and a placebo control group. After the 3-year intervention, no beneficial effects of the omega-3 supplementation and multidomain interventions, either combined or alone, on cognitive function[S15] and IC[S16] were observed. An additional 2-year observation without any intervention was conducted on the MAPT participants after the intervention was completed. The MAPT Study [no. NCT00672685] was approved by the French Ethical Committee located in Toulouse (CPP SOOM II) and authorized by the French Health Authority. All participants signed informed consent.

Study population

The MAPT Study recruited 1679 adults aged 70 years and over with the following inclusion criteria: spontaneous memory complaint, limitations in one instrumental activity of daily living or gait speed $\leq$0.8 m/s. Patients were excluded if presenting with a Mini-Mental State Examination (MMSE)[S17] score $<$24, diagnosis of dementia, limitation in any basic activities of daily living and taking omega-3 supplements within the past 6 months before study enrolment. The current study included 1238 subjects with data for plasma biomarkers and IC from the 12- to 60-month visits. Among them, 535 participants in the MAPT multidomain intervention groups (either combined omega-3 supplementation or not) underwent vision and hearing assessments at the 12-month and 24-month visits and were included to estimate the five-domain IC as an exploratory outcome.

Measurement of plasma biomarkers

Plasma biomarkers were measured during the 12-month visit in the MAPT Study. CRP was measured (mg/L) by immunoturbidity according to standard protocols. Plasma levels of IL-6, TNFR-1, MCP-1 and GDF-15 were assessed using the fully automated immunoassay platform, Ella (ProteinSimple/Bio-Techne, San Jose, CA, USA). Proteins were

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quantified using a single disposable microfluidic SimplePlexTM cartridge. The plasma samples were thawed on ice, diluted 1:4 in sample diluent (SD 13) and loaded into cartridges with high and low control concentrates. Each plasma sample was divided into four unique microfluidic parallel channels within the cartridge, which were specific for each of the four proteins being analysed. Each protein channel contains three analyte-specific glass nanoreactors (GNRs), allowing each plasma sample to be run in triplicates for each of the four protein samples. Cartridges included a built-in lot-specific standard curve for each defined protein. All steps in the procedure were conducted automatically by the instrument without user activity. The obtained protein concentrations were calculated by the internal instrument software and displayed in pg/mL.

Measurement of IC

The primary outcome was the four-domain IC composed of cognition, locomotion, psychological and vitality. Cognition was assessed by the 30-item MMSE (a higher score indicates better cognitive function).[S17] Locomotion was evaluated by the Short Physical Performance Battery (SPPB; ranging from 0 to 12; higher means better performance).[S18] The psychological domain was measured by the 15-item Geriatric Depression Scale (GDS), with scores varying from 0 to 15 (higher is worse).[S19] We used the handgrip strength of the dominant hand, measured with a hydraulic dynamometer (Jamar, Bolingbrook, IL), for vitality. Participants were asked to hold the dynamometer in a standing position with the arm straight down and squeeze it as hard as possible. The maximum value of three attempts was recorded [in kilograms (kg)]. All four domains were annually evaluated for the entire study population from the 12-month visit (defined as the baseline in this study) to the 60-month visit (the average length of follow-up: 3.1 ± 1.3 years). To obtain the global IC score, we first rescaled the measurements of each domain into the 100-scale system (higher indicates better function); the original scores of GDS were weighted as -1 before rescaling. We further calculated the global IC score by the arithmetic mean of these four domains.

Considering the sensory function was only measured in half of the MAPT population at 12 and 24 months, we evaluated the five-domain IC as an exploratory outcome. This outcome consisted of the prior four domains plus the sensory according to the original IC construct.[2] The sensory domain included the near-vision acuity and the hearing impairment indicated by the Screening Version of Hearing Handicap Inventory for the Elderly (HHIE-S).[S20,S21] The near-vision acuity was measured by a Monoyer chart with a score ranging from 0 to 10 (a higher score indicates better performance); the average performance of the left and right eyes was applied. The HHIE-S scale comprises 10 questions about the perception of hearing difficulty in older adults' daily lives. Participants were scored as 0 if they perceived no difficulty, 2 if they reported sometimes and 4 if they reported difficulty. The overall HHIE-S score ranged from 0 to 40; a higher score indicates worse hearing performance.[S20,S21] We rescaled the HHIE-S score and visual acuity following the abovementioned method; the original scores of HHIE-S were weighted as -1 before rescaling. The score of the sensory domain was calculated by averaging the rescaled values of the HHIE-S and visual acuity. The five-domain IC score was determined as the mean value of the five IC domains (cognition, locomotion, psychological, vitality and sensory).

Trajectories of IC

Participants were classified into three IC trajectory groups according to their IC rate of change over time (details described in the 'Statistical analysis' section): (1) 'Accelerated IC decline', if their IC slopes were < -1.78 points per year (the lowest 20%); (2) 'Slight IC decline', if their IC slopes were within -1.78 to -0.53 (middle 60%); and (3) 'Stable IC', if their IC slopes were > -0.53 (the highest 20%).

Covariates

The demographic covariates used for the analyses were age, sex, MAPT group allocation and educational level (no diploma, primary school certificate, secondary education, high school diploma, university level).

Statistical analysis

Descriptive data were presented as means and standard deviations (SD), medians and interquartile ranges (IQRs) or frequencies and percentages, as appropriate. We first used linear mixed-effects regressions to identify the longitudinal trajectories of IC in our study population. When examining the primary outcome (i.e. repeated four-domain IC measurements from 12 to 60 months), a random effect at participant level and a random slope on time were included in the mixed-effects models. For the exploratory outcome (i.e. repeated five-domain IC measurements at 12 and 24 months), only a random effect at participant level was considered (insignificant random effect on time). The linear mixed-effects models showed that IC trajectories were linear in our population; the time coefficient represented the slope of IC change over time.

To evaluate the associations of IC with each plasma biomarker, we introduced plasma biomarkers into the linear mixed-effects models (a separate model per biomarker). IC was evaluated as a global score and as separate IC domains. The biomarker coefficients in the models indicated

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cross-sectional associations, and the coefficients for biomarker–time interaction indicated longitudinal associations. All models were adjusted for the covariates mentioned above. We kept all biomarker values in our main analysis because there are no established cut-offs for extreme values of plasma biomarkers in the literature. We further conducted a sensitivity analysis with biomarker outliers (defined as >4 SD from the mean) removed to test whether such values have altered the associations.

We estimated each participant's IC trajectory slope based on their longitudinal measurements of four-domain IC and demographic covariates using the linear mixed-effects model aforementioned (without plasma biomarkers). The differences in plasma biomarker concentrations between IC trajectory groups were compared using analysis of covariance (ANCOVA) with adjustment for age and sex, followed by the Tukey–Kramer post hoc test for pairwise comparisons. All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC) and STATA version 17 (College Station, TX), with a significance level of 0.05.

Table 1 Characteristics of study population at the 12-month visit of the MAPT Study

	N	Median (IQR) or *n* (%)
Age (year), *mean (SD)*	1238	76.2 (4.3)
Female	1238	789 (63.7%)
MAPT group		
Multidomain intervention + omega-3	1238	309 (25.0%)
Omega-3		303 (24.5%)
Multidomain intervention		317 (25.6%)
Placebo		309 (25.0%)
Education		
No diploma	1238	62 (5.0%)
Primary school certificate		210 (17.0%)
Secondary education		408 (33.0%)
High school diploma		185 (14.9%)
University level		373 (30.1%)
Measurement for IC domain[a]		
Cognitive: MMSE, 0–30	1238	29 (27, 29)
Locomotion: SPPB, 0–12	1217	11 (10, 12)
Psychological: GDS, 0–15	1233	3 (1, 4)
Vitality: Handgrip strength (kg)	1135	25 (20, 34)
Sensory		
Visual acuity—Monoyer scale, 0–10	510	7 (5.5, 9)
HHIE-S, 0–40	536	4 (0, 12)
Global IC score, 0-100[b], *mean (SD)*		
4 domains (without sensory)	1115	78.9 (9.3)
5 domains (with sensory)	462	78.9 (8.3)
Plasma biomarker		
CRP, mg/L	1060	1.8 (1, 3.7)
IL-6, pg/mL	1062	2.57 (1.81, 3.8)
TNFR-1, pg/mL	1063	1142 (957, 1386)
MCP-1, pg/mL	1063	204 (170, 252)
GDF-15, pg/mL	1062	1003.5 (804, 1309)

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; GDS, Geriatric Depression Scale; HHIE-S, Hearing Handicap Inventory for the Elderly—the Screening Version; IC, intrinsic capacity; IL-6, interleukin-6; MAPT, Multidomain Alzheimer Preventive Trial; MCP-1, monocyte chemoattractant protein-1; MMSE, Mini-Mental State Examination; SPPB, Short Physical Performance Battery; TNFR-1, tumour necrosis factor receptor-1.
Value presented in median (IQR) for continuous variables or number (%) for categorical variables except where indicated other.
[a]Higher value indicates better function except for GDS and HHIE-S.
[b]Higher value indicates better IC.

Results

Characteristics of the study population and IC trajectories

Of 1238 participants, the mean age was 76.2 (SD = 4.3) years, and 63.7% were female. The mean value of the baseline four-domain IC score was 78.9 (SD = 9.3) of 100 points possible (*Table* 1), and the annual IC decline rate was 1.2 points per year on average (adjusted $\beta = -1.17$; 95% CI: -1.30 to -1.05; $P < 0.001$; *Table* S1). Similar baseline IC score and annual IC decline rate were found using the five-domain measure (*Tables* 1 and S1).

Association between plasma biomarkers and global IC score

Table 2 displays the cross-sectional and longitudinal associations between plasma inflammation-related biomarkers and IC. Regarding the four-domain IC, higher CRP, IL-6, TNFR-1 and GDF-15 were associated with lower IC scores at baseline. In addition, participants with higher TNFR-1, MCP-1 and GDF-15 had faster IC declines over time. Significant associations of IL-6, TNFR-1 and GDF-15 with IC were observed with five-domain IC at the cross-sectional level, but none of these biomarkers was associated with IC change over time. Sensitivity analysis removing the extreme plasma biomarkers values provided similar results, except for plasma TNFR-1 (*Table* S2); plasma TNFR-1 was not associated with baseline five-domain IC score after removing one outlier (adjusted $\beta = -3.20$; 95% CI: -8.38 to 1.98; $P = 0.225$; *Table* S2).

Association between plasma biomarkers and individual IC domain

After adjusting for covariates, all plasma biomarkers showed associations with locomotion change. Furthermore, elevated MCP-1 and GDF-15 were associated with worsening function in the psychological domain. Increasing MCP-1 levels were also associated with worse vitality over time. None of the five plasma biomarkers predicted the change of cognitive domain (*Figure* 1 and *Table* S3).

Table 2 Associations between plasma inflammation-related biomarkers and IC

Plasma biomarker[a]	Four-domain IC score[b]					Five-domain IC score[c]				
	N	β	95% CI		*P*	*N*	β	95% CI		*P*
Cross-sectional										
CRP	1060	−1.56	−2.64	−0.48	0.005	453	−1.13	−2.70	0.43	0.156
IL-6	1062	−3.16	−4.82	−1.50	<0.001	460	−2.76	−5.19	−0.32	0.026
TNFR-1	1063	−6.86	−10.25	−3.47	<0.001	461	−5.01	−9.99	−0.02	0.049
MCP-1	1063	1.08	−1.99	4.16	0.490	461	0.01	−4.28	4.29	0.998
GDF-15	1062	−7.07	−10.02	−4.12	<0.001	460	−5.20	−9.48	−0.92	0.017
Longitudinal										
CRP	1060	−0.13	−0.45	0.19	0.429	453	0.40	−0.96	1.76	0.566
IL-6	1062	−0.36	−0.85	0.13	0.150	460	1.59	−0.52	3.70	0.139
TNFR-1	1063	−1.28	−2.29	−0.27	0.013	461	−2.03	−6.20	2.14	0.341
MCP-1	1063	−1.33	−2.24	−0.42	0.004	461	0.25	−3.52	4.03	0.895
GDF-15	1062	−1.42	−2.26	−0.58	0.001	460	−1.24	−4.66	2.19	0.479

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IC, intrinsic capacity; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; TNFR-1, tumour necrosis factor receptor-1.
[a]All values of biomarkers were log-transformed.
[b]Composed of cognitive, locomotive, psychological and vitality domains; evaluated by linear mixed-effects regression with adjustment for age, sex, Multidomain Alzheimer Preventive Trial (MAPT) group allocation and educational level.
[c]Composed of cognitive, locomotive, psychological, vitality and sensory (vision + hearing) domains; evaluated by linear mixed-effects regression with adjustment for age, sex, MAPT group allocation and educational level.

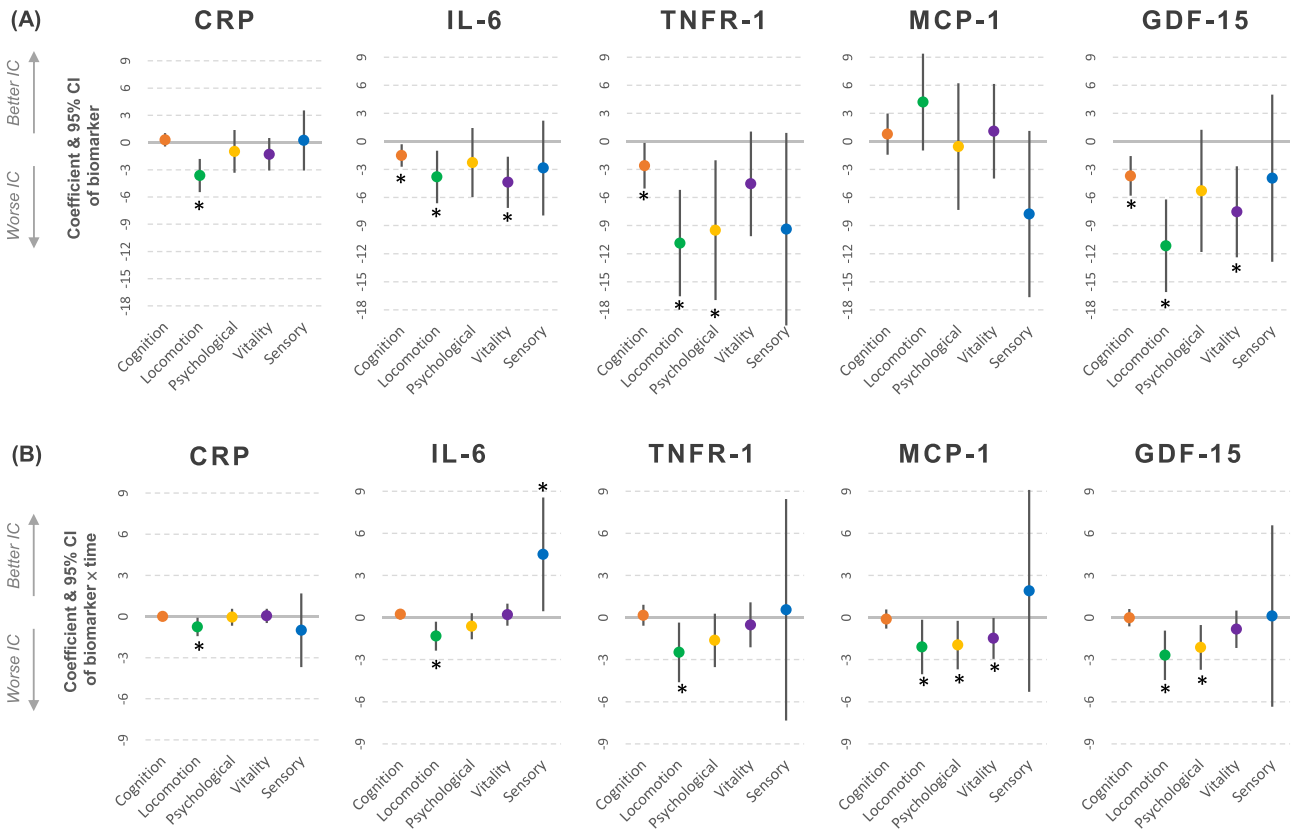

Figure 1 (A) Cross-sectional; (B) longitudinal associations between plasma inflammation-related biomarkers and intrinsic capacity (IC) domains examining by linear mixed-effects regression. The sensory domain is composed of vision and hearing capacities. *$P < 0.05$.

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Descriptive inflammatory profile according to different IC trajectory groups

Plasma levels of TNFR-1, MCP-1 and GDF-15 presented significant differences between participants with different IC trajectories (adjusted *P* value for difference <0.05; *Table* 3). A gradient in the levels of all biomarkers was observed, with the highest levels obtained in the IC accelerated group and the lowest in the IC stable group. Compared with participants with stable or slight declined IC, those with accelerated IC decline were more likely to have higher plasma TNFR-1, MCP-1 and GDF-15 at baseline (post hoc *P* value < 0.05 for all pairwise comparisons; *Table* 3), indicating that these three plasma biomarkers had potential to identify older adults with worse IC trajectories.

Discussion

This study investigated the associations of five circulating inflammation-related markers with IC in older adults. We demonstrated that higher TNFR-1 and GDF-15 were consistently associated with four-domain IC, consisting of cognition, locomotion, psychological and vitality capacities, at both cross-sectional and longitudinal levels. Domain-specific analysis revealed that all inflammatory biomarker concentrations predicted the locomotion change over time; higher plasma GDF-15 levels were associated with faster psychological declines, and higher plasma MCP-1 levels were related to the impairment of psychological and vitality domains. Furthermore, plasma levels of TNFR-1, MCP-1 and GDF-15 showed a gradient in reference to the IC longitudinal trajectories, with the highest levels observed in the accelerated group and the lowest in the IC stable group. Taken together, these findings supported our hypothesis that higher levels of inflammation-related biomarkers were associated with lower initial IC levels and faster IC declining rates in older adults.

Despite the slight differences in the measurement for each IC domain, our study population presented similar baseline IC levels (78.9 ± 9.3 points) to the PEP cohort from the USA (77 ± 11 points; mean age 78.4 years)[3] and was relatively higher compared with the Mexican population in the SAGE study (range from 36.9 to 61.2 points in a cohort aged ≥50 years).[5] We demonstrated that IC decreased 1.2 points per year in older adults. Furthermore, a 10-fold increase in plasma levels of TNFR-1, MCP-1 and GDF-15 was associated with an additional 1.3–1.4 points of IC decline per year. Although there is no established cut-off for clinically meaningful IC decline, Stolz et al.[3] found that a 1-point decrease in IC was associated with a 7% increased risk of ADL disability and a 5% increased risk of mortality in older adults. Taken together, the inflammation-related IC decline in older individuals may indicate a doubling or even higher risk of adverse health outcomes in the future.

Age-related inflammation can be derived from extrinsic causes—diet, smoking, chronic infections, etc.[20]—and intrinsic mechanisms, including cellular senescence and associated secretory phenotype (SASP)[20-22] and increased oxidative stress due to mitochondrial dysfunction.[22] IL-6,[23] TNFR-1,[24] MCP-1[25] and GDF-15[26,27] have been identified as the SASP components in different senescence cells. Elevated GDF-15 levels were also observed in response to impaired mitochondria, despite the unknown function remaining.[27] The persistent stimulation from the inflammation sources and age-associated immune dysregulation may lead to chronically

Table 3 Descriptive inflammatory profile according to categories of IC trajectory

		IC slopes (point per year)[a]				
Mean (SD)	Whole study population	Accelerated IC decline (lowest 20%, <−1.78)	Slight IC decline (middle 60%, −1.78 to −0.53)	Stable IC (highest 20%, > − 0.53)	*P* value for difference[b]	Post hoc analysis[c]
IC at baseline, 0–100	78.9 (9.3)	78.2 (8.9)	79.3 (9.4)	78.5 (9.4)	0.512	
IC slopes (change per year)	−1.17 (0.86)	−2.46 (0.68)	−1.10 (0.32)	−0.10 (0.36)	<0.001	* ** ***
Inflammation-related biomarkers						
CRP, mg/L	3.3 (5.2)	3.4 (5.4)	3.4 (5.4)	2.9 (4.6)	0.419	
IL-6, pg/mL	3.9 (12.4)	4.0 (9.6)	4.1 (14.9)	3.2 (3.0)	0.324	
TNFR-1, pg/mL	1223.7 (441.2)	1282.3 (455.6)	1239.8 (459.3)	1119.1 (346.5)	0.001	* ** ***
MCP-1, pg/mL	221.7 (86.2)	231.7 (80.1)	222.5 (88.9)	209.7 (82.6)	0.035	* ** ***
GDF-15, pg/mL	1125.7 (504.9)	1201.7 (550.2)	1145.9 (524.1)	991.8 (356.2)	0.002	* ** ***

CRP, C-reactive protein; GDF-15, growth differentiation factor-15; IC, intrinsic capacity; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1; TNFR-1, tumour necrosis factor receptor-1.
Values are presented as mean (SD); raw biomarker levels are provided in the table and log-transformed values were used in the parametric analysis.
[a]IC was composed of cognitive, locomotive, psychological and vitality domains (four-domain measure).
[b]*P* value was calculated by one-way analysis of covariance (ANCOVA) with adjustment for age and sex.
[c]*P* value was determined by the Tukey–Kramer post hoc test for pairwise comparisons; *$P < 0.05$ between groups with slight IC decline and with accelerated IC decline; **$P < 0.05$ between groups with stable IC and with accelerated IC decline; ***$P < 0.05$ between groups with stable IC and slight IC decline.

activated inflammation,[21] which promotes an overall catabolic state in tissues and organs[21] that eventually declines functional capacities. For example, the up-regulated cytokines can interfere with muscle[6,7] and bone anabolism,[28] further inducing decreased physical performance.[8,29] Moreover, neuroinflammation in the brain with activated microglia and astrocytes and over-producing pro-inflammatory cytokines may reduce neurogenesis and impair synaptic plasticity, resulting in cognitive decline.[30]

Circulating CRP, IL-6 and TNFR-1 have been commonly regarded as markers of systemic inflammatory response in literature.[11] In the current study, only plasma TNFR-1 was associated with longitudinal IC change, suggesting that TNFR-1 may be a more reliable marker of inflammation-related functional decline than CRP and IL-6. Indeed, TNFR-1 levels are relatively stable in circulation.[16,31] Similar results were observed in the prior study, which found significant associations of cognitive decline with TNFR-1 but not with IL-6.[32] Given that IL-6 had been strongly associated with mortality[33,34] and disability[35] in older people, it is also probable that plasma TNFR-1 is more sensitive to early functional decline, whereas IL-6 may be more related to long-term adverse outcomes. More mechanistic studies on testing this hypothesis, however, are acquired.

In the present study, higher plasma GDF-15 levels showed both cross-sectional and longitudinal associations with IC. GDF-15 had shown a protective effect against tissue inflammation.[12,36] Increased GDF-15 levels facilitated survival during acute infections by promoting tolerance to cardiac tissue damage in mice models.[36] In addition, GDF-15 depletion in old mice presented increased inflammatory responses in liver and adipose tissues.[12] On the other hand, elevated GDF-15 levels were associated with age-related inflammatory diseases,[37] and long-term adverse outcomes included mortality.[13,38] The exact biological function of GDF-15 during chronic inflammation is still unclear; however, our results contribute to the current body of evidence by showing higher GDF-15 levels were detrimental to IC evolution and suggesting plasma GDF-15 as a promising measure of biological age in older adults.

Despite lack of association with baseline IC, higher plasma MCP-1 levels were associated with longitudinal IC declines, particularly in the locomotion, psychological and vitality domains. MCP-1 has been known to enhance neuroinflammation and leads to cognitive impairment[39]; however, studies on plasma MCP-1 with other functional outcomes are poorly investigated. To the best of our knowledge, this is the first study that found that higher MCP-1 was prospectively associated with physical function and handgrip strength (as vitality). MCP-1 had been recognized as a marker of ageing through cellular senescence.[40] However, compared with other inflammation-related biomarkers in this study, the evidence on MCP-1, cellular senescence and age-related outcomes is scarce and remains to be clarified by more updated research.

Concerning the domain-specific analysis, all inflammation-related biomarkers were associated with locomotion decline, suggesting an early effect of inflammation on physical performance. This finding was in line with previous works investigating the associations between physical function and individual inflammatory biomarkers.[8,9,17] On the other hand, none of the plasma biomarker levels predicted cognitive capacity changes. This result was compatible with available research, which showed associations between IL-6 and cognitive declines in the studies with longer follow-up (7–9 years)[14,41] but not in the one with short observation (median 2.7-year follow-up).[15] It is worth noting that we observed the marginal association between increasing IL-6 levels and improved sensory capacity; however, we did not find individual associations of IL-6 levels with vision (adjusted $\beta = 4.38$; 95% CI: -1.69 to 10.45; $P = 0.158$) and hearing (adjusted $\beta = 3.54$; 95% CI: -1.32 to 8.41; $P = 0.153$).

Our exploratory study observed no significant associations between inflammatory biomarkers and IC evolution when the sensory capacity was considered, suggesting that the sensory domain might be at a more 'distal' link with biological ageing than the other 'core' four domains. Further studies exploring the hierarchy of the IC domains are needed. The lack of association could be explained by the short follow-up period because the substantial change in functional performance may take several years.[15] Moreover, the hearing capacity assessed by the handicap questionnaire (i.e. HHIE-S) may not be equivalent to the actual hearing ability of older individuals, and it can become less varied once they adapt to daily-life disabilities. Finally, it is not excluded that MAPT interventions may have affected this finding because all participants investigated for five-domain IC had received multidomain intervention during the follow-up period.

This is one of the first works to investigate the associations between plasma inflammation-related markers and IC using a longitudinal approach and multiple biomarkers. However, some limitations should be raised. First, there is no standard IC measurement yet in the current field. It is worth highlighting that five IC domains are interrelated, with changes in one that may affect others. Our current approach, which used the mean score of the domains to represent the global IC level, could not consider the within-domain interactions. Further research investigating the IC measurement that includes the dynamic interactions within domains can shed light on this issue. Second, our study is a secondary analysis of a randomized controlled trial, in which the sample size calculation was performed considering the primary analysis of the MAPT Study. Third, the MAPT Study enrolled subjects at risk of cognitive decline, which might affect the generalizability of our results to other populations. Fourth, we measured plasma biomarkers in a subset of MAPT participants 1 year after the study enrolment. Because three out of four subjects in the current study had received interventions, it is not excluded that their biomarker levels may be affected by MAPT

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intervention. Although our analyses added MAPT group allocation as a covariate to minimize this bias, the intervention effects cannot be omitted, particularly in the analyses for five-domain IC and sensory domains. In other words, the lack of association in our study may be related to the potential beneficial effect from interventions and require cautious interpretation. Finally, the full-domain IC including sensory was only explored in half of our population for 1-year follow-up. Future longitudinal studies operationalizing the complete IC domains with several years of follow-up and a large sample are required.

Conclusion

In conclusion, our study supported that inflammation, measured via plasma TNFR-1, MCP-1 and GDF-15, may be involved in the faster IC decline of older individuals, indicating a doubling or even higher risk of adverse health outcomes in the future. Further mechanistic studies to understand the importance of the different markers for the decline in functions are encouraged.

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WHL designed and conceptualized the research, performed the analyses, interpreted the data and drafted the manuscript. SG and JW interpreted the data and revised the draft critically for important intellectual content. AL and AP managed data of plasma IL-6, TNFR-1, MCP-1 and GDF-15, interpreted the data and revised the draft critically for important intellectual content. BV conceived the MAPT study, interpreted the data and revised the draft critically for important intellectual content. EG and PSB designed and conceptualized the research, interpreted the data and revised the draft critically for important intellectual content. All authors have read and agreed with the final version to be submitted.

The authors certify that they comply with the ethical guidelines for authorship and publishing of the *Journal of Cachexia, Sarcopenia and Muscle*.[41]

Conflict of interest

The authors have no conflicts of interest.

MAPT/DSA Group

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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