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Vital exhaustion and markers of low-grade inflammation in healthy adults: The Amsterdam Growth and Health Longitudinal Study

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Accepted for publication: Stress and Health

Abstract

This study analyzes distinct trajectories of vital exhaustion (VE; a measure of mental health incorporating signs of stress and depression) over a period of 15 years in healthy adults and investigates further the consequences for markers of low-grade inflammation as indicators of cardiovascular disease-risk (CVD-risk). Data of 341 participants of the Amsterdam Growth and Health Longitudinal Study were utilized. VE was measured by the Maastricht Questionnaire. Markers of low-grade inflammation included Interleukin-6 and -8 and Tumour Necrosis Factor-α. Distinct trajectories of VE were obtained by Latent Class Growth Models and consequences for markers of low-grade inflammation of the trajectories were analyzed by linear regressions. We found comparable trajectories of VE for males and females; a "never vitally exhausted" subgroup (16.9% and 25.1% respectively), a "stable preclinical VE" subgroup (51.7% and 68.1%) and a "chronic VE state" subgroup (31.5% and 6.7%). The subgroups had similar levels of the markers investigated. This study is the first to analyze VE longitudinally in healthy adults and indicates that although distinct trajectories of VE were identified, differential consequences for CVD-risk were unapparent.

Introduction

Psychosocial factors such as stress and depression are believed to influence multiple disease processes, including cardiovascular disease (CVD) and low-grade inflammation processes, through both direct- and indirect pathways. The direct pathway hypothesis maintains that chronic stress leads to the dysregulation of the hypothalamic pituitary adrenocortical and sympatho-adrenomedullary axes [151]. The indirect pathway hypothesis argues that psychosocial variables influence CVD health by modulating the likelihood of an individual carrying out health behaviours, such as exercise and cigarette smoking [152]. Adipose tissue (shown to be a metabolically active organ, and often induced by an unhealthy lifestyle) is thought to mediate this indirect pathway by the secretion of cytokines [107], which in turn induce low-grade inflammatory processes in the human body. These processes have been shown to play crucial roles in atherogenesis, leading to CVD [110]. Further, markers of low-grade inflammation (or cytokines) such as Interleukin-6, Interleukin-8 or Tumour Necrosis Factor-α have by itself been shown to refine quantification of CVD-risk, implying a direct effect between these cytokines and CVD [108]. Studies researching this indirect pathway have predominantly studied well known constructs such as depression and stress. The role of stress in the initiation, maintenance and relapse of cigarette smoking [153] for example, has been highlighted and depression was found to be a significant risk factor for a sedentary lifestyle [154]. Moreover, both stress and depression are strongly related to a range of CVD risk factors including hypertension [155], atherosclerosis [156] and elevated cytokine levels [157].

A less-well known construct, but closely linked to stress and depression is vital exhaustion (VE). VE is thought to coincide with longer periods of work- or emotional stress, but it is also possible that VE follows periods of stress [158]; at least it appears that stress management programs decrease VE-levels [159]. The construct was originally developed by Appels and colleagues [160–163] to best capture heterogeneity in mental precursors for heart disease. A retrospective study [164] revealed that patients who had recently suffered a myocardial infarction reported a variety of stress-like symptoms in combination with fatigue and depression. This cluster of diverse complaints was labelled VE. VE has been shown to predict not only myocardial infarction [165], but also microvascular complications [166] and elevated triglyceride levels [167] in a number of patient populations such as patients with myocardial infarction [168], as well as other cardiovascular disease risk factors such as hypertension and diabetes [169], and chronic obstructive pulmonary disease [170].

Slowly, studies looking into associations between VE and cytokines are emerging gaining more insight in the exact mechanisms described above. For example, Janszky et al. [171] show that vitally exhausted subjects have higher levels of cytokines. Moreover, Chumaeva et al. [172] show that vitally exhausted subject also have higher levels of markers of endothelial dysfunction, important early markers for CVD also [107]. What is lacking, however, are longitudinal studies to confirm the causal pathway (i.e. VE leading to increased secretion of cytokines) that has been proposed in the aforementioned studies. To our knowledge, the course of VE over time has only been studied once [173], but in relation to CVD itself. This study investigated (heterogeneity in) the course of VE in cardiac patients and found different trajectories of VE were associated with differential CVD-risk. Highlighted was the heterogeneous course of VE; the authors unravelled four distinct trajectories: 1) low levels of VE at all time points, 2) decreasing levels of VE over time, 3) increasing levels of VE over time and 4) high levels of VE at all time points. Whether such heterogeneity and the relations found can be translated to the general population, in which extremes make up only a very small proportion of the total, is yet to be studied. The aims of the current study therefore are twofold. First, we will aim to analyze distinct trajectories of VE over a period of 15 years in a cohort of healthy adults to evaluate the degree of heterogeneity in the course of VE by investigating distinct trajectories, hypothesized to exist based on previous research [173]. Second, we will investigate the consequence for cytokine levels as (early) markers of CVD-risk of these distinct trajectories. Knowledge of distinct VE trajectories and its outcomes in healthy individuals allows for a better understanding of the aetiology between VE and low-grade inflammation, which is related to CVD (-risk), in order to provide opportunities for more targeted prevention of cardiovascular diseases, incorporating mental health components in order to increase (long-term) effectiveness.

Materials and methods

The Amsterdam Growth and Health Longitudinal Study (AGAHLS) has been described in detail previously [8, 72, 83]. Briefly, approximately 650 boys and girls (mean age of 13 years) from the first two grades of two secondary schools in the Netherlands were included in the study in 1976. The initial goals of the study were to describe the natural development of growth, health and lifestyle and further to investigate associations between health and lifestyle. Over the past thirty-five years, participants have been followed up ten times. Around

350–375 participants currently still remain in the study. Attrition over the years has been relatively low because rounds of measurement were presented not only for scientific aims, but also because of the social aspects of meeting former school mates during measurement days. Throughout the years, several drop-out analyses have been conducted, which revealed non-selective dropout only [8].

At all times of measurement participants and/or their parents/guardians (during adolescence only) gave written informed consent and each round of measurement was approved by the Medical Ethical Committee of the VU University Medical Centre.

For the current analyses, participants from whom at least three measurements over time were available and who had cytokines measurements at age 42 were included in the analyses; those who attended the measurement wave at age 42 all had their blood drawn (N=344). Only three of the 344 participants did not have at least three measurements of VE available for analyses and were excluded from the analyses. Participants who were pregnant at the most recent round of measurement (N=1) were also excluded from the analyses. If women were pregnant during earlier rounds, that particular measurement was excluded, but the participant herself was not. Pregnant participants were excluded because of validity concerns in the blood parameters and VE measures. In total, 163 (47.8%) females and 178 (52.2%) males were included in the present analysis.

Measurements of Vital Exhaustion

Vital Exhaustion (VE), measured by the Maastricht Questionnaire [160] is a self-reported questionnaire developed to capture a variety of symptoms including irritability, depression and fatigue. The questionnaire consists of 23 items asking the participant about their feelings of dejection, demoralization, stress, fatigue levels, amongst others. Answers include "yes", "undecided", and "no" and according to the instructions of the questionnaire [174] each answer was given a score of two, one, and zero, respectively. The only two positive items (asking about enjoying sexual behaviours and feelings of happiness - questions nine and fourteen) were reverse-scored. All answers were then added up giving a summed score of VE ranging from 0-46.

The distribution of the summed score of VE did not meet the Normality assumption and due to the scores of zero, log transformation was not possible. Therefore, the VE-scores were recoded in variables with three categories 1) not vitally exhausted; a score of zero, 2) "preclinical" vitally exhausted; a score of

1-13 and 3) vitally exhausted; a score of 14 or higher. These cutoff points were decided on according to descriptions by the developers of the questionnaire [165]. The Maastricht Questionnaire has good internal consistency (Cronbach's alpha of the current sample = 0.84), comparable to other studies [171]. Information of VE was available at the five most recent rounds of measurement, i.e. between 27 and 42 years of age and detailed descriptions and applications of the Maastricht Questionnaire in other study populations can also be found elsewhere [162, 163, 175].

Markers of CVD-risk (markers of low-grade inflammation)

Markers of low-grade inflammation were available at the most recent round of measurement when the participants were 42 years old. Blood was drawn from the antecubital vein when the participants were in a fasting state. Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Tumour Necrosis Factor-alpha (TNF-α) were assessed by an electro-chemiluminescence detection system using multi array technology (SECTOR Imager 2400, Meso Scale Discovery). The intra- and interassay CV at the laboratium were for IL-6, 6.3 and 17.5%; for IL-8, 6.9 and 7.3%; and TNF-α, 5.9 and 12.6%, respectively. The methodology of the multi array technology is described in detail elsewhere [176].

Potential covariates

All covariates were determined at the most recent round of measurement when the participants were 42 years old. Covariates (by definition (expected to be) related to both the independent and dependent variables; VE and low-grade inflammation [177, 178]) were selected based on existing literature describing these relationships. Main covariates included smoking behaviour, alcohol use and body fat distribution. Socioeconomic status, a common confounder in relationships between mental health measures and blood parameters, was not considered as a possible confounder because the AGHLS-cohort is a relatively homogenous cohort regarding social economic status [8].

Smoking behaviour [171, 175, 179] was considered a potential covariate following results of previous studies reporting relationships between smoking behaviour and VE and between smoking behaviour and several markers of low-grade inflammation; (long-term) tobacco smoking, following depression, has been found to lead to increased levels of IL-6, Fibrinogen and CRP [179] and the prevalence of smoking is known to be higher among stressed, depressed or vitally exhausted individuals [180].

Smoking behaviour was measured by a validated questionnaire [88], from which participants were categorized in either of three categories; 1) never smoked, 2) ever smoked (but not a current smoker, at age 42) or 3) current smoker (at age 42). Although self-reported, the smoking questionnaire highly agreed with the results from a dipstick test (Nicheck 1; a test measuring the amount of nicotine metabolites in morning urine collected at the measurement site).

Alcohol consumption [175, 181, 182] was considered a covariate because previous studies have reported on independent relationships between markers of low-grade inflammation, and alcohol use in similar study populations [183] and stressed, depressed and vitally exhausted individuals are known to consume more alcoholic beverages [175].

Alcohol consumption was also measured by a validated questionnaire [89], assessing the frequency of consuming alcohol (average per week) and the number of drinks consumed at each occasion (at age 42). These two questions were combined to form the continuous variable frequency*number of drinks. This variable was entered into the regression models as a categorical variable (gender-specific tertiles) to overcome the linearity assumption.

Body fat percentage [171, 175] was measured by means of a DXA-scan (Hologic QDR-4500A, software version 8.21, Hologic Brussels, Belgium). The DXA-scan provides information on the fat- and lean masses of the human body. The relationships between body fat percentage and several markers of low-grade inflammation have been demonstrated in many previous studies [107, 111] and at least one previous study [175] reported that vitally exhausted subjects have higher body weight compared to non-vitally exhausted subjects. For this study, we used the fat percentage of the whole body (%), which was analyzed as a continuous variable in the regression models.

Statistical analyses

All analyses were run using the Mplus 6.12 [34] and PASW 20 software packages. Results of descriptive analyses are shown as means (standard deviations) for continuous variables and as percentages for categorical variables. Analyses were conducted in two steps and for males and females separately because from previous research [175, 184] it was hypothesized that the relationships between VE and markers of low-grade inflammation were different for males and females.

In the first step, Latent Class Growth models, LCGM [4, 41, 42] were applied to obtain distinct developmental trajectories of VE. LCGM is a contemporary longitudinal technique based on structural equation modelling, using both continuous and categorical latent (unobserved) variables. The underlying aim of the technique is to model the heterogeneity in the study sample by identifying *k* number of distinct latent classes (i.e. subgroups) of, in the present study, developmental trajectories of VE. In LCGM, the assumption that all individuals in the study sample come from one underlying population is relaxed, implying that multiple underlying subpopulations might exist. To determine the optimal number of trajectories, a "forward" approach [18, 120] was used, i.e. starting with a model with only 1 trajectory, implying that all participants in the study have the same VE development over time. Subsequently, more trajectories (or classes) were added, and model fit was assessed. Model fit assessment was done in several ways. First, two model fit parameters were assessed; the Bayesian Information Criterion (BIC) [66] and the Bootstrapped Likelihood Ratio Test (BLRT) [17]. The BIC considers both the likelihood of the model as well as the number of parameters in the model. A lower BIC implies a better fitting model (where a difference of at least 10 points [67] is regarded as a sufficient improvement). The BLRT uses bootstrap samples to estimate the more common log likelihood difference test [30]. A *P*-value is provided; a significant *P*-value favours the *k* class model over the *k*-1 class model and a non-significant *P*-value favours the *k*-1 class model.

Second, posterior probabilities were assessed. For each individual in the sample, the probability to belong to each of the *k* classes was estimated. The probability for the class to which the individual was assigned to, should be considerably higher than the other probability/probabilities [18, 185]. In this way, the classes clearly distinguish from each other.

Last, the clinical interpretation of the VE-patterns was assessed. Solutions with uninterpretable patterns were rejected in favour of solutions with clinically interpretable patterns [18]. The procedure for determining the final number of classes, including the optimization of the final solution has been described in detail previously [18, 120]. Taking into account the sample size and the number of hypothesized trajectories from previous research [173], we modelled a maximum of four trajectories.

In the second step, possible associations between class membership and markers of low-grade inflammation were analyzed with two linear regression models: 1) a crude model where class membership was associated with the marker of

low-grade inflammation, 2) a model adjusted for smoking- and drinking habits, and body fat percentage. Results are presented as regression coefficients with corresponding 95% confidence intervals and *P*-values.

Results

For females, the LCGM analyses with VE revealed three fairly stable trajectories, which differed mainly on average VE-scores. A never vitally exhausted subgroup (VE-scores of 0; N=41), a preclinical VE subgroup (VE-scores 1-13; N=111) and a subgroup with chronic VE values of 14 or higher (N=11). For males a similar result was obtained; a fairly stable, never vitally exhausted subgroup (N=30; VE-scores of 0), a stable preclinical VE subgroup (N=92; VE-scores 1-13) and a subgroup with chronic VE values (N=56; VE-scores of 14 or higher).

The average VE levels for males and females separately at each time point are visualized in **Figure 5**.

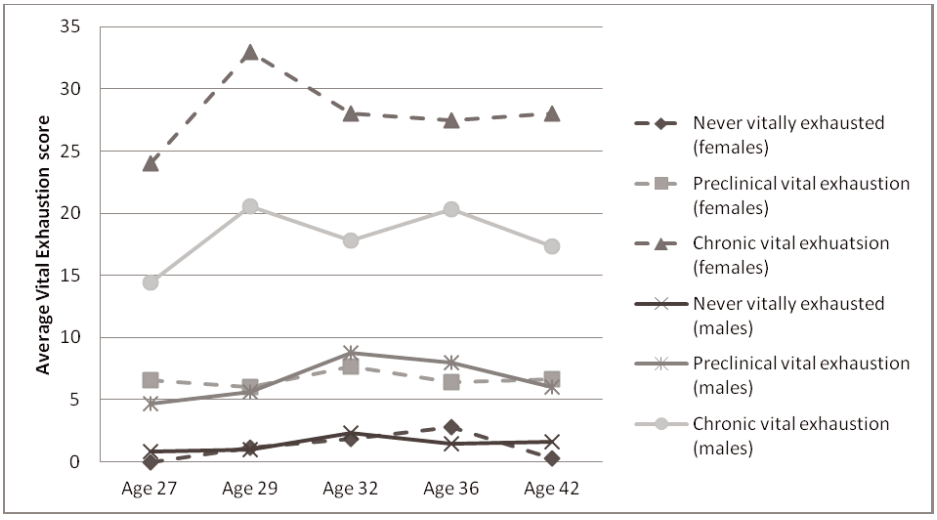

Figure 5 Distinct developmental trajectories of vital exhaustion

Table 12 shows descriptive information of the study sample separated for the gender specific VE-classes. IL-6, CRP and TNF-α were non-normally distributed and are thus presented as medians (IQR). In both analyses and in both males and females, the never vitally exhausted trajectories showed the lowest smoking- and drinking prevalence, whereas the body fat percentages did not differ over the trajectories.

Table 12 Descriptive information of the gender-specific trajectories of vital exhaustion

	Females			Males		
	Never vitally exhausted (N=41)	**Preclinical VE (N=111)**	**Chronic VE (N=11)**	**Never vitally exhausted (N=30)**	**Preclinical VE (N=92)**	**Chronic VE (N=56)**
Interleukin-6 (pg/ml)[1]	2.47 (1.85)	2.27 (1.83)	2.27 (2.69)	2.43 (2.18)	2.53 (1.99)	2.93 (2.69)
Interleukin-8 (pg/ml)[1]	9.33 (3.75)	9.39 (4.17)	9.70 (4.86)	9.26 (3.21)	8.79 (4.36)	8.80 (4.17)
TNF-α (pg/ml)[1]	8.21 (3.18)	8.63 (2.98)	7.41 (3.87)	8.14 (2.64)	8.02 (3.00)	8.50 (2.71)
Percentage body fatness (%)	20.70 (4.53)	20.29 (4.69)	20.52 (4.57)	29.56 (5.59)	29.16 (5.61)	29.96 (6.87)
% smokers at age 42	12.2	19.8	18.2	0.0	16.3	14.3
% previous smokers	34.1	21.6	36.4	23.3	26.1	30.4
% alcohol consumers at age 42	90.2	97.3	90.9	79.3	91.2	89.3

[1] Descriptive information is denoted by median (IQR) due to non-normality

Table 13a and 13b show the results of the linear regression analyses relating class membership to IL-6, IL-8 and TNF-α. Each trajectory was compared to the other two, resulting in three regression coefficients per analysis. In both the crude and adjusted models, no (significant) associations were found between class membership and cytokines and all regression coefficients approached 1.00, indicating that the markers of low-grade inflammation did not differ between the trajectories.

Additional analyses

In order to determine if a continuous assessment of VE provided any further information to our research question, additional regression analyses were performed. For these analyses we calculated the average VE-score between the ages 27 and 42 and related this mean score to the markers of low-grade inflammation using straightforward regression analyses controlling for the same confounders as the previous analyses. This approach was chosen based on the findings of the primary analyses presented above. The classes mainly differed on baseline VE-scores (showing, to a relatively small extent a heterogeneous development) making the splitting in distinct subgroups not necessary. The results of these analyses, again stratified for gender, are visualized in **Table 14** and show similar results; statistically non-significant regression coefficients (which can be interpreted as ratios) approach 1.00.

Discussion

To our knowledge, this study is the first to examine the course of VE in a healthy study population. We identified three distinct trajectories for males and females. For females we found 1) subgroup of individuals who were never vitally exhausted over a period of 15 years, 2) a preclinical VE subgroup and 3) a subgroup in chronic VE state. For males we found fairly similar trajectories. These results show that in a healthy study sample the course of VE is only slightly heterogeneous and stable over time, in part confirming previous studies investigating distinct development of VE [173], depression [186] and anxiety [187, 188] in several *patient* populations. In these populations, the course of depression and anxiety did, however, fluctuate more; in most cases a recovering subgroup could be identified, a subgroup which we were unable to reveal.

As VE is not much investigated longitudinally, in particular in non-patient study populations, an explanation for our findings might lie in the fact that the concept of VE, compared to the concepts of stress, anxiety or depression, is

Table 13a Linear regression models showing associations between class membership and markers of low-grade inflammation for females

	Interleukin-6[1]			Interleukin-8[1]			Tumor Necrosis Factor-α[1]		
Crude model:	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**
Never vitally exhausted versus preclinical	0.99	0.77 to 1.26	0.919	0.99	0.87 to 1.12	0.898	0.99	0.88 to 1.11	0.846
Chronic versus preclinical	0.88	0.57 to 1.33	0.525	0.98	0.79 to 1.21	0.858	0.88	0.72 to 1.08	0.227
Never vitally exhausted versus chronic	1.13	0.72 to 1.78	0.593	1.01	0.80 to 1.27	0.923	1.12	0.90 to 1.39	0.309
Adjusted model[2]:	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	**P-value**
Never vitally exhausted versus preclinical	0.98	0.77 to 1.26	0.897	0.99	0.87 to 1.11	0.854	0.99	0.89 to 1.12	0.917
Chronic versus preclinical	0.87	0.57 to 1.34	0.533	0.98	0.79 to 1.21	0.818	0.90	0.74 to 1.09	0.280
Never vitally exhausted versus chronic	1.12	0.71 to 1.77	0.610	1.01	0.80 to 1.27	0.909	1.11	0.90 to 1.37	0.342

[1] Log transformed due to violation of Normality assumption - regression coefficients interpreted as ratios instead of differences

[2] Adjusted for body fat percentage, smoking behavior and alcohol intake

Table 13b Linear regression models showing associations between class membership and markers of low-grade inflammation for males

	Interleukin-6[1]			Interleukin-8[1]			Tumor Necrosis Factor-α[1]		
Crude model:	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**
Never vitally exhausted versus preclinical	1.03	0.76 to 1.40	0.850	1.01	0.87 to 1.18	0.883	0.97	0.85 to 1.10	0.591
Chronic versus preclinical	1.16	0.91 to 1.49	0.222	0.97	0.86 to 1.10	0.634	1.04	0.94 to 1.15	0.432
Never vitally exhausted versus chronic	0.89	0.64 to 1.23	0.459	1.04	0.89 to 1.22	0.621	0.93	0.81 to 1.06	0.275
Adjusted model[2]:	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**	**b**	**95% CI**	***P*-value**
Never vitally exhausted versus preclinical	1.00	0.73 to 1.37	0.980	1.00	0.86 to 1.16	0.967	0.96	0.84 to 1.09	0.499
Chronic versus preclinical	1.15	0.90 to 1.47	0.271	0.96	0.85 to 1.08	0.451	1.03	0.93 to 1.14	0.598
Never vitally exhausted versus chronic	0.87	0.63 to 1.22	0.424	1.04	0.89 to 1.23	0.600	0.93	0.81 to 1.07	0.305

[1] Log transformed due to violation of Normality assumption - regression coefficients can interpreted as ratios instead of differences
[2] Adjusted for body fat percentage, smoking behavior and alcohol intake

Table 14 Linear regression models showing associations between average VE-scores and markers of low-grade inflammation

	Interleukin-6[1]			Interleukin-8[1]			Tumor Necrosis Factor-α[1]		
	Females								
Crude model:	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**
	1.04	0.97 to 1.12	0.293	0.99	0.96 to 1.03	0.605	1.00	0.97 to 1.03	0.989
Adjusted model[2]:	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**
	1.04	0.96 to 1.11	0.345	1.00	0.96 to 1.03	0.797	1.00	0.97 to 1.03	0.963
	Males								
Crude model:	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**
	0.97	0.91 to 1.05	0.514	1.00	0.96 to 1.03	0.721	0.97	0.94 to 1.01	0.111
Adjusted model[2]:	**b[3]**	**95% CI**	**P-value**	**b[3]**	**95% CI**	***P*-value**	**b[3]**	**95% CI**	***P*-value**
	0.98	0.91 to 1.06	0.573	1.00	0.96 to 1.03	0.699	0.97	0.94 to 1.01	0.187

[1] Log transformed due to violation of Normality assumption - regression coefficients can interpreted as ratios instead of differences
[2] Adjusted for body fat percentage, smoking behavior and alcohol intake
[3] Regression coefficients are calculated for a difference of 5 points on the VE-questionnaire

still a point of discussion. Although the constructs show a considerable degree of overlap [168], clear signs of depression, anxiety or stress are often absent in vitally exhausted individuals [165]. Furthermore, a recent study revealed that, VE as a concept, is heterogeneous in its symptom presentation in cardiac patients [46]. In that study three distinct symptom profiles were found; a subgroup without symptoms of VE and two subgroups with symptoms: one with major signs of fatigue and one with major signs of lack of concentration. The three profiles showed a difference in cardiovascular disease risk. When comparing the endorsed questions of the preclinical VE-subgroups in our study with the chronically VE-subgroups, we noticed the distribution of the endorsed questions was very different for the two subgroups showing (signs of) VE. Of course, by definition (see methods section), the number of endorsed questions was higher for the chronically VE-subgroups, but also the type of questions endorsed differed between the two subgroups. These findings could further suggest that negative health consequences such as elevated CVD-risk proposed to coincide with a vitally exhausted state is (partly) dependent on the *type* of questions endorsed also, besides on the *number* questions endorsed and this particularly is applicable for healthy individuals. Further psychometric studies are therefore warranted for continued examination of the factor structure of VE, including studying the heterogeneous symptom presentation.

Janszky et al. [171] showed that vitally exhausted subjects *cross-sectionally* have higher levels of markers of low-grade inflammation. We did not find elevated levels of these markers in the subgroups showing signs of VE over time, indicating potentially no increased CVD-risk. From the stress and depression literature in particular, it is seen that the adverse cardiovascular consequences are greater in patients with major depression compared to patients with only mild symptoms [189] and similar findings are present in the cortisol-stress literature [190]. Numerous studies show significant health consequences of severe mental health symptom presentation [191–193], but less studies show such strong consequences when only mild symptoms are reported, comparable to those in our study [171, 175]. These findings indicate the possibility of a threshold effect; our participants were relatively young compared to the participants in other studies and the median VE-score at the most recent round of measurement was 21.00 points, where a score of 14-46 indicates VE. Our findings could also point to the existence of differential mechanisms for VE versus depression or stress in healthy adults [169], although it has been shown that there is some degree of overlap between the two constructs, as well as in

the hypothesized pathways that link VE, depression and stress to CVD [158, 168].

In the current study we were unable to confirm existing theories about the interrelationships between markers of low-grade inflammation and VE (or other measures of mental health). Our non-significant findings might be (partly) explained by the relatively small sample sizes, a result of splitting the study sample into three distinct classes. We performed several additional analyses on the complete sample to investigate the possible sample size issues. The results, now conducted with larger sample sizes, show a similar picture; statistically non-significant regression coefficients also approach 1.00.

From previous studies researching similar relationships, effect sizes were expected to be small to moderate, as many factors other than mental health status have shown to influence levels of markers of low-grade inflammation to some degree also (e.g. visceral fat being one of the strongest predictors [107]). From previous studies researching for example depression in relation to Interleukin-6 levels in community-based study samples, differences in markers of low-grade inflammation between patients with and without depression were found to be 0.25 mg/L [194]. In another study [195], however, levels of IL-6 differed only by 0.18 mg/L when comparing patients with- and without posttraumatic stress disorder. Although community-based, these studies still compared fairly extreme subgroups with each other making our small effect sizes not very unexpected. However, the importance of markers of low-grade inflammation for the refinement of cardiovascular disease risk continues to be acknowledged in the literature [107, 108], and research in community-based samples, where extremes are relatively uncommon is crucial to better understand the underlying mechanisms.

Strengths and limitations

Vital exhaustion is a relatively understudied construct, especially in non-patient populations. We used VE-scores measured at five measurement occasions over a period of 15 years to be able to analyze the natural course of VE, more specifically, heterogeneity in this course. To our knowledge, we are the first to analyze VE over such a long follow-up period. Moreover, LCGM are contemporary statistical techniques useful to unravel heterogeneity in the course of VE. LCGM categorize individuals based on their development of VE over time, instead of on an a-priori classification in predefined subgroups [6, 18]. These techniques have shown their merit in particular in the fields of

psychology and criminology and their potential for more clinically oriented research is highlighted more and more [29]. Applying the techniques within mental health research and beyond provides researchers with new insights for future research, in particular due to their data-driven nature.
A limitation concerns our sample size. Based on previous research [175], we hypothesized gender to be an effect modifier. Therefore, we had to stratify our analyses by gender, creating two samples of just under 200 participants. Although these sample sizes are relatively small, they do meet the minimum requirements for conducting analyses based on structural equation modelling [30, 31]. Moreover, there were no indications that our relatively small sample sizes negatively influenced our results; although issues with statistical power are possible, our regression coefficients generally approached 1.00, indicating no to small relationships between VE and the markers of low-grade inflammation anyway. Moreover, as described in the methods section, the AGAHLS is fairly homogeneous regarding socio-economic status. The participants are generally highly educated [8] and therefore not really representative for the whole Dutch population. Moreover, the findings in this study might not be generalisable to other socioeconomic classes for this reason.

Conclusion
It appears as though the current study is one of the first studies to focus on VE and low-grade inflammation as risk factors for elevated CVD-risk *longitudinally* in a non-patient study population. Studying this relationship in a longitudinal setting makes it possible to draw conclusions about the individual development of VE in relation to CVD risk. We showed the development of VE to be fairly stable over time, although three distinct courses were identified for both females and males. Current hypotheses about the interrelations between VE, inflammation and CVD that have been studied in predominantly cardiac patients did not seem to hold in our non-patient sample. Therefore, further research studying the presentation of VE as well as other measures of mental health such as stress and depression and subsequent consequences for CVD is needed in non-patient study populations.

Acknowledgements
This work was partly supported by the VU University in the form of a grant awarded by the EMGO$^+$ Institute for Health and Care Research (TH).