Inflammatory marker trajectories associated with frailty and aging in a 20-year longitudinal study

Leonard Daniël Samson[1,2], Anne-Marie Buisman[1], José A. Ferreira[1], H. Susan J. Picavet[1], W. M. Monique Verschuren[1,3], A. Mieke H. Boots[2], Peter Engelfriet[1]

[1]: *National Institute of Public Health and the Environment, Bilthoven, The Netherlands*

[2]: *Department of Rheumatology and Clinical Immunology, University of Groningen, University Medical Center Groningen, The Netherlands*

[3]: *Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands*

Supplementary table 1: Summary characteristics of the cytokines and chemokines in the cohort

Protein	Baseline concentration	Endpoint concentration	AUC
C-reactive protein			
CRP	$1.04(0.85-1.27)*10^6$	$1.28(1.08-1.51)*10^6$	$1.56(1.32-1.85)*10^6$
CC chemokines			
CCL1/I-309	$33.20(30.54-36.09)$	$33.96(31.47-36.65)$	$34.07(31.67-36.65)$
CCL2/MCP-1	$1.02(0.96-1.09)*10^2$	$1.10(1.03-1.17)*10^2$	$1.08(1.03-1.14)*10^2$
CCL5/RANTES	$8.76(7.91-9.71)*10^4$	$7.69(7.03-8.41)*10^4$	$7.97(7.40-8.58)*10^4$
CCL11/Eotaxin	$47.59(44.88-50.47)$	$53.22(50.26-56.35)$	$50.73(48.38-53.19)$
CCL27/C-TACK	$9.44(8.63-10.33)*10^2$	$1.18(1.07-1.29)*10^3$	$1.09(1.01-1.17)*10^3$
CXC chemokines			
CXCL9/MIG	$30.68(27.84-33.81)$	$31.58(28.85-34.57)$	$31.50(29.31-33.85)$
CXCL10/IP-10	$2.38(2.20-2.57)*10^2$	$3.35(3.11-3.61)*10^2$	$3.00(2.81-3.21)*10^2$
CXCL11/I-TAC	$59.49(50.64-69.89)$	$72.40(63.00-83.20)$	$69.44(61.38-78.56)$
Interleukins			
IL-6	$24.43(20.35-29.33)$	$24.58(20.53-29.43)$	$26.30(22.95-30.14)$
IL-10	$7.17(6.11-8.41)$	$6.87(5.73-8.22)$	$7.59(6.72-8.57)$
Soluble receptors			
sCD14	$2.16(2.04-2.28)*10^6$	$2.19(2.07-2.32)*10^6$	$2.26(2.15-2.37)*10^6$
sCD40L	$4.06(3.55-4.65)*10^2$	$3.66(3.21-4.18)*10^2$	$4.02(3.63-4.45)*10^2$
sIL-6R	$2.29(2.18-2.40)*10^4$	$2.41(2.29-2.54)*10^4$	$2.38(2.28-2.49)*10^4$
Other			
P selectin	$7.77(6.71-8.99)*10^4$	$6.83(6.06-7.70)*10^4$	$8.00(7.34-8.72)*10^4$
sGP130	$2.19(2.12-2.26)*10^4$	$2.24(2.17-2.30)*10^4$	$2.24(2.19-2.30)*10^4$
C5a	$4.68(4.11-5.32)*10^3$	$4.99(4.41-5.65)*10^3$	$5.09(4.52-5.72)*10^3$
BDNF	$1.83(1.70-1.97)*10^4$	$1.68(1.53-1.84)*10^4$	$1.73(1.61-1.87)*10^4$

Note:

Concentrations ($pg*mL^{-1}$) and AUC values ($years*pg*mL^{-1}$) are geometric mean values with 95% confidence intervals.

Supplementary table 2: Association of inflammatory markers with change in frailty index score in men and women

Sex	Marker	Comparison with	n	P value	ρ	FDR	Selected
Men	CRP	Change in FI (last two rounds)	52	0.03	0.43	0.47	–
Men	CCL27/C-TACK	Change in FI (last two rounds)	52	0.07	0.13	0.63	–
Men	P selectin	Change in FI (last two rounds)	52	0.11	-0.22	0.64	–
Men	IL-6	Change in FI (last two rounds)	47	0.28	-0.04	1.24	–
Men	CXCL11/I-TAC	Change in FI (last two rounds)	52	0.45	-0.07	1.63	–
Men	sCD14	Change in FI (last two rounds)	52	0.45	0.10	1.36	–
Men	C5a	Change in FI (last two rounds)	52	0.47	0.12	1.22	–
Men	CCL11/Eotaxin	Change in FI (last two rounds)	52	0.48	0.15	1.09	–
Men	CXCL9/MIG	Change in FI (last two rounds)	52	0.49	0.05	0.98	–
Men	sGP130	Change in FI (last two rounds)	47	0.60	-0.13	1.09	–
Men	CXCL10/IP-10	Change in FI (last two rounds)	52	0.64	-0.04	1.05	–
Men	IL-10	Change in FI (last two rounds)	47	0.70	-0.02	1.05	–
Men	CCL1/I-309	Change in FI (last two rounds)	52	0.77	0.10	1.07	–
Men	CCL5/RANTES	Change in FI (last two rounds)	52	0.82	0.09	1.06	–
Men	sCD40L	Change in FI (last two rounds)	52	0.87	-0.02	1.04	–
Men	sIL-6R	Change in FI (last two rounds)	52	0.89	-0.01	1.00	–
Men	BDNF	Change in FI (last two rounds)	52	0.95	0.01	1.00	–
Men	CCL2/MCP-1	Change in FI (last two rounds)	52	0.99	0.13	0.99	–
Women	sCD40L	Change in FI (last two rounds)	58	0.02	-0.25	0.32	–
Women	sCD14	Change in FI (last two rounds)	58	0.05	0.31	0.41	–
Women	IL-10	Change in FI (last two rounds)	52	0.06	-0.36	0.38	–
Women	IL-6	Change in FI (last two rounds)	52	0.06	-0.37	0.29	–
Women	CRP	Change in FI (last two rounds)	58	0.09	0.18	0.33	–
Women	P selectin	Change in FI (last two rounds)	58	0.18	-0.14	0.54	–
Women	CCL5/RANTES	Change in FI (last two rounds)	58	0.18	-0.20	0.47	–
Women	CCL11/Eotaxin	Change in FI (last two rounds)	58	0.19	-0.16	0.42	–
Women	BDNF	Change in FI (last two rounds)	58	0.27	-0.23	0.54	–
Women	CXCL9/MIG	Change in FI (last two rounds)	58	0.33	-0.21	0.59	–
Women	sIL-6R	Change in FI (last two rounds)	58	0.36	0.06	0.59	–
Women	CXCL11/I-TAC	Change in FI (last two rounds)	58	0.37	-0.14	0.55	–
Women	C5a	Change in FI (last two rounds)	58	0.47	0.16	0.66	–
Women	CCL1/I-309	Change in FI (last two rounds)	58	0.70	-0.17	0.90	–
Women	CCL2/MCP-1	Change in FI (last two rounds)	58	0.79	-0.01	0.95	–
Women	CXCL10/IP-10	Change in FI (last two rounds)	58	0.80	0.04	0.90	–
Women	sGP130	Change in FI (last two rounds)	53	0.90	0.04	0.95	–
Women	CCL27/C-TACK	Change in FI (last two rounds)	58	1.00	0.00	1.00	–

Note:
Permutation version of the Spearman test was used. Tests are stratified by the tertile of the initial frailty index (FI) scores and by age. FDR: (estimated) False Discovery Rate. Selected: condition selected based on FDR <0.15

Supplementary table 3: Frailty index components

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
1	RR	High (systolic) blood pressure	$RR < 160$		$RR \geq 160$
2	Cardiologic Disease	One or more of the following conditions: myocardial infarction, bypass-surgery, balloon dilatation, cardiac catheterization, pacemaker implantation, large blood vessel surgery, hospitalization due to cardiac failure	No		One or more prevalent
3	Diabetes	Prevalence of Diabetes	No		Yes
4	Hearing	Inability to maintain a conversation in a group of 3 or more people due to hearing impairment (with hearing aid if needed)	Yes or with some effort		No or with great effort
5	Malignancy	(History of) any form of malignancy	No		Yes
6	Joint Inflammation	Chronic joint inflammation in the past year	No		Yes
7	Osteoporosis	Osteoporosis diagnosed by a medical doctor, in the past year,	No		Yes
8	Lower Back Pain	Severe lower back complaints in the past year (including lumbar herniated nucleus pulposus)	No		Yes
9	CVA	(History of) stroke	No		Yes
10	Migraine	Migraine prevalence in the past year	No		Yes

Supplementary table 3: Frailty index components *(continued)*

No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
11	Neurologic Disease	One or more of the following neurological diseases, diagnosed by a medical doctor: m. Parkinson, Multiple Sclerosis, epilepsy	No		Yes
12	Asthma	Asthma diagnosed by a doctor and one or more asthma attacks in the past year	No		Yes
13	Spirometry Ratio	Poor lung function quantified by spirometry measurements: first second of forced expiration divided by the forced vital capacity (FEV1/FVC)	$FEV1/FVC >$ 0.70		$FEV1/FVC \leq$ 0.70
14	Digestive Tract	Severe bowel disorders in the past year, diagnosed by a medical doctor	No		Yes
15	Vertigo	Vertigo with falling the past 12 months	No vertigo	Some vertigo	Severe vertigo
16	Pain	Limited in daily activities due to pain	No limitation	Some limitation	Severe limitation
17	Incontinence	Unintentional urine incontinence past 12 months	No		Yes
18	Ankle Brachial Index	Poor ankle brachial index (ABI, the ratio of the systolic blood pressure in the ankle to the blood pressure in the arm)	$ABI > 0.9$		$ABI \leq 0.9$

Supplementary table 3: Frailty index components *(continued)*

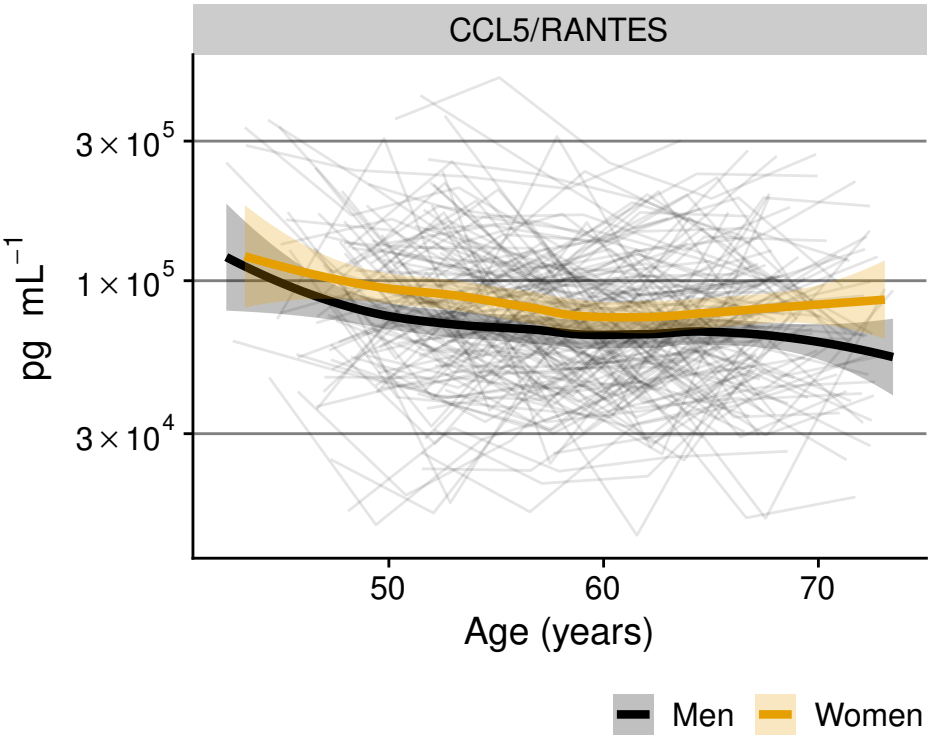
No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
19	Health Perception	(Subjective) perception of poor health	No or some impairment		Severe impairment
20	Eyesight	Bad eyesight perception, facial recognition within 4 meters (with glasses/ contact lenses if needed)	No		Yes
21	Renal Function	Poor renal function (estimated Glomular Filtration Rate (eGFR), calculated with plasma creatinine concentrations) (Inker et al., 2012)	$eGFR \geq 60$		$eGFR < 60$
22	Cognitive Speed	Poor cognitive speed. Z-scores corrected for measurements per person and for education level. Scores derived from the Stroop Color-Word Test and the Letter-Digit Substitution Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort
23	Cognitive Memory	Poor cognitive memory. Z-scores corrected for measurements per person and for education level. Scores derived from the Verbal Learning Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort

Supplementary table 3: Frailty index components *(continued)*

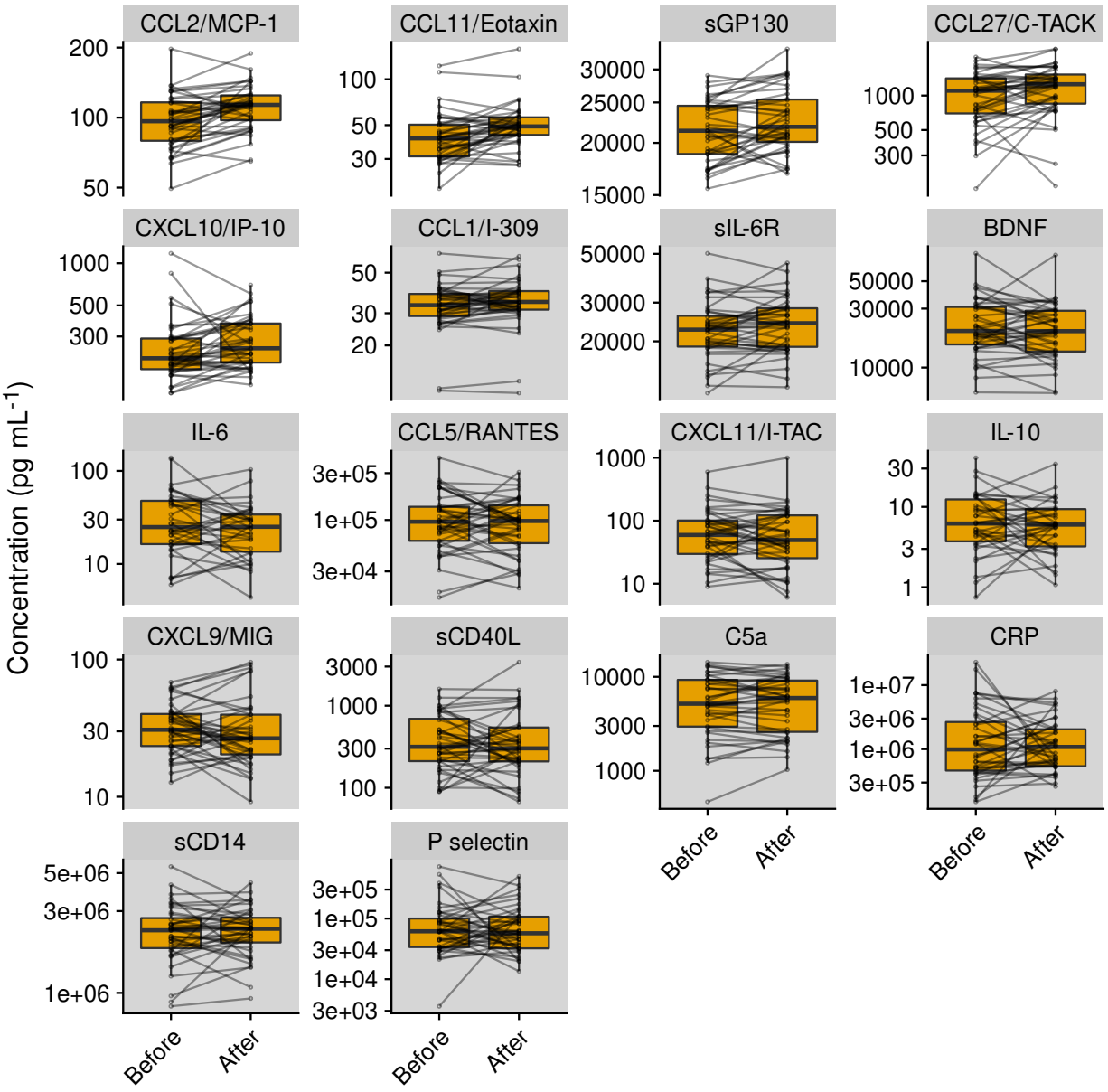
No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
24	Cognitive Flexibility	Poor cognitive flexibility. Z-scores corrected for measurements per person and education level. Scores derived from the Stroop Color-Word Test, as described previously (Nooyens et al., 2011)	Not beloging to 10% participants with lowest z-score within the Doetinchem cohort		Belonging to 10% participants with lowest z-score within the Doetinchem cohort
25	Physical Inactive	Not meeting the Dutch healthy exercise norm.(Kemper, 2000). In addition: belonging to the 25th lowest percentile of walking activity and the 10th percentile lowest low/medium/high intensive activities in the Doetinchem cohort	No		Yes
26	ADL	Limited in washing and dressing due to health	No limitation	Some limitation	Severe limitation
27	Household	Limited in daily activities (cooking, cleaning) due to health	No limitatlion	Some limitation	Severe limitation
28	Walking	Limited in in walking 100 meters	No limitatlion	Some limitation	Severe limitation
29	Lifting	Limited in lifting or carrying groceries due to health	No limitatlion	Some limitation	Severe limitation

Supplementary table 3: Frailty index components *(continued)*

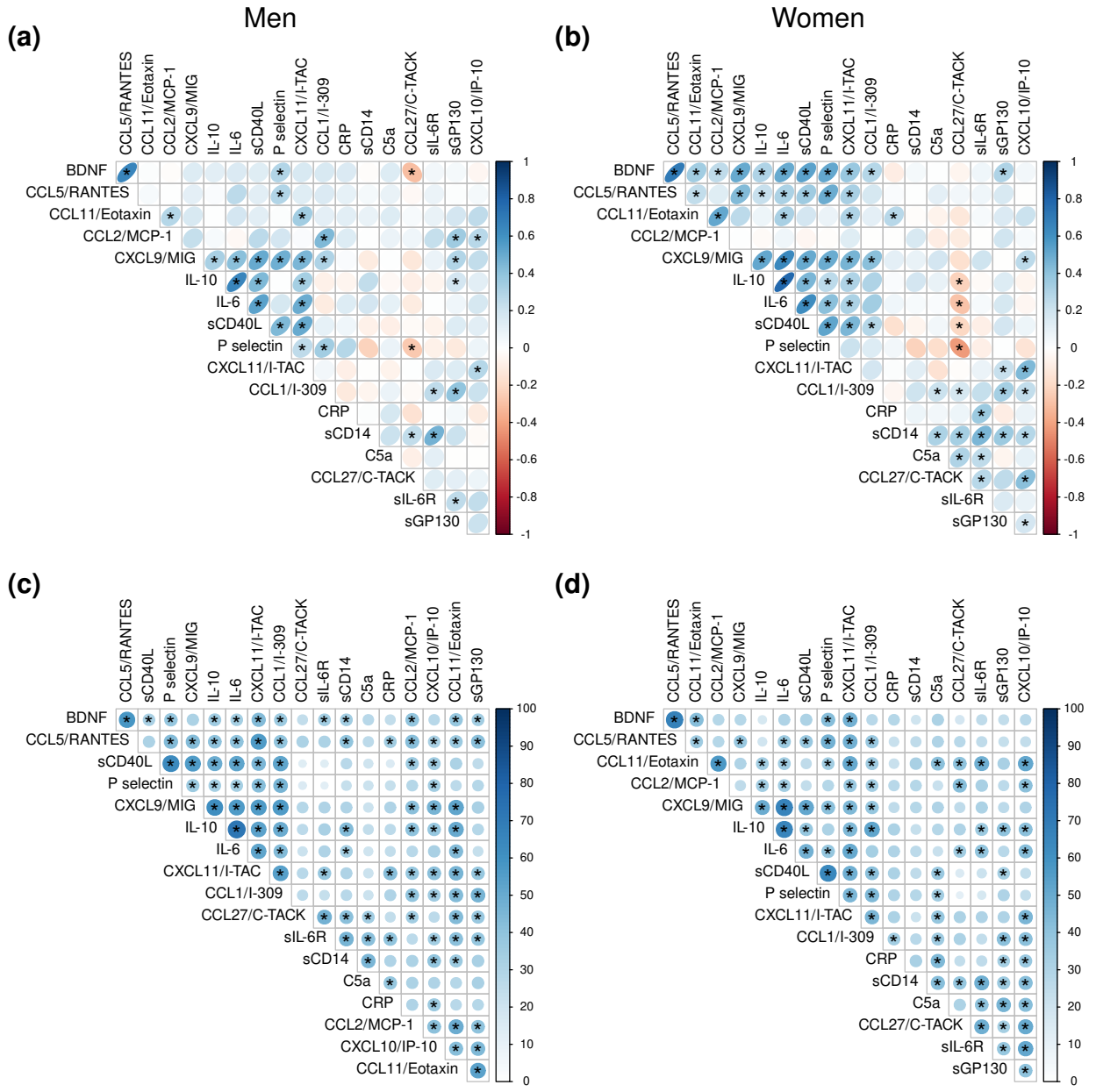
No	Frailty index component	Description	Value: 0	Value: 0.5	Value: 1
30	Walking Stairs	Limited in climbing stairs	No limitatlion	Some limitation	Severe limitation
31	Grip Strength	Poor grip strength (cutoff points as described previously) (Fried et al., 2001)			
		$Men, BMI \leq 24$	> 29		≤ 29
		$Men, 24 < BMI \leq 26$	> 30		≤ 30
		$Men, 26 < BMI \leq 28$	> 30		≤ 30
		$Men, BMI > 28$	> 32		≤ 32
		$Women, BMI \leq 24$	> 17		≤ 17
		$Women, 23 < BMI \leq 26$	> 17.3		≤ 17.3
		$Women, 26 < BMI \leq 29$	> 18		≤ 18
		$Women, BMI > 29$	> 21		≤ 21
32	Depressed	Feeling depressed the past week	No limitatlion	Some limitation	Severe limitation
33	Happiness	Feeling unhappy the past 4 weeks	No limitatlion	Some limitation	Severe limitation
34	Mental Effort	Feeling as if every activity costs effort during the past week	No limitatlion	Some limitation	Severe limitation
35	Getting Going	Not being able to get going the past week	No limitatlion	Some limitation	Severe limitation



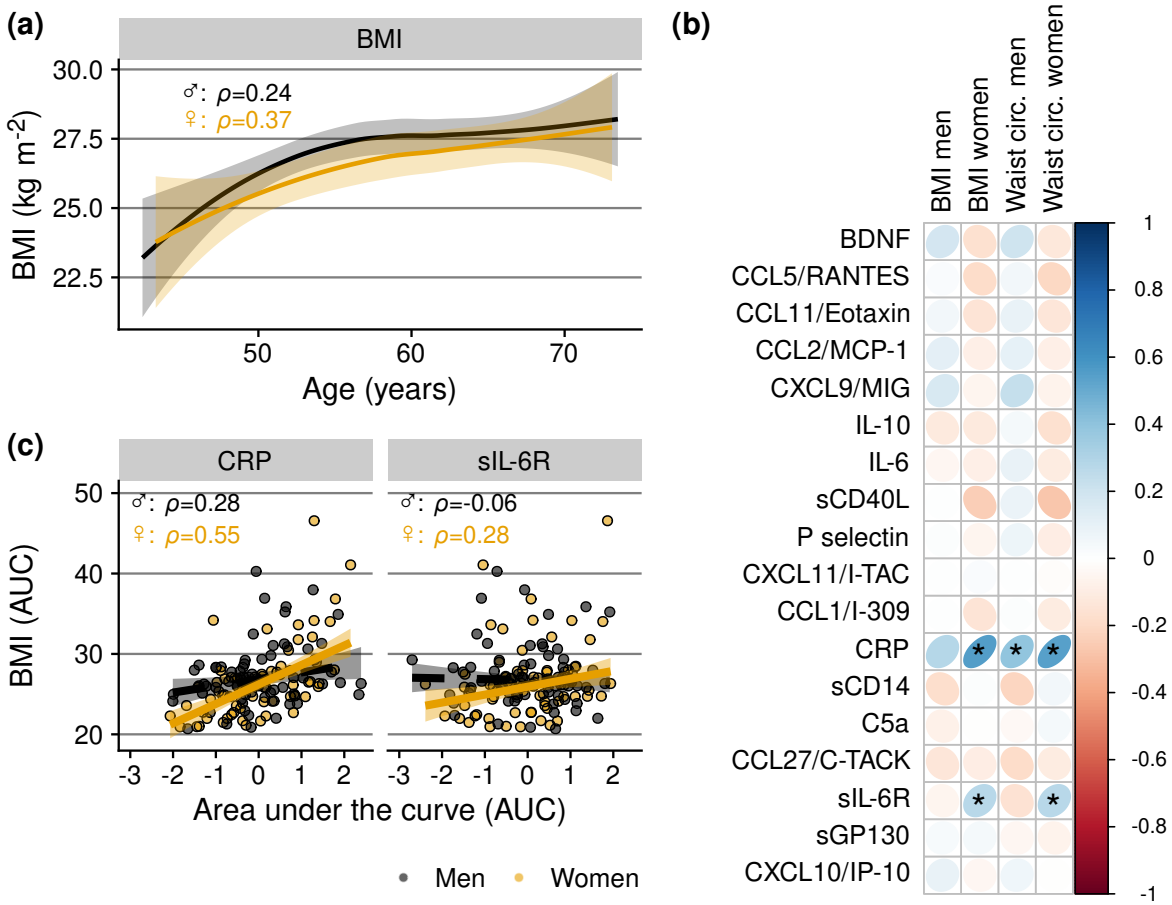
Supplementary figure 1: The concentration of CCL5/RANTES over 20 years in men and women (showing that this is continuously higher in women).



Supplementary figure 2: Menopause is related to changes in inflammatory marker profile. Inflammatory marker concentrations are shown shortly before and shortly after menopause (average difference: 5.3 years) in women of whom data at both timepoints are available (n=40/70). Tiles of biomarkers in which an association was found with menopause are shown in a white background, others in a grey background.



Supplementary figure 3: Relationships between inflammatory markers shown as ((**a**), (**b**)) correlation between pairs of inflammatory markers at study endpoint and ((**c**), (**d**)) similarity between pairs of inflammatory marker trajectories during about 20years of follow-up. In **(a)** and **(b)** the direction and strength of the association in is visualized with an oval shape and a color gradient. In **(c)** and **(d)** the blue gradient color and the size of the circles shows the percentage of participants of which a pair of biomarkers had the highest increase in concentration at the same moment in 20 years of follow-up. *= an association between two inflammatory markers, with false discovery rate being set at a maximum of 15%. n=71 women, n=73 men.



Supplementary figure 4: Relationship between body mass and inflammatory markers. **(a)** Local polynomial regression lines showing change in BMI over time in men and women. **(b)** Relationship of inflammatory marker levels with BMI and waist circumference of men (n=73) and women (n=71). Direction and strength of the associations in **(b)** are visualized with an oval shape and a color gradient. To capture the cumulative "exposure", both BMI and inflammatory markers in **(b)** and **(c)** are expressed as area under the curve of levels/concentrations versus time, standardized to take into account different follow-up periods, and transformed into z-values. **(c)** BMI values (AUC) related to the AUC of CRP and sIL-6R. Trendlines in **(c)** are (robust) linear regression lines with 95% confidence interval.