Early life cardiovascular risk factors and midlife cognitive performance: The Cardiovascular Risk in Young Finns Study

Short title: Childhood vascular factors and midlife cognition

Suvi P. Rovio, PhD,* Katja Pahkala, PhD,*† Jaakko Nevalainen, PhD,‡ Markus Juonala, MD, PhD,§ Pia Salo, MD, PhD,* Mika Kähönen, MD, PhD,‖ Nina Hutri-Kähönen, MD, PhD,¶ Terho Lehtimäki, MD, PhD,# Eero Jokinen, MD, PhD,†† Tomi Laitinen, MD, PhD,‡‡ Leena Taittonen, MD, PhD,§§‖‖ Päivi Tossavainen, MD, PhD,‖‖ Jorma S.A. Viikari, MD, PhD,§ Juha O. Rinne, MD, PhD,¶¶ Olli T. Raitakari, MD, PhD*##

*Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; †Paavo Nurmi Centre, Sports & Exercise Medicine Unit, Department of Physical Activity and Health, University of Turku, Turku, Finland; ‡School of Health Sciences, University of Tampere, Tampere, Finland; §Department of Medicine, University of Turku and Division of Medicine, Turku University Hospital, Turku, Finland; ‖Department of Clinical Physiology, University of Tampere and Tampere University Hospital, Tampere, Finland; ¶Department of Pediatrics, University of Tampere and Tampere University Hospital, Tampere, Finland; #Department of Clinical Chemistry, Fimlab Laboratories and School of Medicine, University of Tampere, Tampere, Finland; ††Department of Paediatric Cardiology, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; ‡‡Department of Clinical Physiology, University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; §§Vaasa Central Hospital, Vaasa, Finland; ‖‖Department of Pediatrics, PEDEGO Research Unit and Medical Research Center Oulu, University of Oulu, Oulu, Finland; ¶¶Turku PET Centre and Department of Neurology, University of Turku and Turku University Hospital, Turku, Finland; ##Department of Clinical Physiology and Nuclear Medicine, University of Turku, Turku, Finland.

Address for correspondence:
Suvi Rovio
Research Centre of Applied and Preventive Cardiovascular Medicine
University of Turku
Kiinamyllynkatu 10
20520 Turku, Finland
Telephone: +358 40 5665036
Fax: 23337477
Email: suvi.rovio@utu.fi

Acknowledgements: Expert technical assistance in data management and statistical analyses by Irina Lisinen and Johanna Ikonen is gratefully acknowledged. **Sources of funding:** The Young Finns Study has been financially supported by the Academy of Finland: grants 286284 (T.L.), 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117797 (Gendi), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds, Juho Vainio Foundation, Sigrid Juselius Foundation, Yrjö Jahnsson Foundation, Paavo Nurmi Foundation, Finnish Foundation for Cardiovascular Research, Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation. The authors

report no relationships with industry. The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit the manuscript for publication.

ABSTRACT
Background: In adults, high blood pressure (BP), adverse serum lipids, and smoking associate with cognitive deficits. The effects of these risk factors from childhood on midlife cognitive performance are unknown.
Objectives: The aim of this study was to investigate the associations between childhood/adolescence cardiovascular risk factors and midlife cognitive performance.
Methods: From 1980, a population-based cohort of 3596 children (baseline age 3-18 years) have been followed-up for 31 years in 3-9 year intervals. BP, serum lipids, body mass index and smoking were assessed in all follow-ups. Cumulative exposure as the area under the curve for each risk factor was determined in childhood (6-12 years), adolescence (12-18 years) and young adulthood (18-24 years). In 2011, cognitive testing was performed in 2026 participants aged 34-49 years.
Results: High systolic BP, elevated serum total-cholesterol and smoking from childhood were independently associated with worse midlife cognitive performance, especially memory and learning. The number of early life risk factors, including high levels (extreme 75th percentile for cumulative risk exposure between ages 6-24) of systolic BP, total-cholesterol and smoking associated inversely with midlife visual and episodic memory and visuospatial associative learning (-0.140 standard deviations per risk factor, $p<0.0001$), and remained significant after adjustment for contemporaneous risk factors. Individuals with all risk factors within recommended levels between ages 6-24 performed 0.29 standard deviations better ($p=0.006$) on this cognitive domain than those exceeding all risk factor guidelines at least twice. This difference corresponds to the effect of six years aging on this cognitive domain.
Conclusions: Cumulative burden of cardiovascular risk factors from childhood/adolescence associate with worse midlife cognitive performance independent of adulthood exposure.

Key words: cognitive performance, blood pressure, serum cholesterol, body mass index, smoking

Abbreviations
ApoE = Apolipoprotein E
AUC = Area under the curve
BMI = Body mass index
BP = Blood pressure
HDL = High density lipoprotein
LDL = Low density lipoprotein
PAL = Paired associates learning test
RTI = Reaction time test
RVP = Rapid visual information processing test
SE = Standard error
SD = Standard deviation
SWM = Spatial working memory test
YFS = The Cardiovascular Risk in Young Finns Study

Condensed Abstract: In adults, high blood pressure (BP), adverse serum lipids, and smoking associate with cognitive deficits, but the effects from childhood are unknown. From 1980, a population-based cohort of 3596 children (age 3-18 years) have been regularly followed-up for

31 years. BP, serum lipids, body mass index and smoking were assessed in all follow-ups. In 2011, cognitive testing was performed in 2026 participants aged 34-49 years. High systolic BP, elevated serum total-cholesterol and smoking from childhood were independently associated with worse midlife cognitive performance, especially memory and learning. Our findings support active controlling of cardiovascular risk factors from childhood.

Introduction

Epidemiological evidence indicates that exposure to midlife high blood pressure (BP), adverse serum lipids, and smoking associate with cognitive decline later in life (1-4). Studies in animal models have also observed associations between these risk factors and cognitive performance. Spontaneously hypertensive rats show cognitive decline compared to normotensive strains (5). In rodents (6,7), the high-fat diet induced hypercholesterolemia leads to memory deficits. In addition, adolescent rats exposed to nicotine show long-lasting cognitive deficits (8). Although the mechanisms underlying these associations are largely unclear, experimental studies have suggested that cardiovascular risk factors may damage both neuronal and vascular tissues of the brain. Studies in rodents have shown that hypertension may alter cerebral vasculature, and eventually lead to restrained function of the blood-brain barrier (9). Moreover, experiments on rodents have indicated that diet induced hypercholesterolemia may influence the expression of genes in the brain relevant for cellular mechanisms for learning, memory and neurodegeneration (10). Simultaneously, experiments on rodent and rabbit brains have shown evidence that hypercholesterolemia may induce inflammatory changes (6,7,11), and associate with disturbed beta-amyloid metabolism (6,7,11). Furthermore, experimental evidence on rodent brain have suggested that smoking exposure may induce oxidative stress (12-14) which may trigger cerebral inflammatory changes (12), and lead to neuropathological changes related to cognitive decline such as accumulation of beta-amyloid peptide and phosphorylation of tau protein (13).

It is therefore plausible that exposure to cardiovascular risk factors in early life may affect later cognitive performance. In support of this hypothesis, a link between cumulative burden of young adulthood cardiovascular risk factors and cognitive performance was shown in young and middle aged adults in the CARDIA cohort (15). Whether a similar link exists between

early life risk exposure and adulthood cognitive function is unknown. We addressed this knowledge gap in the Cardiovascular Risk in Young Finns Study (YFS) that has followed a population-based sample of individuals from childhood to adulthood. As a part of the 31-year follow-up study, cognitive testing was performed using a test battery focusing on several cognitive domains that are related to brain structures typically affected in the early stages of cognitive decline (16). We hypothesized that a greater burden of cardiovascular risk factors in childhood, adolescence and young adulthood, including repeatedly assessed BP, serum lipids, and smoking, associate with worse cognitive performance in midlife.

Methods

Population

This study is a part of the national YFS, which is an ongoing longitudinal population-based study on cardiovascular risk factors from childhood to adulthood. The first cross-sectional study including 3596 randomly selected children and adolescents (boys and girls; aged 3, 6, 9, 12, 15 and 18 years) was performed in 1980. The cohort has been followed-up in regular intervals in 1983, 1986, 1989, 2001, 2007, and the latest follow-up study was conducted in 2011. Detailed information on the population and protocol is reported elsewhere (17).

Procedures and measurements

Outcome measure cognition

In 2011, a computerized cognitive testing battery (CANTAB®) was used to assess cognitive performance in 2026 participants. The YFS test battery included: 1) **motor screening test** used as a training/screening tool to indicate difficulties in test execution, 2) **paired associates learning test** (PAL) measured visual and episodic memory and visuospatial associative learning, 3) **spatial working memory test** (SWM) measured short term and spatial

working memory and problem solving, 4) **reaction time test** (RTI) measured reaction and movement speed and attention, and 5) **rapid visual information test** (RVP) measured visual processing, recognition and sustained attention.

Each test produced several variables. Principal component analysis was conducted to identify components accounting for the majority of the variation within the dataset. Principal components were created for each test for performance in specific cognitive domains. The motor screening test component was excluded from further analyses due to ceiling effect (*i.e.* all participants had the maximum score in this test). Other components were normalized using rank order normalization procedure resulting in four variables, each with mean 0 and standard deviation (SD) 1. All available data for each cognitive test was used in the analyses, and therefore, the number of participants varies between the models (N=177 were excluded due to technical reasons in some of the test domains and N=51 refused to participate in all or some of the tests). Detailed description and validation of the cognitive data has been reported previously (18).

Exposure variables blood pressure, serum lipids, body mass index and smoking

Standard methods were used for measuring BP, serum total-cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides at baseline and all follow-up studies. Low density lipoprotein (LDL) cholesterol was calculated according to Friedewald's (19). Details of these methods have been described previously (20). At all study phases, the participants' weight and height were measured and BMI was calculated. To utilize all available repeatedly measured exposure data for continuous variables, we estimated subject-specific curves for cardiovascular risk factors by mixed model regression splines (21). The area under the curve (AUC) for continuous risk variables was evaluated to indicate a long term burden of each measured attribute

(22). The AUC variables were defined separately for childhood (6-12 years), adolescence (12-18 years), young adulthood (18-24 years) and early life (6-24 years). For interpretability, the AUC variables were standardized resulting in variables with mean 0 and SD 1. Smoking exposure was queried throughout the follow-up time. Smoking status was dichotomized into daily smokers and non-smokers, defined as current daily smoking (yes/no) at the baseline or at any of follow-up studies when the participants were aged 12-24 years.

In addition to studying the associations using continuous AUC variables and the dichotomized smoking variable, the combined effect of several risk factors from childhood to young adulthood on midlife cognitive performance was analysed. For that purpose, a 3-level variable indicating the number of cardiovascular risk factors during early life (6-24 years) was created (1=no risk factors, 2=one risk factor, 3=two or three risk factors) including the variables that showed significant association with cognitive performance in the multivariate analyses (*i.e.* systolic blood pressure, serum total-cholesterol and smoking). To define risk factors using the continuous AUC variables, the distributions of BP and serum total-cholesterol were dichotomized into high ($\geq 75^{th}$ percentile) and low ($<75^{th}$ percentile) risk factor levels (sensitivity analyses were additionally performed by using cut-points of 70^{th}, 80^{th} and 85^{th} percentiles). Such dichotomized variables and the binary smoking variable were summed to create the variable indicating the number of risk factors (range 0-3) during early life. The age and sex specific mean values of systolic blood pressure, total- and LDL-cholesterol corresponding to the highest 75^{th} percentile of the AUC variables between ages 6-24 years are presented in Online Table 1. A similar variable indicating the number of midlife risk factors at the time of cognitive testing was constructed. Current midlife daily smoking was queried at the time of cognitive testing and categorized as non-smokers vs. daily smokers.

In addition to the arbitrarily selected risk factor cut-points, we examined whether the effect of early life risk exposure is attributable to levels of risk factors repeatedly exceeding the recommended guidelines. The age and sex specific cut-points were considered for variables showing significant effect for cognitive performance in the multivariate analyses *i.e.* systolic BP (23), LDL-cholesterol (24), and smoking (25). The exact cut-points for these risk factors are presented in Online Table 2. The participants were classified into: 1) no risk factor levels exceeding guidelines or levels exceeding at most once per risk factor, 2) risk factor levels exceeding guidelines on one risk factor at least twice, 3) risk factor levels exceeding guidelines at least twice on two risk factors, 4) risk factor levels exceeding guidelines at least twice on all risk factors.

Covariates

The following primary covariates were used in the analyses: age, sex, baseline household income, antihypertensive and dyslipidemia medication, diagnoses of cardiovascular diseases and type 1 and 2 diabetes mellitus. Altogether 1,901 individuals with cognitive test data had complete data on exposure variables and primary covariates. Age was defined in full years at the end of the year 2011. Baseline household income, use of antihypertensive (N=192) or dyslipidemia (N=72) medication and type 1 diabetes diagnoses (N=12) were queried. Participants were classified as having type 2 diabetes based on self-reported and register confirmed diagnosis, self-reported glucose lowering medication or abnormal plasma glucose or haemoglobin A1c values (N=75). Diagnoses of cardiovascular diseases (N=13) were adjudicated from the national hospital discharge register. Additionally, data on following covariates were available for restricted numbers of participants, and were therefore used in additional analyses: childhood academic performance (N=1684), adulthood education (N=1894), apolipoprotein E

(apoE) genotype (N=1803), and adulthood physical activity (N=1884). Childhood academic performance expressed as grade point average (*i.e.* mean of grades in all individual school subjects at baseline or either of the two subsequent follow-ups for those participants who were not of school age at baseline) was queried and used as a proxy for childhood cognitive ability. Adulthood education was assessed with questionnaires at the follow-up studies in 2001, 2007 and 2011. The maximum years of education was determined as a continuous variable from self-reported data concerning total years of education. ApoE genotypes were analysed with standard methods and the individuals were divided into apoEε4 carriers (≥one ε4 allele) and non-carriers (no ε4 alleles). Physical activity was queried and the level of physical activity was calculated as the mean of the adulthood (ages 24-49 years) physical activity indexes (range 5-15). Detailed description of the covariates is presented in the Online Appendix.

Statistical analyses

Student's t-test was applied for continuous variables and χ^2-test for categorical variables. Linear regression analyses were conducted to examine the associations between childhood/adolescence/young adulthood cardiovascular risk factors and midlife cognitive performance. First, age and sex adjusted regression analyses were conducted separately for cumulative burden of each cardiovascular risk factor in childhood, adolescence and young adulthood using each cognitive domain as outcome. After that, family income, antihypertension and dyslipidemia medication and diagnoses of cardiovascular diseases and diabetes mellitus were entered as covariates in these analyses.

Second, further analyses were conducted for visual and episodic memory and visuospatial associative learning (PAL-test) which was the cognitive domain showing most consistent results in the analyses separately for each early life cardiovascular risk factors. These analyses were

conducted first unadjusted, and then entering age and sex as covariates. After that, all early life cardiovascular risk factors were entered in the same age and sex adjusted model. Then, the analyses were additionally adjusted for family income, antihypertension and dyslipidemia medication and diagnoses of cardiovascular diseases and diabetes mellitus. Due to high intercorrelation (r=0.94) between total- and LDL-cholesterol, these variables showed essentially similar relations with the cognitive domains, and were not considered simultaneously in the multivariable models. Finally, all analyses were further adjusted for childhood academic performance, adulthood education, apoEε4 and adulthood physical activity. Additionally, we tested the possible effect modification caused by sex or age on the associations between cardiovascular risk factors and performance on those cognitive domains that showed significant results in the multivariate models. The possible effect modification was analysed by adding interaction terms in the fully adjusted multivariate models. No significant interactions were found (data not shown). All statistical analyses were performed using SAS 9.4 and $p<0.05$ as the level of significance. Detailed description of all statistical methods is presented in the Online Appendix.

Results

Characteristics of the population

The characteristics of the study population and numbers of participants in each separate cognitive test are shown in Online Table 3. The representativeness of the study population participating in the cognitive testing was examined by comparing the baseline differences between participants and non-participants (Online Table 4). Participants were more often women and older than non-participants. In addition, they originated from families with higher income

and had better childhood academic performance. No differences were observed in the exposure variables.

Cardiovascular risk factors in childhood, adolescence and young adulthood

Associations between childhood (6-12 years), adolescence (12-18 years) and young adulthood (18-24 years) cardiovascular risk factors and midlife cognitive performance are shown in Online Table 5. The consistent finding was that elevated systolic BP and adverse lipids (high total- or LDL-cholesterol) in childhood, adolescence and young adulthood, as well as cigarette smoking in adolescence and young adulthood, associated systematically with lower midlife visual and episodic memory and visuospatial associative learning (PAL-test) (detailed results in the Online Table 5). In general, the effect estimates of cardiovascular risk factors for the PAL-test were similar across all exposure age categories. Additionally, we found some links of serum total-cholesterol, triglycerides, smoking and BMI to the test measuring sustained attention and visual processing (RVP-test). However, only the results for the PAL-test remained consistently unchanged after further adjustments for family income, antihypertension and dyslipidemia medication and diagnoses of cardiovascular diseases and diabetes mellitus. Therefore, detailed results are presented to examine the independent effects of early life cumulative burden of systolic BP, serum total-cholesterol, BMI and smoking on PAL-test performance: First, we calculated the mean values of the midlife PAL-test performance and each cardiovascular risk factor in childhood (age 6-9 years), adolescence (age 12-15 years) and early adulthood (age 18-24 years) within the quartiles of the early life AUC variables for the same risk factors (**Table 1**). We also calculated the difference in *cognitive age* across the quartiles in the PAL-test performance based on our previous study that showed a -0.05 SD decline per year in the YFS population.[18] For example, the table shows a 0.42 SD difference between the extreme systolic

blood pressure quartiles. This corresponds to 8.4 years difference in *cognitive age*. Similarly, we see a 0.33 SD difference between the extreme serum total-cholesterol quartiles (6.6 years) and a 0.17 SD difference between smokers and non-smokers (3.4 years).

Second, analyses were conducted to examine the independent effects of early life (between ages 6-24 years) cumulative burden of systolic BP, serum total-cholesterol, BMI and smoking on midlife visual and episodic memory and visuospatial learning (PAL-test) (Table 2). In the unadjusted bivariate analyses (Table 2, row A), early life exposure to elevated systolic blood pressure, serum total-cholesterol and smoking were inversely related with the PAL-test performance. These associations were attenuated but remained significant when the effects of age and sex were taken into account (Table 2, row B), when mutually adjusted for in a multivariate model (Table 2, row C) and when additionally adjusted for family income, antihypertension and dyslipidemia medications and diagnoses of cardiovascular disease and diabetes mellitus (Table 2, row D). This indicates that all three risk factors were independently associated with the PAL-test performance and that none of the other covariates had significant confounding effects.

Further multivariate analyses were conducted including childhood academic performance, adulthood education, apoEε4 genotype, and adulthood physical activity level as covariates in the model. In this sample, the results for BP and serum total-cholesterol remained essentially similar for visual and episodic memory and visuospatial associative learning (PAL-test, N=1450, systolic BP: β=-0.065, SE=0.031, p=0.034; serum total-cholesterol: β=-0.076, SE=0.028, p=0.006), but the additional adjustments diluted the effects of smoking for visual and episodic memory and visuospatial associative learning (PAL-test, N=1450, smoking: β=-0.032, SE=0.060, p=0.60) and for recognition, visual processing and sustained attention (RVP-test,

N=1545, smoking: β=-0.033, SE=0.057, p=0.568). The covariate that was mainly responsible for the dilution of the effect of smoking was childhood academic performance, which was directly and highly significantly (p-value always <0.008) associated with all midlife cognitive domains.

Multiple cardiovascular risk factors

Because early life cardiovascular risk factors associated systematically with visual and episodic memory and visuospatial associative learning (PAL-test), the effects on that cognitive domain were investigated in relation to 1) the number of early and midlife cardiovascular risk factors, and 2) the number of early life risk factors exceeding the recommended guidelines.

Number of early and midlife cardiovascular risk factors

Persons with none or one risk factor (defined as continuous AUC values exceeding the 75th percentile and frequent smoking) during their early life (age 6-24 years) performed better in the PAL-test than participants with 2-3 early life cardiovascular risk factors. After adjusting for age, sex, family income, antihypertension and dyslipidemia medications and diagnoses of cardiovascular diseases and diabetes mellitus, the association between the number of early life cardiovascular risk factors and midlife cognitive performance was highly significant (N=1733; β=-0.135, SE=0.034, p<0.0001). Similar but weaker association between the number of risk factors and PAL-test was found for current midlife cardiovascular risk factors (N=1781; β=-0.078, SE=0.034, p=0.022).

To examine whether the effect of early life risk factors on PAL-test were independent of the effect of contemporaneous risk factors, we stratified the cohort into groups according to the number of early and midlife risk factors (**Figure 1**). When simultaneously entered in a multivariable model, the effect of early life risk factors remained significant (N=1733; β=-0.119, SE=0.036, p=0.001), but the effect of the midlife risk factors was diluted (β=-0.045, SE=0.037,

p=0.220). The 'independent' effect of early risk exposure is illustrated in **Figure 1**. For example, individuals who have been exposed to several risk factors in early life have consistently of about 0.25 SD's lower memory and learning compared to the population mean regardless of the number of contemporary risk factors (red dots in **Figure 1**). This corresponds about 5 years in *cognitive aging* in our population. These results remained essentially similar after further adjustments with childhood academic performance, adulthood education, apoEε4 genotype, and physical activity level (N=1450; early life risk factors: β=-0.116, SE=0.040, p=0.004; midlife risk factors: β=0.004, SE=0.040, p=0.916).

In addition to the 75th percentile cut-point used in the main analyses, we performed sensitivity analyses for the association between the number of risk factors and cognitive performance using cut-points of 70th, 80th, and 85th percentiles for determining the risk factor levels. The associations between the number of early and midlife cardiovascular risk factors and midlife cognitive performance remained virtually unchanged regardless the cut-point used (Online Table 6). Thus, by using several risk factor percentile cut-points, the number of early-life risk factors remained robustly associated with mid-life memory and learning. And when the models were simultaneously controlled for the mid-life risk factors, the effect of early life risk factors remained significant, but the effects of the midlife risk factors were always diluted. Furthermore, all models gave identical results if high total-cholesterol was replaced with high LDL-cholesterol in the risk scores.

<u>Number of early life risk factors exceeding the recommended guidelines</u>

In addition to the risk factor levels based on percentile cut-points, we examined whether the effect of early life risk exposure is attributable to levels of risk factors repeatedly exceeding the recommended guidelines for pediatric atherosclerosis prevention using cut-points shown in

Online Table 2. The analyses considering risk factors exceeding recommended guidelines indicated that the persons with early life risk factors within the recommended guidelines had 0.29 SD better visual and episodic memory and visuospatial associative learning (PAL-test) than those exceeding the guidelines at least twice on all risk factors (model adjusted for age, sex, family income, antihypertension and dyslipidemia medications and diagnoses of cardiovascular diseases and diabetes mellitus: N=1568; β=-0.088, SE=0.032, p=0.007) (**Figure 2**). This effect remained identical after further adjustments with childhood academic performance, adulthood education, apoEε4 genotype, and physical activity level (N=1341; β=-0.077, SE=0.035, p=0.029).

Discussion

We found that increasing cumulative burden of systolic BP, serum total- and LDL-cholesterol and smoking in childhood/adolescence associated with worse visual and episodic memory and visuospatial associative learning at midlife (PAL-test). Importantly, the associations were independent of midlife exposures to the same risk factors. These findings suggest that the associations between early life cardiovascular risk factors and midlife cognitive performance do not only reflect tracking of risk factor levels from childhood to adulthood, but that the risk factors potentially start to exert their influence on cognitive performance already in childhood.

Previous studies have observed inverse associations between adulthood systolic BP, serum cholesterol, BMI and smoking and midlife cognitive performance (15) or risk of late-life cognitive deficits (1-4). To the best of our knowledge, this is the first study examining the cumulative burden of cardiovascular risk factors from childhood in relation to cognitive performance in midlife. Of the risk factors examined, we were able to demonstrate the independent effects of early life systolic BP, serum total- and LDL-cholesterol, and smoking.

Participants with these early life risk factors, factored as continuous or binary variables, defined either by using several arbitrary cut-points or by using current guidelines for atherosclerosis prevention, had worse midlife cognitive performance than those with low risk factor levels. Although the observational nature of our study precludes making clinical recommendations, it provides evidence on cognitive benefits gained by maintenance of cardiovascular risk factors at low levels already from early life. If this hypothesis is correct, adopting active monitoring and treatment strategies against cardiovascular risk factors from childhood would be needed to turn the focus of cognitive deficits prevention from secondary and tertiary prevention to primordial prevention. With the future YFS follow-up data on cognitive performance, we will be able to estimate using observational data whether changes in risk factor levels from childhood to adulthood associate with the deterioration of cognitive function between mid- to old adulthood.

The four cognitive domains were selected as outcomes in order to obtain a comprehensive outlook to cognitive performance. Based on previous experimental studies in animals, we especially expected to see links between cardiovascular risk factors and tests indicating memory and learning. Indeed, we found that the effects of early life risk exposure was strongly and robustly associated with the visual and episodic memory and visuospatial associative learning (PAL-test). Similarly, the CARDIA study found strong associations between serum total-cholesterol, blood pressure and verbal memory (15). Additionally, we found weak links of smoking and BMI to the test measuring sustained attention and visual processing (RVP-test) that were of borderline significant or not did survive adjustments. The CARDIA study used Stroop test that resembled the RVP-test used in our study, and found some associations with systolic blood pressure (15). We did not find associations between risk exposures and working memory (SWM-test) or reaction time (RTI-test). Effects on the reaction time were not expected

based on previous animal data. Additionally, the lack of effects of risk factor exposure on working memory in midlife is perhaps also not surprising because difficulties in encoding and recall might become visible prior to difficulties in other type of memory functions (*e.g.* working memory, recognition) (26). Thus, our results are consistent with studies in animal models that have observed associations between cardiovascular risk factors and memory and learning.

The neural networks related to the PAL-test localize mainly to medial temporal lobes, specifically to hippocampus and parahippocampal gyrus (16). These anatomical structures are responsible for learning and memory (27). Imaging studies have reported that exposure to cardiovascular risk factors is associated with increased amount of cerebral white matter alterations and structural brain changes in the elderly (28-31) and augment the effect of age on volume loss in hippocampus, entorhinal cortex and medial temporal lobes in healthy adults (32). These findings may offer insights into the associations between early cardiovascular risk factors and medial temporal lobe related brain functions. Furthermore, in elderly patients with mild cognitive impairment, deficits in the PAL-test have been linked to preclinical Alzheimer's disease pathology (33-36).

The main neuropathological mechanisms underlying associations between cardiovascular risk factors and old-age cognitive deficits are suggested to be subclinical ischemia causing cerebrovascular damage (27,37), structural brain changes and atrophy (28-31). Additionally, risk factors may influence cerebral β-amyloid protein metabolism (38,39). In young populations, however, the mechanisms remain unknown. Cardiovascular risk factors cause systemic atherosclerosis, loss of distensibility in the vasculature, vessel fibrosis, plasma protein leakage, and accumulation of lipid-containing macrophages in the vessels (40-42). In the brain, these changes may lead to cerebral hypoperfusion and local inflammation which disrupt the vulnerable

surrounding neuronal milieu (37). Additionally, by inducing cerebral hypoperfusion atherosclerotic changes may initiate and/or accelerate neurodegenerative changes (*e.g.* β-amyloid deposition and clearance, synaptic and neuronal dysfunction/loss) that ultimately lead to alterations in cognitive performance (38,39).

We found that the participants with several early life cardiovascular risk factors, including elevated BP, high LDL-cholesterol and smoking, exceeding the recommended guidelines had ~0.3 SD's lower performance in the PAL-test than those participants whose risk factors remained always within the guidelines. When using arbitrary cut-points for BP and total-cholesterol (exceeding 75^{th} percentiles), we similarly found that individuals who had been exposed to all three risk factors in early life had ~0.4 SD's lower performance in the PAL-test than individuals without any early life risk factor exposures. In the YFS population, we see a linear decline in the PAL-test performance that equals to 0.05 SD's per year between ages 34 and 49 (18). Therefore, the present study suggests that the effect of early life risk factor clustering on the PAL-test performance corresponds to 6-8 year difference in *cognitive age*. Furthermore, we found that the cumulative effect of the early life risk exposure on the PAL-test performance was stronger and independent of the effect of current midlife risk exposure. This observation emphasizes the role of early life risk exposure on later cognitive performance, and may be in line with the existence of differential sensitive periods and age windows of vulnerability to environmental factors and conditions during brain maturation (43).

Limitations of this study include that cognitive performance was measured once. Currently, this prevents us from studying the role of early life cardiovascular risk factors on changes in midlife cognitive performance. Furthermore, we were unable to examine the role of glucose levels as an early life risk factor because we did not have data to construct a cumulative

exposure variable similarly to systolic blood pressure and serum total-cholesterol. However, the CARDIA study found no association between young adulthood/midlife cumulative blood glucose burden and midlife cognitive performance among normoglycemic population (15). Another limitation is the possibility of residual confounding due to unmeasured factors, which is always possible in observational studies like the YFS. Our results remained unchanged after controlling the analyses for a wide array of possible confounding factors including the adulthood levels of cardiovascular risk factors. It remains possible, however, that some unmeasured factors contribute to the association between cardiovascular risk factors and cognitive performance. Furthermore, computerized cognitive tests are not routinely used in clinical settings to diagnose cognitive performance. In this study, the test battery was not used for clinical decision making, but as a tool to evaluate cognitive performance among healthy young and middle aged adults on a population level. Previous studies have shown that these tests are useful in capturing variation in cognitive performance in healthy populations (44-46). Therefore, the tests used in the YFS may be considered adequate in discriminating the cognitive variation among the participants. Another potential limitation is a possible selection in the follow-up study - the participants were more often women and older than non-participants, and they originated from families with higher income and had better childhood academic performance. However, no differences were observed in the levels of early life exposure variables. Furthermore, we conducted several statistical tests in our study, which may increase the probability of false positive findings. However, as the main analyses were based on *a priori* hypotheses, we did not apply multiple testing correction. Moreover, with respect to the establishment of causality, observational studies are prone to bias caused by reverse causation. Nevertheless, the use of existing population cohorts from childhood to adulthood is the only realistic approach to test the hypothesis that early life risk exposure is

causally linked with adult cognitive performance, as it is not possible to perform life-long randomized control trials to test causal relations between childhood risk factors and adult outcomes. The most important competing explanation for these associations is that cognitive function in early life might determine (or might associate with other factors determining) the emergence of vascular risk factors. If that hypotheses was true, midlife cognitive performance would simply be a marker of tracked early life cognitive function. We were able to test this hypotheses in a restricted number of the YFS participants by using available data on academic performance as a proxy for their childhood cognitive performance. Academic performance was defined as grade point average, which indicates the mean of all school grades during one school year. As an overall measure of academic performance it may be considered as an indicator of the participants' cognitive ability at baseline. Indeed, when introduced as a covariate, the effect of smoking was diluted. This suggests that the association between early life smoking and midlife cognitive performance might be confounded by baseline cognitive performance. However, the effects of other risk factors as well as the effect of the early life risk factor clustering remained essentially similar after taking account the childhood academic performance. Nevertheless, as the possibility of residual confounding remains, the results in YFS should be replicated in other longitudinal cohorts with follow-up from childhood to adulthood.

Conclusions

In summary, these data indicate that the cumulative burden of BP, serum total- and LDL-cholesterol, and smoking from childhood and adolescence associate independently and combined with midlife cognitive performance. The findings give support to active monitoring/treatment strategies against cardiovascular risk factors from childhood in order to turn the focus of cognitive deficits prevention to primary prevention.

Perspectives

Competency in medical knowledge

Cumulative burden of systolic BP, serum total- and LDL-cholesterol and smoking in childhood/adolescence associate with worse cognitive performance, especially memory and learning, at midlife. Importantly, the associations were independent of midlife exposures to the same risk factors. These results give support to active monitoring and treatment strategies against cardiovascular risk factors already earlier during the lifespan in order to promote adulthood cognitive health. The findings from the current study elucidate the possibilities to move the focus of cognitive decline prevention from secondary and tertiary prevention to primordial prevention through affecting cardiovascular risk factors already from childhood and adolescence.

Translational outlook

The molecular mechanisms of childhood/adolescence cardiovascular risk factors on cognitive decline from young adulthood to middle and old age require further investigation.

References

1. Knopman D, Boland LL, Mosley T, et al. Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology. 2001;56:42-48.

2. Tolppanen A-M, Solomon A, Soininen H, Kivipelto M. Midlife vascular risk factors and Alzheimer's disease: evidence from epidemiological studies. J Alzheimers Dis. 2012;32:531-540.

3. Rusanen M, Rovio S, Ngandu T, et al. Midlife smoking, apolipoprotein E and risk of dementia and Alzheimer's disease: a population-based cardiovascular risk factors, aging and dementia study. Dement Geriatr Cogn Disord. 2010;30:277-284.

4. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. Stroke. 2011;42:2672-2713.

5. Grünblatt E, Bartl J, Iuhos D-I, et al. Characterization of cognitive deficits in spontaneously hypertensive rats, accompanied by brain insulin receptor dysfunction. J Mol psychiatry. 2015;3:6.

6. Ullrich C, Pirchl M, Humpel C. Hypercholesterolemia in rats impairs the cholinergic system and leads to memory deficits. Mol Cell Neurosci. 2010;45:408-417.

7. Thirumangalakudi L, Prakasam A, Zhang R, et al. High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. J Neurochem. 2008;106:475-485.

8. Counotte DS, Spijker S, Van de Burgwal LH, et al. Long-lasting cognitive deficits resulting from adolescent nicotine exposure in rats. Neuropsychopharmacology. 2009;34:299-306.

9. Ueno M, Sakamoto H, Tomimoto H, et al. Blood-brain barrier is impaired in the hippocampus of young adult spontaneously hypertensive rats. Acta Neuropathol. 2004;107:532-538.

10. Mateos L, Akterin S, Gil-Bea F-J, et al. Activity-regulated cytoskeleton-associated protein in rodent brain is down-regulated by high fat diet in vivo and by 27-hydroxycholesterol in vitro. Brain Pathol. 2009;19:69-80.

11. Sparks DL, Kuo YM, Roher A, Martin T, Lukas RJ. Alterations of Alzheimer's disease in the cholesterol-fed rabbit, including vascular inflammation. Preliminary observations. Ann N Y Acad Sci. 2000;903:335-344.

12. Khanna A, Guo M, Mehra M, Royal W. Inflammation and oxidative stress induced by cigarette smoke in Lewis rat brains. J Neuroimmunol. 2013;254:69-75.

13. Ho Y-S, Yang X, Yeung S-C, et al. Cigarette smoking accelerated brain aging and induced pre-Alzheimer-like neuropathology in rats. PLoS One. 2012;7:e36752.

14. Rueff-Barroso CR, Trajano ETL, Alves JN, et al. Organ-related cigarette smoke-induced oxidative stress is strain-dependent. Med Sci Monit. 2010;16:BR218-26.

15. Yaffe K, Vittinghoff E, Pletcher MJ, et al. Early adult to midlife cardiovascular risk factors and cognitive function. Circulation. 2014;129:1560-1567.

16. de Rover M, Pironti VA, McCabe JA, et al. Hippocampal dysfunction in patients with mild cognitive impairment: a functional neuroimaging study of a visuospatial paired associates learning task. Neuropsychologia. 2011;49:2060-2070.

17. Raitakari OT, Juonala M, Rönnemaa T, et al. Cohort profile: the cardiovascular risk in Young Finns Study. Int J Epidemiol. 2008;37:1220-1226.

18. Rovio SP, Pahkala K, Nevalainen J, et al. Cognitive Performance in Young Adulthood and Midlife: Relations With Age, Sex, and Education-The Cardiovascular Risk in Young Finns Study. Neuropsychology. 2016:30;532-42.

19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.

20. Porkka K V, Raitakari OT, Leino A, et al. Trends in serum lipid levels during 1980-1992 in children and young adults. The Cardiovascular Risk in Young Finns Study. Am J Epidemiol. 1997;146:64-77.

21. Welham S. Longitudinal Data Analysis. In: Fitzmaurice G, Davidian M, Verbeke G, Molenberghs G, eds. Longitudinal Data Analysis. Chapman & Hall /CRC; 2009:253-289.

22. Lai C-C, Sun D, Cen R, et al. Impact of long-term burden of excessive adiposity and elevated blood pressure from childhood on adulthood left ventricular remodeling patterns: the Bogalusa Heart Study. J Am Coll Cardiol. 2014;64:1580-1587.

23. Kaelber DC, Pickett F. Simple table to identify children and adolescents needing further evaluation of blood pressure. Pediatrics. 2009;123:e972-4.

24. Jolliffe CJ, Janssen I. Distribution of lipoproteins by age and gender in adolescents. Circulation. 2006;114:1056-1062.

25. Kavey R-EW, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K. American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation. 2003;107:1562-1566.

26. Drag LL Bieliauskas LA. Contemporary Review 2009: Cognitive Aging. J Geriatr Psychiatry Neurol. 2010;23:75-93.

27. Blennow K, de Leon MJ, Zetterberg H. Alzheimer's disease. Lancet. 2006;368:387-403.

28. Vuorinen M, Solomon A, Rovio S, et al. Changes in vascular risk factors from midlife to late life and white matter lesions: a 20-year follow-up study. Dement Geriatr Cogn Disord. 2011;31:119-125.

29. Knopman DS, Penman AD, Catellier DJ, et al. Vascular risk factors and longitudinal changes on brain MRI: the ARIC study. Neurology. 2011;76:1879-1885.

30. Debette S, Seshadri S, Beiser A, et al. Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology. 2011;77:461-468.

31. Launer LJ, Lewis CE, Schreiner PJ, et al. Vascular factors and multiple measures of early brain health: CARDIA brain MRI study. PLoS One. 2015;10:e0122138.

32. Raz N, Lindenberger U, Rodrigue KM, et al. Regional brain changes in aging healthy adults: general trends, individual differences and modifiers. Cereb Cortex. 2005;15:1676-1689.

33. Blackwell AD, Sahakian BJ, Vesey R, Semple JM, Robbins TW, Hodges JR. Detecting dementia: novel neuropsychological markers of preclinical Alzheimer's disease. Dement Geriatr Cogn Disord. 2004;17:42-48.

34. Swainson R, Hodges JR, Galton CJ, et al. Early detection and differential diagnosis of Alzheimer's disease and depression with neuropsychological tasks. Dement Geriatr Cogn Disord. 12:265-280.

35. Fowler KS, Saling MM, Conway EL, Semple JM, Louis WJ. Computerized neuropsychological tests in the early detection of dementia: prospective findings. J Int Neuropsychol Soc. 1997;3:139-146.

36. Fowler KS, Saling MM, Conway EL, Semple JM, Louis WJ. Paired associate performance in the early detection of DAT. J Int Neuropsychol Soc. 2002;8:58-71.

37. Kalaria RN. Vascular basis for brain degeneration: faltering controls and risk factors for dementia. Nutr Rev. 2010;68 Suppl 2:S74-87.

38. Reed BR, Marchant NL, Jagust WJ, DeCarli CC, Mack W, Chui HC. Coronary risk correlates with cerebral amyloid deposition. Neurobiol Aging. 2012;33:1979-1987.

39. Rodrigue KM, Rieck JR, Kennedy KM, Devous MD, Diaz-Arrastia R, Park DC. Risk factors for β-amyloid deposition in healthy aging: vascular and genetic effects. JAMA Neurol. 2013;70:600-606.

40. Altman R, Rutledge JC. The vascular contribution to Alzheimer's disease. Clin Sci (Lond). 2010;119:407-421.

41. Grinberg LT, Thal DR. Vascular pathology in the aged human brain. Acta Neuropathol. 2010;119:277-290.

42. Vasilevko V, Passos GF, Quiring D, et al. Aging and cerebrovascular dysfunction: contribution of hypertension, cerebral amyloid angiopathy, and immunotherapy. Ann N Y Acad Sci. 2010;1207:58-70.

43. Marco EM, Macrì S, Laviola G. Critical age windows for neurodevelopmental psychiatric disorders: evidence from animal models. Neurotox Res. 2011;19:286-307.

44. Koepsell TD, Kurland BF, Harel O, Johnson EA, Zhou X-H, Kukull WA. Education, cognitive function, and severity of neuropathology in Alzheimer disease. Neurology. 2008;70:1732-1739.

45. Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge Neuropsychological Test Automated Battery (CANTAB): a factor analytic study of a large sample of normal elderly volunteers. Dementia. 5:266-281.

46. De Luca CR, Wood SJ, Anderson V, et al. Normative data from the CANTAB. I: development of executive function over the lifespan. J Clin Exp Neuropsychol. 2003;25:242-254.

Figure Legends

Central Illustration: Previous studies have shown that childhood cardiovascular risk factors, including high blood pressure, altered serum lipids, high body mass index and smoking associate with adulthood levels of these risk factors and also with adulthood cardiovascular health. Additionally, previous evidence suggests a link between adulthood levels of cardiovascular risk factors and middle/old age cognitive performance. (Previous evidence shown with grey arrows in the illustration.) Our present study elucidates the associations between cardiovascular risk factors from childhood to early adulthood and midlife cognitive performance in the unique population of the Cardiovascular Risk in Young Finns Study with 31 years follow-up time (Novel evidence shown with red arrow in the illustration). Our results showed that high systolic BP, elevated serum total-cholesterol and smoking from childhood were independently associated with worse midlife cognitive performance, especially memory and learning. These findings give support to active monitoring/treatment strategies against cardiovascular risk factors from childhood in order to turn the focus of cognitive deficits prevention to primary prevention by maintenance of cardiovascular risk factors.

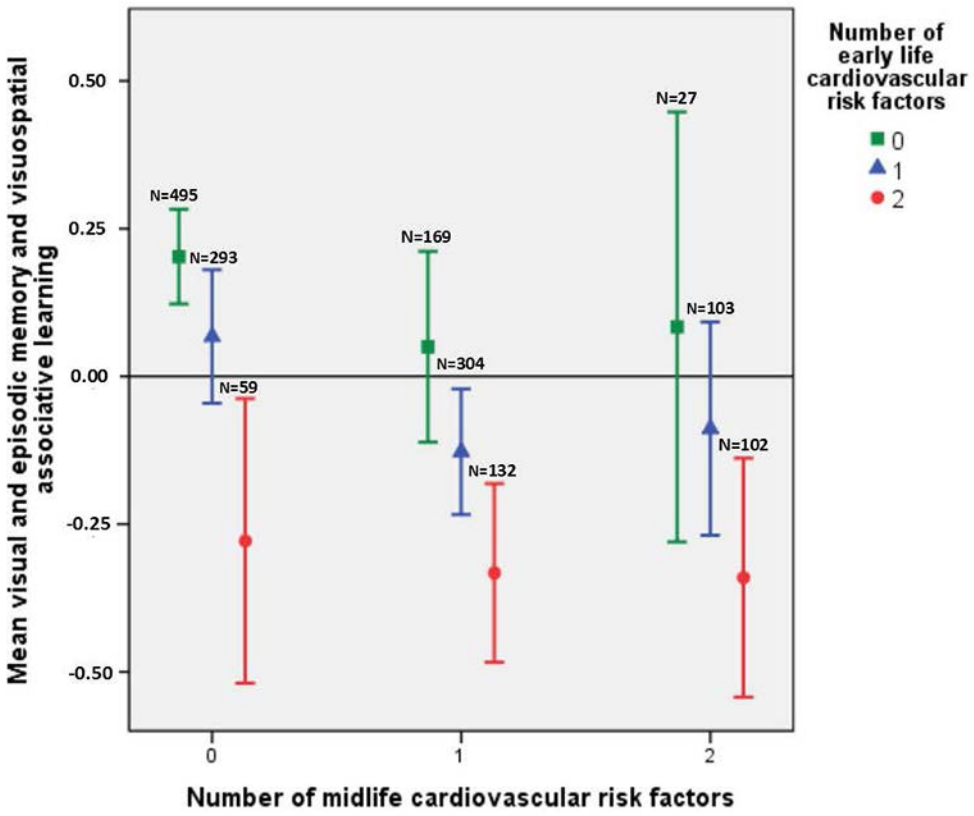

Figure 1. Midlife performance on episodic memory and visual associative learning according to the number of early life and midlife cardiovascular risk factors (N=1733). The values represent means and 95% confidence intervals indicating cognitive performance on visual and episodic memory and visuospatial associative learning in subgroups classified according to the number of risk factors in early- and midlife. The variables showing significant association for cognitive performance in the multivariate analyses (*i.e.* systolic blood pressure, serum total-cholesterol and smoking) were included in the variable for the cardiovascular risk factor clustering. In a multivariable model, the association between the number of early life cardiovascular risk factors and midlife cognitive performance was significant (p=0.004) after adjustments for age, sex, family income and the number of midlife cardiovascular risk factors. The reference line is set on the population mean. A 3-level variable indicating the number of risk

factors during early life was created from the area under the curve (AUC) variables for each cardiovascular risk factor (1=no risk factors, 2=one risk factor, 3=two or three risk factors) and the categories were indicated with colors in the figure. The AUC variables for BP and serum total-cholesterol were dichotomized into high risk factor level (≥ 75th percentile) and low risk factor level (<75th percentile). Smoking status was dichotomized into smokers and non-smokers. The dichotomical variables were summed to create the variable indicating the number of risk factors (range 0-3) during early life (6-24 years). A similar variable indicating the number of cardiovascular risk factors at the time of cognitive testing was formed and placed in the x-axis in the figure.

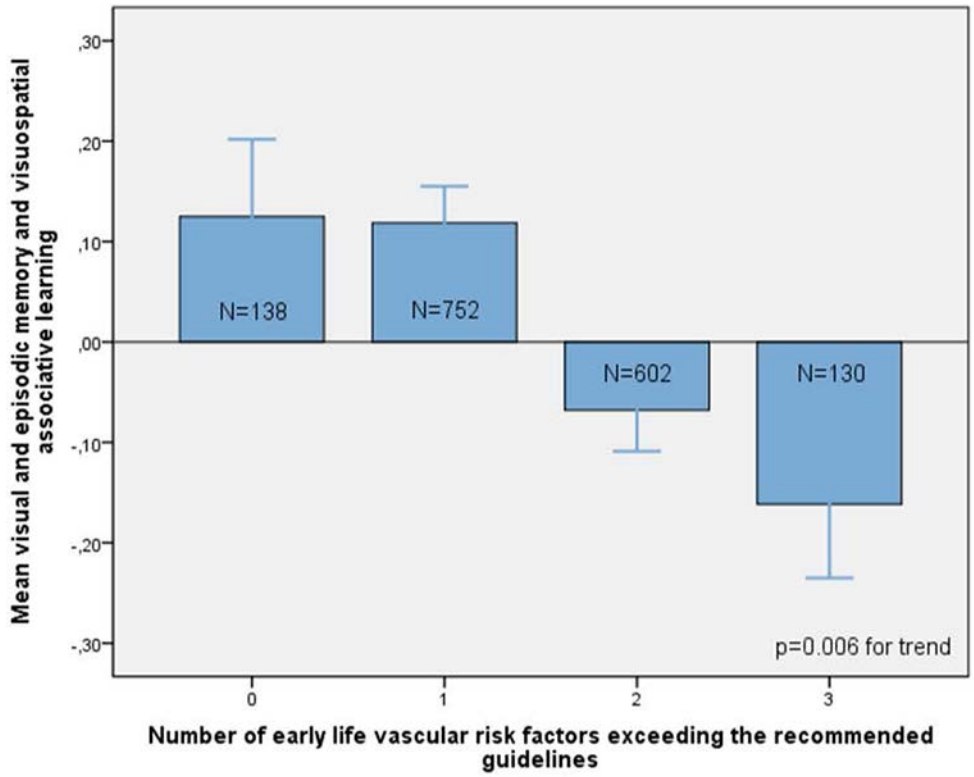

Figure 2. Midlife performance on episodic memory and visual associative learning according to the number of risk factors (high LDL-cholesterol, elevated systolic BP, and cigarette smoking) with levels exceeding the recommended guidelines (16-18). The variables

showing significant association for cognitive performance and with guideline recommendations for children/adolescence (*i.e.* LDL-cholesterol, systolic blood pressure and smoking) were included in the variable for the cardiovascular risk factor clustering. The bars indicate the mean values of the principal component for visual and episodic memory and visuospatial associative learning and the whiskers are standard errors. The individuals were classified into: 0) no risk factor levels exceeding guidelines or levels exceeding at most once per risk factor, 1) risk factor levels exceeding guidelines twice or more on one risk factor, 2) risk factor levels exceeding guidelines twice or more on two risk factors, 3) risk factor levels exceeding guidelines twice or more on all risk factors. The inverse dose-response relation with cognitive performance was significant (β=-0.088, p=0.007), adjusted for age, sex, family income, antihypertension and dyslipidemia medications and diagnoses of cardiovascular diseases and diabetes mellitus. The reference line is set on the population mean.

Table 1. Performance in the Paired Associates Learning-test, estimated difference in cognitive age and mean values of risk factor variables across the early life cumulative cardiovascular risk factor burden.

Early life risk factor burden (between ages 6 and 24 years).	**PAL-test**	**Difference in *cognitive age****	**Mean (SD) systolic blood pressure at age 6-9 years**	**Mean (SD) systolic blood pressure at age 12-15 years**	**Mean (SD) systolic blood pressure at age 18-24 years**
The area under the curve variable for systolic blood pressure					
1st quartile (n=462)	0.21 (1.00)	ref.	101.8 (6.1)	102.9 (6.3)	107.1 (7.4)
2nd quartile (n=461)	0.09 (0.99)	+2.4 years	108.3 (6.0)	110.0 (5.4)	114.9 (6.1)
3rd quartile (n=462)	-0.09 (0.98)	+6.0 years	111.1 (6.3)	115.3 (5.5)	122.6 (5.9)
4th quartile (n=463)	-0.21 (0.98)	+8.4 years	117.5 (6.8)	124.3 (8.1)	132.6 (8.4)
			Mean (SD) serum total-cholesterol at age 6-9 years	**Mean (SD) serum total-cholesterol at age 12-15 years**	**Mean (SD) serum total-cholesterol at age 18-24 years**
The area under the curve variable for serum total-cholesterol					
1st quartile (n=462)	0.16 (0.96)	ref.	4.7 (0.5)	4.1 (0.5)	3.9 (0.5)
2nd quartile (n=461)	-0.02 (0.98)	+3.6 years	5.3 (0.4)	4.8 (0.4)	4.6 (0.4)
3rd quartile (n=462)	0.03 (0.98)	+2.6 years	5.8 (0.4)	5.2 (0.4)	5.1 (0.5)
4th quartile (n=463)	-0.17 (1.04)	+6.6 years	6.6 (0.6)	6.1 (0.7)	6.0 (0.7)
			Daily smoking age 6-12 years	**Daily smoking age 12-18 years**	**Daily smoking age 18-24 years**
Daily smoking (between ages 12-24 years)					
No (n=1306)	0.04 (1.00)	ref.	*All participants were non-smokers*	0 %	0 %
Yes (n=491)	-0.13 (0.97)	+3.4 years		71.3 %	94.5 %

Values are means (standard deviations) of the performance in the paired associates learning (PAL)-test indicating learning and memory and of the cardiovascular risk factor values in the quartiles of the area under the curve (AUC)-variables for systolic blood pressure and serum total-cholesterol and for dichotomized early life (age 12-24 years) smoking. For the PAL component higher values indicate better performance.

*The difference in *cognitive age* has been calculated comparing the difference in the PAL-test performance between the risk factor quartiles to our previous finding on the effect of age (-0.0.5 SD per year) on the PAL-test.[18] The lowest quartile has been used as the reference category for all comparisons for *cognitive age*.

Table 2. Associations between cumulative burden of early life vascular risk factors (age 6-24 years) and midlife visual and episodic memory and visuospatial associative learning (PAL-test) in 1733 YFS participants.

ROW	MODEL	β coefficient (SE)	p-value
A	**Unadjusted bivariate models**		
	Systolic blood pressure	**-0.152 (0.023)**	**<0.0001**
	Serum total-cholesterol	**-0.122 (0.024)**	**<0.0001**
	Body mass index	0.018 (0.026)	0.490
	Smoking	**-0.182 (0.054)**	**0.001**
B	**Age and sex adjusted models**		
	Systolic blood pressure	**-0.067 (0.026)**	**0.010**
	Serum total-cholesterol	**-0.064 (0.025)**	**0.010**
	Body mass index	-0.004 (0.025)	0.870
	Smoking	**-0.123 (0.053)**	**0.001**
C	**Multivariate model 1**		
	Systolic blood pressure	**-0.076 (0.028)**	**0.006**
	Serum total-cholesterol	**-0.059 (0.025)**	**0.018**
	Body mass index	0.026 (0.026)	0.315
	Smoking	-0.140 (0.053)	**0.008**
D	**Multivariate model 2**		
	Systolic blood pressure	**-0.064 (0.028)**	**0.023**
	Serum total-cholesterol	**-0.053 (0.025)**	**0.037**
	Body mass index	0.029 (0.026)	0.281

Smoking	**-0.140 (0.053)**	**0.008**

Values are β coefficients (standard errors) and p-values from linear models. Unadjusted models are conducted separately for variables indicating cumulative burden of early life (6-24 years) cardiovascular risk factors (*i.e.* systolic blood pressure, serum total-cholesterol and body mass index, adolescence and young adulthood smoking at the age of 12 to 24 years) without covariates. Age and sex adjusted models are conducted separately for each early life cardiovascular risk factor. In the multivariate model 1, all variables indicating cumulative burden of early life (6-24 years) cardiovascular risk factors are entered simultaneously in an age and sex adjusted model. The multivariate model 2 is adjusted additionally for childhood family income, adulthood antihypertension and dyslipidemia medications, and diagnoses of cardiovascular diseases and diabetes mellitus. Variables for cognitive performance (PAL-test) and cardiovascular risk factors are standardized (mean 0, standard deviation 1), thus the beta coefficients indicate the amount of change in the PAL-test performance in standard deviations when the cumulative burden (6-24 years) of an early life risk factor increases one standard deviation. One standard deviation unit is equivalent to ~6 mmHg for systolic blood pressure, ~0.7 mmol/l (~27 mg/dL) for total-cholesterol and ~2.4 kg/m^2 for body mass index. For smoking, the beta coefficient estimates the effect of daily smoking between ages 12-24 years. For the PAL-test, lower values indicate lower cognitive performance. In all models, the participants with missing data on any of the variables in the fully adjusted model were excluded from the analyses.

ONLINE APPENDIX

SUPPLEMENTAL METHODS

Cognition

During the follow-up examination in 2011, a cognitive testing battery developed by the Cambridge Cognition (CANTAB®) was used to assess cognitive function among the YFS participants. The CANTAB® test is a computerized, predominantly non-linguistic and culturally neutral test focusing on a wide range of cognitive domains. The test is performed using a validated touch-screen computer system. The full test battery includes 25 individual tests from which, a suitable test battery for each particular study may be selected. In YFS, the test battery was selected so that it could be accomplished in 20-30 minutes, and included tests that are sensitive to aging.[1,2] The tests included in the test battery measured several cognitive domains: 1) short term memory, 2) spatial working memory, 3) problem solving, 4) reaction time, 5) attention, 6) rapid visual processing, 7) visual memory, 8) episodic memory, and 9) visuospatial learning.

Cognitive testing was performed during clinical examination. Due to the blood sampling included in the study protocol, the subjects came to the examinations after fasting at least 12 hours. They were instructed to avoid smoking, heavy physical activity as well as drinking alcohol and coffee during the previous evening and the morning before the examinations. Before the cognitive testing the subjects were provided with a light snack including a whole meal oat-based snack biscuit, a small portion of fruit/berry oatmeal and weak fruit/berry squash.

During cognitive testing the participants first conducted **a motor screening test** (MOT test) measuring psychomotor speed and accuracy. In this study, the motor screening test was considered as a training procedure where the participants were introduced to the equipment

used in the testing, and a screening tool to point out any difficulties in vision, movement, comprehension or ability to follow simple instructions. **Paired associates learning test** (PAL-test) was used to assess visual and episodic memory as well as visuospatial associative learning containing aspects of both delayed response procedure and conditional learning. **Spatial working memory test** (SWM-test) was used to measure ability to retain spatial information and to manipulate items stored in the working memory, problem solving as well as the ability to conduct a self-organized search strategy. **Reaction time test** (RTI-test) assessed speed of response and movement on tasks where the stimulus was either predictable (simple location task) or unpredictable (five-choice location task). **Rapid visual information test** (RVP-test) was used to assess, visual processing, recognition and sustained attention.

Each of the CANTAB® tests produced several variables. For this study, principal component analysis was conducted as a multivariate technique for data reduction and to identify components accounting for the majority of the variation within the cognition dataset. Principal component analysis was selected since it allows analyzing the major sources of variation in a multi-dimensional data without introducing inherent bias. Principal component analyses were performed separately for all individual tests. The first components resulting from these analyses were considered to represent cognitive performance related to the particular cognitive domain. After creating the test wise principal components their distributions were analyzed. The component for motor screening test was excluded from further analyses because it did not discriminate the subjects indicating a ceiling effect. All other components were normalized based on the rank order normalization procedure resulting in five separate variables, each with mean value of 0 and standard deviation (SD) of 1.

Covariates

Age was defined in full years at the end of the year 2011. At the baseline, the sum of household income was assessed with an eight-category question and used as an indicator of the socio-economic status of the family (hereafter family income). For this study, the original income categories were converted to correspond to the value of money in 2011, and after that combined into four categories: 1) <17 000 euros/year, 2) 17 000–27 000, 3) 27 000–37 000 euros/year, and 4) >37 000 euros/year. Data on antihypertensive (N=192) and dyslipidemia (N=72) medications were obtained from the questionnaires in the latest follow-up study (2011). Diagnoses of cardiovascular diseases (N=13) were adjudicated from the national hospital discharge register. Diagnoses of type 1 diabetes were self-reported in the last follow-up study (year 2011). Participants were classified as having type 2 diabetes if, at any of the follow-up visits (2001, 2007 or 2011-2012), their fasting plasma glucose value was equal or greater than 7 mmol/l, or if they reported having the diagnosis made by a physician. In addition, individuals whose hemoglobin A1c was equal or greater than 6.5% (48 mmol/l) at 2011 follow-up or who reported taking glucose lowering medication at 2007 or 2011 follow-up were classified as having type 2 diabetes. Finally, type 2 diabetes diagnoses were obtained from the National Social Insurance Institution's Drug Reimbursement Registry (N=75). Furthermore, childhood academic performance of the study participant was expressed by grade point average that was calculated as a mean of grades in all individual school subjects reported in a questionnaire. In Finland, the school grades vary on a scale between 4 indicating failure (US grade: F) and 10 indicating excellent knowledge and skills (US grade: A). In this study, the grade point average values were used as a proxy for childhood cognitive ability. Adulthood education was assessed with questionnaires at the follow-up studies in 2001, 2007 and 2011. For this study, the maximum years of education was determined as a continuous variable from self-reported data concerning total years of education. Apolipoprotein E (APOE) genotypes were analyzed with

2 single nucleotide polymorphisms (rs429358 and rs7412), and the APOE promoter polymorphisms −219 and + 113 (rs405509 and rs440446, respectively). In this study, subjects were divided into 1) apoEε4 carriers (at least one ε4 allele) and 2) non-carriers (no ε4 alleles). Physical activity was measured with a self-administered questionnaire at baseline and in all follow-ups. The questionnaire consisted of items on the frequency and intensity of physical activity, frequency of vigorous physical activity, hours spent on vigorous physical activity, average duration of a physical activity session, and participation in organized physical activity. A comprehensive physical activity index was calculated for baseline and each follow-up study similarly to previous studies.[3] Mean of the adulthood (ages 24-49 years) physical activity indexes was considered as an indicator of physical activity level in this study.

Statistical analyses

Subject-specific curves for systolic BP, serum total-, HDL- and LDL-cholesterols, triglycerides and BMI were estimated by mixed model regression splines.[4] The covariance structure for the longitudinal setting was modelled by allowing for subject specific regression spline coefficients, which were incorporated as random effects to the model. To avoid overfitting we reduced the number of knots (two knots on the calendar time from 1980 to 2011) for the subject-specific part from that of the fixed effects part (four knots on age from 3 to 34 years). The mean profile was allowed to vary across birth cohorts and sex in terms of possibly different fixed effects parts. Similar to the approach of Lai et al (2014), we then evaluated the area under the curve (AUC) as a measure of a long term burden of each of the measured attributes.[5] For this study, the AUC variables for BP, serum total-, HDL- and LDL-cholesterols, triglycerides and BMI were defined separately for childhood (age 6-12 years), adolescence (12-18 years), young adulthood (18-24 years) and early life (6-24 years). For interpretability, the AUC variables were standardized resulting in normally distributed variables with mean 0 and SD 1.

To analyze the effect of risk factor clustering from childhood to young adulthood on cognitive performance at midlife, a variable indicating the number of risk factors during early life (6-24 years) was created. First, the AUC variables for BP and serum total-cholesterol were dichotomized into 1) high risk factor level ($\geq 75^{th}$ percentile; coded as 1) and 2) low risk factor level ($<75^{th}$ percentile; coded as 0). Smoking status was dichotomized similarly into 1) smokers (coded as 1) and 2) non-smokers (coded as 0). Finally, the dichotomic variables for BP, serum total-cholesterol and smoking were summed to create the variable indicating the number of risk factors (range 0-3) during early life. The number of persons with three early life cardiovascular risk factors was substantially low (n=62) and therefore, the persons with 2 or 3 risk factors were considered as the highest group. A similar variable indicating the number of cardiovascular risk factors at the time of cognitive testing was formed based on the BP and serum total-cholesterol values (dichotomized from the 75^{th} percentile similarly to the early life AUC variables) and smoking status during the latest follow-up study (range of the sum variable 0-3). The number of participants with three cardiovascular risk factors in the latest follow-up study was low (n=28), and therefore, the persons with 2 or 3 risk factors were again addressed to the same category. Sensitivity analyses were additionally performed using cut-points of 70^{th}, 80^{th} and 85^{th} percentiles for continuous variables (Online table 5).

To investigate whether the effect of cardiovascular risk factor clustering is attributable to risk factor exposure levels exceeding recommended guidelines, the age and sex specific guideline values were considered for systolic BP[6], LDL-cholesterol[7] and smoking.[8] Each subject was classified as having risk factor levels below/above the recommended guidelines at each age point. Subsequently, the subjects were classified according to the number of risk factors (*i.e.* systolic BP, LDL-cholesterol and smoking) and times when they had risk factor levels exceeding the guidelines between ages 6-24 into: 1) no risk factor levels exceeding guidelines or levels exceeding guidelines at most once on each risk factor, 2) risk factor levels exceeding

guidelines on one risk factor twice or more, 3) risk factor levels exceeding guidelines twice or more on two risk factors, 4) risk factor levels exceeding guidelines twice or more on all risk factors.

Associations between two categorical variables were studied with χ^2-test, while Student's t-test was applied for analyses for continuous variables. Linear regression analyses were conducted to investigate the associations between childhood / adolescence / young adulthood BP, serum total-, HDL- and LDL-cholesterols, serum triglycerides, BMI and principal components for midlife cognitive performance. First, age and sex adjusted regression analyses were conducted separately for cumulative burden of each cardiovascular risk factor in childhood, adolescence and young adulthood using each cognitive domain as outcome. After that, these analyses were adjusted additionally for family income, antihypertension and dyslipidaemia medication and diagnoses of cardiovascular diseases and diabetes mellitus.

Second, further analyses were conducted for visual and episodic memory and visuospatial associative learning (PAL-test) which was the cognitive domain showing most consistent results in the analyses separately for each early life cardiovascular risk factors. These analyses were conducted first unadjusted, and then entering age and sex as covariates. After that, all early life cardiovascular risk factors were entered in the same age and sex adjusted model. Finally, the analyses were additionally adjusted for family income, antihypertension and dyslipidaemia medication and diagnoses of cardiovascular diseases and diabetes mellitus. Additional analyses were conducted adjusting the models additionally for childhood academic performance, apoEε4 genotype, and adulthood physical activity level to analyze whether these factors act as mediators in the found associations. Additionally, we analyzed the possible interaction between cardiovascular risk factors and sex or age by adding interaction terms in the fully adjusted models (*e.g.* 'sex*serum total-cholesterol' or 'age*serum total-cholesterol').

All statistical analyses were performed using SAS 9.4 and the level of statistical significance was set at $p<0.05$.

SUPPLEMENTAL REFERENCES

1 Robbins TW, James M, Owen AM, Sahakian BJ, McInnes L, Rabbitt P. Cambridge Neuropsychological Test Automated Battery (CANTAB): A Factor Analytic Study of a Large Sample of Normal Elderly Volunteers. *Dementia* 1994;5:266-81.

2 De Luca CR, Wood SJ, Anderson V, Buchanan J-A, Proffitt TM, Mahony K, Pantelis C. Normative Data From the Cantab. I: Development of Executive Function Over the Lifespan. *J Clin Exp Neuropsych* 2003;25:242-54.

3 Telama R, Yang X, Viikari J, Välimäki I, Wanne O, Raitakari O. Physical activity from childhood to adulthood: a 21-year tracking study. *Am J Prev Med* 2005;28:267–73.

4 Welham, S. J. Smoothing spline models for longitudinal data. In: Fitzmaurice et al. (editors), Longitudinal Data Analysis, Chapman & Hall/CRC, 2009.

5 Lai C-C, Sun D, Cen R, Wang J, Li S, Fernandez-Alonso C, Chen W, Srinivasan SR, Berenson GS. Impact of long-term burden of excessive adiposity and elevated blood pressure from childhood on adulthood left ventricular remodeling patterns: the Bogalusa Heart Study. *J Am Coll Cardiol* 2014;64:1580-7.

6 Kaelber DC, Pickett F. Simple Table to Identify Children and Adolescents Needing Further Evaluation of Blood Pressure. *Pediatrics* 2009;123:e972-e974.

7 Jolliffe CJ, Janssen I. Distribution of Lipoproteins by Age and Gender in Adolescents. *Circulation* 2996;114:1056-1062.

8 Kavey R-EW. Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K. American Heart Association Guidelines for Primary Prevention of Atherosclerotic Cardiovascular Disease Beginning in Childhood. *Circulation* 2003;107:1562-1566.

9 Rovio SP, Pahkala K, Nevalainen J, Juonala M, Salo P, Kähönen M, Hutri-Kähönen N, Lehtimäki T, Jokinen E, Laitinen T, Taittonen L, Tossavainen P, Viikari J, Rinne JO, Raitakari OT. Cognitive performance in young adulthood and midlife: Relateions with age, sex, and education – The Cardiovascular Risk in Young Finns Study. *Neuropsychology* 2016;30:532-42.

Online Table 1. Age and sex specific mean values of systolic blood pressure, total-and LDL-cholesterol among participants in the highest 75th percentile in the area under the curve (AUC) variables between ages 6-24 years.

	Mean (SD) **Boys/Girls**
Systolic blood pressure, mmHg	
Age 6 years	112.16 (7.00) / 118.11 (9.31)
Age 9 years	117.79 (8.76) / 120.39 (8.92)
Age 12 years	117.35 (8.78) / 122.30 (9.04)
Age 15 years	127.36 (10.16) / 127.67 (9.17)
Age 18 years	131.82 (9.99) / 128.37 (9.94)
Age 21 years	135.28 (9.39) / 131.96 (7.08)
Age 24 years	133.94 (8.78) / 131.88 (10.33)
Serum total-cholesterol, mmol/l	
Age 6 years	5.38 (0.94) / 5.71 (0.98)
Age 9 years	5.44 (0.88) / 5.64 (1.17)
Age 12 years	5.29 (0.94) / 5.42 (1.07)
Age 15 years	4.74 (0.90) / 5.29 (1.18)
Age 18 years	4.89 (0.97) / 5.32 (1.21)
Age 21 years	5.04 (1.03) / 5.26 (0.94)
Age 24 years	5.00 (0.98) / 5.13 (0.92)
Serum LDL-cholesterol, mmol/l	
Age 6 years	3.44 (0.90) / 3.76 (0.92)
Age 9 years	3.39 (0.79) / 3.63 (1.12)
Age 12 years	3.29 (0.85) / 3.44 (0.99)
Age 15 years	2.91 (0.84) / 3.29 (1.10)
Age 18 years	3.07 (0.93) / 3.33 (1.16)
Age 21 years	3.07 (0.94) / 3.15 (0.82)
Age 24 years	3.06 (0.94) / 3.03 (0.79)

The values of serum total- and LDL-cholesterol are converted into mg/dl by multiplying the values in mmol/l by 39.

Online Table 2. Cut-points for the recommended guidelines for systolic blood pressure and low density lipoprotein cholesterol.

	Cut-point values (boys / girls)
Systolic blood pressure, mmHg	
Age 6 years	105.0 / 104.0
Age 9 years	109.0 / 110.0
Age 12 years	115.0 / 116.0
Age 15 years	120.0 / 120.0
Age 18 years	120.0 / 120.0
Age 21 years	120.0 / 120.0
Age 24 years	120.0 / 120.0
Serum LDL-cholesterol, mmol/l	
Age 6 years	2.38 / 2.38
Age 9 years	2.38 / 2.38
Age 12 years	2.50 / 2.38
Age 15 years	2.38 / 2.43
Age 18 years	2.51 / 2.52
Age 21 years	2.59 / 2.59
Age 24 years	2.59 / 2.59

Values are age and sex specific cut-point values for recommended guidelines for systolic blood pressure (Kaelber DC et al. Pediatrics. 2009;123(6):e972-4) and low density lipoprotein cholesterol (Jolliffe CJ et al. Circulation. 2006;114(10):1056-1062). The values of serum LDL-cholesterol are converted into mg/dl by multiplying the values in mmol/l by 39.

Online Table 3. Characteristics of the study population.

	All (N=2026)	Men (N=922)	Women (N=1104)
Background characteristics			
Age, years (N=2026)			
At baseline	10.8 (5.0)	10.7 (5.1)	10.9 (5.0)
At cognitive testing	41.8 (5.0)	41.7 (5.1)	41.9 (5.0)
Family income at baseline, N (%), (N=1956)			
<17 000 euros/year	512 (26.2)	229 (25.7)	283 (26.6)
17 000–27 000 euros/year	575 (29.4)	270 (30.3)	305 (28.6)
27 000–37 000 euros/year	425 (21.7)	191 (21.5)	234 (22.0)
>37 000 euros/year	444 (22.7)	200 (22.5)	244 (22.9)
Childhood academic performance (N=1777)	7.77 (0.7)	7.57 (0.7)	7.94 (0.7)
ApoE ε4 carriers (≥one ε4 allele) (N=1909)	680 (35.6)	314 (36.6)	366 (34.8)
Early life smoking, N (%), yes (N=1968)	544 (27.6)	291 (32.6)	253 (23.5)
Physical activity index (range 5-15), (N=2005)	9.21 (1.72)	9.14 (1.84)	9.26 (1.62)
Cardiovascular risk factors at baseline			
Systolic blood pressure, mmHg (N=2009)	112.8 (11.9)	113.8 (13.0)	112.0 (10.9)
Diastolic blood pressure, mmHg (N=1732)	68.6 (9.4)	68.9 (9.6)	68.3 (9.2)
Total-cholesterol, mmol/l (N=2003)	5.3 (0.9)	5.2 (0.9)	5.4 (0.9)
HDL-cholesterol, mmol/l (N=2002)	1.6 (0.3)	1.6 (0.3)	1.6 (0.3)
LDL-cholesterol, mmol/l (N=2002)	3.4 (0.8)	3.4 (0.8)	3.5 (0.8)
Triglycerides, mmol/l (N=2003)	0.7 (0.3)	0.7 (0.3)	0.7 (0.3)
Body mass index, kg/m^2 (N=2011)	18.0 (3.1)	18.0 (3.2)	17.9 (3.1)
Cardiovascular risk factors at cognitive testing			
Systolic blood pressure, mmHg (N=2019)	118.9 (14.1)	122.9 (13.3)	115.6 (13.8)
Diastolic blood pressure, mmHg (N=2019)	74.9 (10.5)	77.8 (10.8)	72.4 (9.5)
Total-cholesterol, mmol/l (N=2008)	5.2 (1.0)	5.3 (1.0)	5.1 (0.9)
HDL-cholesterol, mmol/l (N=2006)	1.3 (0.3)	1.2 (0.3)	1.4 (0.3)
LDL-cholesterol, mmol/l (N=1961)	3.3 (0.8)	3.4 (0.9)	3.1 (0.8)

Triglycerides, mmol/l (N=2008)	1.3 (1.2)	1.6 (1.2)	1.1 (1.2)
Body mass index, kg/m^2 (N=2020)	26.5 (5.1)	27.0 (4.4)	26.1 (5.5)
Area under the curve (AUC) variables for cardiovascular risk factors			
Childhood (6-12 years), mean (SD)			
Systolic blood pressure, mmHg*yrs (N=2026)	647.5 (31.9)	646.0 (32.4)	648.8 (31.5)
Total-cholesterol, mmol/l*yrs (N=2026)	33.1 (4.0)	32.8 (4.1)	33.3 (3.9)
HDL-cholesterol, mmol/l*yrs (N=2026)	9.1 (1.4)	9.2 (1.4)	8.9 (1.4)
LDL-cholesterol, mmol/l*yrs (N=2026)	22.1 (4.3)	21.6 (4.3)	22.5 (4.2)
Triglycerides, mmol/l*yrs (N=2026)	3.8 (1.1)	3.6 (1.1)	3.9 (1.1)
Body mass index, kg/m^2*yrs (N=2026)	99.7 (9.7)	100.0 (9.8)	99.4 (9.6)
Adolescence (12-18 years), mean (SD)			
Systolic blood pressure, mmhg*yrs (N=2026)	685.8 (40.6)	699.3 (40.0)	674.5 (37.6)
Total-cholesterol, mmol/l*yrs (N=2026)	29.4 (4.1)	28.4 (4.0)	30.2 (4.0)
HDL-cholesterol, mmol/l*yrs (N=2026)	8.7 (1.4)	8.3 (1.3)	9.0 (1.4)
LDL-cholesterol, mmol/l*yrs (N=2026)	18.4 (3.9)	17.9 (3.9)	18.9 (3.8)
Triglycerides, mmol/l*yrs (N=2026)	4.9 (1.4)	4.8 (1.4)	5.0 (1.3)
Body mass index, kg/m^2*yrs (N=2026)	120.6 (14.3)	120.5 (14.4)	120.6 (14.2)
Young adulthood (18-24 years), mean (SD)			
Systolic blood pressure, mmHg*yrs (N=2026)	713.2 (53.1)	746.2 (45.6)	685.7 (42.1)
Total-cholesterol, mmol/l*yrs (N=2026)	29.4 (4.2)	28.3 (4.1)	30.4 (4.1)
HDL-cholesterol, mmol/l*yrs (N=2026)	8.1 (1.6)	7.2 (1.3)	8.8 (1.5)
LDL-cholesterol, mmol/l*yrs (N=2026)	17.7 (3.7)	17.2 (3.7)	18.1 (3.7)
Triglycerides, mmol/l*yrs (N=2026)	6.2 (1.6)	6.2 (1.7)	6.2 (1.5)
Body mass index, kg/m^2*yrs (N=2026)	135.2 (17.3)	138.1 (16.9)	132.8 (17.2)
Cognitive components, mean (SD)			
PAL-test (N=1848)	*Cognitive*	-0.1 (1.0)	0.1 (1.0)
SWM-test (N=2011)	*components*	0.2 (1.0)	-0.2 (1.0)
RTI-test (N=1822)	*were*	0.2 (1.0)	-0.2 (0.9)
RVP-test (N=1975)	*standardized;*	0.1 (1.0)	-0.1 (1.0)
	mean 0, SD 1		

Values are means (standard deviations) for continuous variables and numbers (percentages) for categorical variables. Student's t-test and χ^2-test were used. Early life smoking was dichotomized into those smoking at any follow-up phase between ages 12-24. Family income was measured as sum of participants' parents' income and transformed to correspond with the current value of money. Physical activity index was calculated as a mean of adulthood (ages 24-29 years) physical activity indexes that were created based on data queried at each follow-up study on frequency and intensity of physical activity, frequency of vigorous physical activity, hours spent on vigorous physical activity, average duration of a physical activity session, and participation in organized physical activity (range 5-15). Principal component analyses was used to calculate components indicating visual and episodic memory and visuospatial associative learning (PAL-test), working memory and problem solving (SWM-test), reaction time (RTI-test) and recognition, visual processing and sustained attention (RVP-test) based on CANTAB® cognitive test battery. All comparisons between men and women were statistically significant ($p<0.05$) except for age at baseline and at the follow-up, family income, apoE ε4 allele, adulthood physical activity index, baseline diastolic blood pressure, baseline HDL-cholesterol, baseline body mass index, childhood and adolescence AUC's for body mass index, and young adulthood triglycerides for which the comparisons were non-significant. The values of serum total-, HDL- and LDL-cholesterol are converted into mg/dl by multiplying the values in mmol/l by 39. The values for triglycerides are converted into mg/dl by multiplying the values in mmol/l by 89.

Online Table 4. Baseline and early life characteristics of participants and non-participants.

	Participants (N=2026)	**Non-participants (N=1570)**	**P-value**
Sex, male	922 (45.51)	842 (53.63)	<0.0001
Age at baseline, years	10.84 (5.01)	9.92 (4.92)	<0.0001
Family income at baseline, N (%)			
<17 000 euros/year	512 (26.18)	438 (29.26)	
17 000–27 000 euros/year	575 (29.40)	479 (32.00)	
27 000–37 000 euros/year	425 (21.73)	309 (20.64)	0.003
>37 000 euros/year	444 (22.70)	271 (18.10)	
Early life smoking, N (%)	544 (27.64)	397 (28.14)	0.752
Childhood academic performance	7.77 (0.73)	7.65 (0.74)	<0.0001
ApoE ε4 carriers (≥one ε4 allele)	680 (35.62)	273 (37.19)	0.451
Systolic blood pressure, mmHg	112.80 (11.92)	112.21 (12.53)	0.165
Diastolic blood pressure, mmHg	68.58 (9.39)	69.02 (9.84)	0.221
Total-cholesterol, mmol/l	5.29 (0.90)	5.31 (0.93)	0.551
HDL-cholesterol, mmol/l	1.56 (0.31)	1.56 (0.31)	0.939
LDL-cholesterol, mmol/l	3.42 (0.82)	3.45 (0.86)	0.427
Triglycerides, mmol/l	0.67 (0.31)	0.66 (0.32)	0.434
Body mass index, kg/m^2	17.97 (3.12)	17.69 (3.10)	0.009

Values are means (standard deviations) for continuous variables and number of subjects (percentages) for categorical variables. Age and sex adjusted p-values were calculated using linear models. The participants are subjects who participated in the cognitive testing during the latest follow-up study of the Cardiovascular Risk in Young Finns Study. The non-participants are subjects who did not participate in cognitive testing. Blood pressure, serum lipids and body mass index are measured at the baseline. Early life smoking was dichotomized into those smoking at any follow-up phase between ages 12-24. Family income was measured as sum of

participants' parents' income, transformed to correspond with the current value of money. Grade point average (range from 4.0 to 10.0) is calculated as the mean of grades on all school subjects at baseline or either of the two following follow-ups (for those participants who were not of school age at the baseline). Grade point average is considered as a measure of childhood academic performance. The values of serum total-, HDL- and LDL-cholesterol are converted into mg/dl by multiplying the values in mmol/l by 39. The values for triglycerides are converted into mg/dl by multiplying the values in mmol/l by 89.

Online Table 5. Associations between childhood, adolescence and young adulthood cardiovascular risk factors and midlife cognitive performance.

	Childhood (6-12 years)		Adolescence (12-18 years)		Young adulthood (18-24 years)	
	β coefficient (SE)	**p-value**	**β coefficient (SE)**	**p-value**	**β coefficient (SE)**	**p-value**
Systolic blood pressure						
PAL-test	**-0.058 (0.023)**	**0.013**	**-0.067 (0.026)**	**0.011**	**-0.097 (0.030)**	**0.001**
SWM-test	0.006(0.022)	0.770	-0.001 (0.025)	0.956	-0.012 (0.029)	0.681
RTI-test	0.039 (0.024)	0.103	0.036 (0.027)	0.180	0.028 (0.030)	0.360
RVP-test	-0.020 (0.023)	0.393	-0.034 (0.026)	0.196	-0.046 (0.030)	0.121
Diastolic blood pressure						
PAL-test	-0.024 (0.027)	0.382	-0.035 (0.027)	0.185	**-0.053 (0.025)**	**0.035**
SWM-test	0.032 (0.026)	0.216	0.029 (0.026)	0.264	0.020 (0.024)	0.406
RTI-test	0.027 (0.028)	0.328	-0.008 (0.027)	0.761	-0.009 (0.025)	0.728
RVP-test	-0.030 (0.027)	0.266	-0.025 (0.027)	0.348	-0.028 (0.025)	0.266
Serum total-cholesterol						
PAL-test	**-0.063(0.025)**	**0.010**	**-0.066 (0.025)**	**0.009**	**-0.066 (0.024)**	**0.007**
SWM-test	-0.031 (0.023)	0.180	-0.027(0.024)	0.263	-0.019 (0.023)	0.427
RTI-test	0.003 (0.025)	0.917	-0.001 (0.026)	0.976	-0.005 (0.025)	0.836
RVP-test	-0.046 (0.025)	0.063	-0.047 (0.025)	0.062	**-0.050 (0.024)**	**0.041**
Serum LDL-cholesterol						
PAL-test	**-0.059 (0.028)**	**0.031**	**-0.056 (0.025)**	**0.025**	**-0.052 (0.023)**	**0.024**
SWM-test	-0.044 (0.026)	0.091	-0.036 (0.024)	0.133	-0.021 (0.022)	0.333
RTI-test	-0.013 (0.028)	0.635	-0.016 (0.025)	0.515	-0.012 (0.024)	0.613
RVP-test	-0.047 (0.027)	0.084	-0.039(0.025)	0.119	-0.041 (0.023)	0.078

Online Table 5. Associations between childhood, adolescence and young adulthood vascular risk factors and midlife cognitive performance *(continued)*.

	Childhood (6-12 years)		Adolescence (12-18 years)		Young adulthood (18-24 years)	
	β coefficient (SE)	**p-value**	**β coefficient (SE)**	**p-value**	**β coefficient (SE)**	**p-value**
Serum HDL-cholesterol						
PAL-test	-0.064 (0.037)	0.087	-0.059 (0.035)	0.098	-0.048 (0.031)	0.117
SWM-test	-0.020 (0.036)	0.576	-0.016 (0.034)	0.640	-0.019 (0.029)	0.519
RTI-test	0.020 (0.038)	0.594	0.014 (0.036)	0.708	-0.003 (0.031)	0.919
RVP-test	-0.014 (0.038)	0.706	-0.012 (0.036)	0.746	-0.001 (0.031)	0.980
Serum triglycerides						
PAL-test	-0.045 (0.027)	0.093	**-0.053 (0.027)**	**0.049**	-0.042 (0.022)	0.055
SWM-test	-0.034 (0.026)	0.182	-0.012 (0.025)	0.647	-0.001 (0.021)	0.972
RTI-test	-0.030 (0.028)	0.283	0.001 (0.027)	0.976	-0.010 (0.022)	0.646
RVP-test	-0.031 (0.027)	0.242	**-0.053 (0.027)**	**0.045**	-0.040 (0.022)	0.070
Body mass index						
PAL-test	-0.020 (0.024)	0.415	-0.014 (0.025)	0.570	-0.009 (0.024)	0.709
SWM-test	0.027 (0.023)	0.240	0.026 (0.023)	0.272	0.034 (0.023	0.149
RTI-test	0.024 (0.025)	0.324	0.020 (0.025)	0.414	0.005 (0.025)	0.850
RVP-test	-0.036 (0.024)	0.136	**-0.052 (0.024)**	**0.034**	**-0.076 (0.024)**	**0.024**

		Adolescence and young adulthood (12-24 years)	
Smoking		**β coefficient (SE)**	**p-value**
PAL-test	*All 6-12-year-old participants were non-smokers*	**-0.123 (0.053)**	**0.020**
SWM-test		0.020 (0.050)	0.692
RTI-test		-0.037 (0.053)	0.489

RVP-test	**-0.116 (0.052)**	**0.027**

1

The values are β coefficients (standard errors) and p-values from linear models. Variables for cognitive performance and cardiovascular risk factors are standardized (mean 0, standard deviation 1) and thus the beta coefficients indicate the amount of change in standard deviations when risk factors increase one standard deviation. All models were adjusted for age and sex. Systolic and diastolic blood pressure, serum lipids and body mass index were treated as continuous variables. Principal component analyses was used to calculate components indicating visual and episodic memory and visuospatial associative learning (PAL-test), working memory and problem solving (SWM-test), reaction time (RTI-test) and recognition, visual processing and sustained attention (RVP-test) based on CANTAB cognitive test battery. In the variables for cognitive performance, lower values indicate lower cognitive performance. The analyses were conducted using all available data for each cognitive test; in the analyses for systolic and diastolic blood pressure, serum total- and LDL-cholesterol, serum triglycerides, and body mass index N=1781 in the PAL-test, N=1941 in the SWM-test, N=1756 in the RTI-test, and N=1906 in the RVP-test. In the analyses for HDL-cholesterol N=1780 in the PAL-test, N=1940 in the SWM-test, N=1755 in the RTI-test, and N=1905 in the RVP-test and in the analyses for smoking N=1733 in the PAL-test, N=1886 in the SWM-test, N=1708 in the RTI-test, and N=1851 in the RVP-test.

Online Table 6. Sensitivity analyses for the number of early and midlife cardiovascular risk factors.

	Extreme 70th percentile		**Extreme 75th percentile**		**Extreme 80th percentile**		**Extreme 85th percentile**	
	β estimate (SE)	**p-value**	**β estimate (SE)**	**p-value**	**β estimate (SE)**	**p-value**	**β estimate (SE)**	**p-value**
Univariate models								
Number of early life risk factors	**-0.136 (0.033)**	**<0.001**	**-0.135 (0.034)**	**<0.001**	**-0.120 (0.035)**	**<0.001**	**-0.111 (0.037)**	**0.002**
Number of midlife risk factors	**-0.090 (0.032)**	**0.006**	**-0.078 (0.034)**	**0.022**	-0.063 (0.035)	0.071	-0.061 (0.037)	0.103
Multivariate model								
Number of early life risk factors	**-0.115 (0.035)**	**0.001**	**-0.119 (0.036)**	**0.001**	**-0.107 (0.038)**	**0.004**	**-0.099 (0.039)**	**0.011**
Number of midlife risk factors	-0.056 (0.035)	0.110	-0.045 (0.037)	0.220	-0.035 (0.038)	0.351	-0.038 (0.040)	0.345

A 3-level variable indicating the number of risk factors during early (age 6-24 years) and midlife (at the latest follow-up in 2011; age 34-49 years) was created from the area under the curve (AUC) variables for each cardiovascular risk factor (1=no risk factors, 2=one risk factor, 3=two or three risk factors). The AUC variables for BP and serum total-cholesterol were dichotomized into high risk factor level ($\geq 70^{th}/75^{th}/80^{th}/85^{th}$ percentile)

and low risk factor level (<70th/75th/80th/85th percentile). Smoking status was dichotomized into smokers and non-smokers. The dichotomical variables were summed to create the variable indicating the number of risk factors (range 0-3) during early life and at midlife. The univariate models were conducted separately for variables indicating the number of early life and midlife risk factors. In the multivariate model, the variables indicating the number of early life and midlife risk factors were entered simultaneously in the analysis. All analyses were adjusted for age, sex, family income, hypertension and dyslipidemia medications and diagnoses of cardiovascular diseases and diabetes mellitus.