Roles of allostatic load, lifestyle and clinical risk factors in mediating the association between education and coronary heart disease risk in Europe.

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ABSTRACT

Background: Previous studies have shown that differential exposure to lifestyle factors may mediate the association between education and coronary heart diseases (CHD). However, few studies have examined the potential roles of allostatic load (AL) or differential susceptibility.

Methods: 25,310 men and 26,018 women aged 35-74 and CHD-free at baseline were identified from 21 European cohorts and followed for a median of 10 years, to investigate the mediating role of AL, as well as of smoking, alcohol use and body mass index (BMI), on educational differences in CHD incidence, applying marginal structural models and three-way decomposition.

Results: AL is a mediator of the association between educational status and CHD incidence, with the highest proportion mediated observed among women and largely attributable to differential exposure, (28% [95%CI 19% to 44%]), with 8% [95%CI 0% to 16%] attributable to differential susceptibility. The mediating effects of smoking, alcohol and BMI, compared to AL, were relatively small for both men and women.

Conclusion : Overall, the educational inequalities in CHD incidence were partially mediated through differential exposure to AL. By contrast, the mediation of the educational gradient in CHD by investigated lifestyle risk factors was limited. As differential susceptibility in men was found to have a predominant role in accumulation of AL in low educational classes, the investigation of AL-related risk factors is warranted.

What is already known on this subject?

Differential exposure to behavioural and biological risk factors accounts for a limited proportion of social inequalities in cardiovascular diseases

Allostatic load**,** a measure of the physiological "cost" resulting from chronic stress, has been associated with socioeconomic gradients in coronary heart disease but no studies have investigated the potential role of susceptibility to allostatic load.

We applied modern counterfactual mediation methods to examine the role of differential exposure and differential susceptibility to allostatic load, and other lifestyle factors on educational gradients in coronary heart disease in Europe.

What this study adds?

Allostatic load contributes to the educational inequalities observed in coronary heart disease incidence in Europe.

Overall, in men and women, allostatic load mediated inequalities in coronary heart disease were largely driven by differential exposure (16% and 28%, respectively) while effects of differential susceptibility were modest (3% and 8%, respectively).

There was limited evidence of differential susceptibility to other mediators (smoking, body mass index and alcohol use).

INTRODUCTION

The degree to which social, biological and behavioural risk factors explain socioeconomic inequalities in cardiovascular disease (CVD) remains uncertain. Two (non-mutually exclusive) mechanisms are hypothesized to play a role [1]. Firstly, risk factors for disease are unequally distributed across socioeconomic groups (*differential exposure*), accounting for approximately half of the inequalities observed, with large heterogeneity across populations [2,3]. Second, *differential susceptibility* posits that effects of risk factors on CVD may differ across socioeconomic groups [1].

One early study aiming to distinguish between *differential exposure* and susceptibility, reported an increased susceptibility of manual workers to job strain and myocardial infarction, however these were not investigated simultaneously [4]. Others have explored these mechanisms simultaneously for observed socio-economic status (SES) inequalities in CVD, reporting mixed findings, likely reflecting variable methodologies and heterogeneity across populations. Two studies by Nordahl *et al* have applied three-way decomposition models one of which found a substantial contribution of smoking to educational gradients in CVD mortality [5,6]. Hussein *et al*, using Oaxaca Blinder Decomposition, concluded *differential exposure* to a number of factors accounted for most of the inequality in CVD incidence, but *differential susceptibility* to neighbourhood socioeconomic conditions was observed [7].

However, there remains a lack of insight on possible causal pathways invoked by *differential susceptibility*. Furthermore, analyses have not investigated the potential role of susceptibility to allostatic load (AL), a measure of the physiological "cost" resulting from exposure to chronic stress [8]. AL has been associated with coronary heart disease (CHD) [9] and is proposed as a candidate pathway linking low education to coronary heart disease through a *differential susceptibility* mechanism [1,10,11]. Specifically, it has been proposed that the relationship between socioeconomic status (SES) and AL accumulation may be

mediated by psychosocial and behavioural risk factors and in turn AL may act as a mediator between SES and CHD incidence.[12,13] Thus, *differential susceptibility* may contribute to a greater accumulation of AL in individuals with lower education and a disproportionate effect of AL on CHD incidence. Indeed, we recently disentangled the contribution of lifestyle factors to the educational class gradient in AL in terms of *differential exposure and susceptibility* [14]. Following on from this work, based on the same 21 European population-based cohorts, we aim to examine this second pathway, disentangling the contribution of *differential exposure* and *susceptibility* to AL and other mediators in CHD educational gradients applying modern counterfactual mediation models.

METHODS

Study Population

We utilized data from the BiomarCaRE project which includes population-based cohort studies harmonized in the MORGAM (MONICA Risk Genetics Archiving and Monograph) Project with stored serum/plasma [15,16]. 21 European cohorts (from 6 countries as outlined in **Supplementary Table S1**) with harmonised data on education, lifestyle factors and markers required for the allostatic risk score, were included. Individuals aged 35-74 years (Belfast [men aged 49 to 60] and Brianza and Catalonia [individuals aged up to 66 and 67 at baseline, respectively]) with no history of acute coronary events or stroke cardiovascular disease (CVD) at baseline and with complete data for calculation of the AL score were included. All participating studies received approval by local ethics review boards.

Educational Level

The number of years of schooling was collected at baseline and education levels were classified into three categories (high, intermediate and low) as population-, sex- and birth cohort-specific thirds of years of schooling [17].

Allostatic Load

AL comprises primary measures (stress hormones), intermediary outcomes (response of metabolic, cardiovascular and inflammation systems to stress), resulting in allostatic overload. Finally, chronic stress dysregulation, leads to the manifestation of disease (e.g. hypertension, diabetes and obesity) [18,19]. AL score was computed using eight selected biomarkers corresponding to three different physiological systems: inflammation (C reactive protein; CRP), metabolism (high-density lipoprotein [HDL] cholesterol, total cholesterol,

triglycerides, blood glucose [HbA1C in MONICA/KORA Augsburg] and body mass index [BMI]); cardiovascular (systolic blood pressure and diastolic blood pressure). Measurement details for biomarkers are outlined in **Supplementary Table S2**. Markers of the neuroendocrine system (e.g. epinephrine and cortisol) were not available. AL score was calculated based on the sum of an individual's Z-score for each of the 8 individual biomarker components. Before standardization, markers with a skewed distribution were log-transformed. As HDL cholesterol is inversely associated with CHD, this was inverted. The Z-scores were derived from population-, sex- and fasting status-specific mean and standard deviation values.

Secondary analyses calculated AL sub-scores for the three individual physiological systems by summing Z-scores for the relevant markers only.

Other Covariates

Cigarette smoking, ascertained by interview or self-reported questionnaire, was categorised as a 5-class variable as non-smokers (never and former smokers) and current smokers, which were categorised into three levels of cigarettes/day (≤10, 11–20, >20). Daily alcohol intake (in grams) was converted to average drinks per day, with 12.5g of alcohol considered a standard drink [20]. Alcohol use was categorised as abstainers (0 drinks per day), 1–2, 3–4 and ≥5 drinks per day (0, 1-2, ≥3 drinks per day for women). Body mass index (BMI) was categorised as normal (<24.9kg/m^2), overweight (25-29.9kg/m^2) and obese (>30kg/m^2).

Outcome

Hospitalizations for coronary heart disease and death were identified through linkage to national or regional death registries, hospital discharge records, population-based

registries, or via participants. Most centres used standard epidemiological criteria to define coronary events, while some relied on routine cause of death diagnoses and hospital discharge diagnoses. There was variation in codes used depending on local ICD-coding practices (ICD-9/10). The main endpoint for this study was the occurrence of CHD (first fatal and non-fatal myocardial infarction, unstable angina pectoris, coronary death or unclassifiable death).

Statistical Analyses

59,065 participants had valid data on education and were free of previous CVD at recruitment. 8,737 were excluded because of missing data for AL score markers; no follow-up information; or missing data on lifestyle factors. 51,328 participants (87% of the original sample) were included, with no substantial differences in this percentage across educational classes (data not shown). Cohorts are described in **Supplementary Table S1.**

To assess the mediatory role of AL score and behavioural factors (smoking, alcohol and BMI) on educational class differences in CHD incidence through *differential exposure* and *differential susceptibility* (accounting for measured confounding of age, sex and study population) we applied 3-way decomposition. This method decomposes the difference in CHD outcome between two educational classes (i.e. the "total effect" of education) into the sum of three components: the pure direct effect (PDE), the pure indirect effect (PIE), and the mediated interaction (MI) [21]. To estimate these three components, we used marginal structural models by fitting sex-specific additive hazards regression models with age as the time scale [22,23]. The additive hazards model allows for the estimation of the "total effect" as the additional number of CHD events (per 100,000 person-years) in individuals in one education class as compared to the reference group (high education). The estimated average PDE can be interpreted as the additional number of CHD events in the low *vs.* high

educational class that is not mediated by the mediator; the estimated average PIE is the additional number of CHD events due to the different distribution of the mediator (indicating *differential exposure*); and the estimated average MI is the additional number of CHD events in the low class due to the interaction between education and the mediator on the outcome (*differential susceptibility*). The sum of the two components is the total proportion of inequalities mediated by the mediator [5]. Marginal equation models are described in detail in **Supplementary Methods**. We report coefficients (**Supplementary Tables S3-S4**) and weights distribution (**Figures S1-S4**) for the underlying propensity score models, to document the positivity assumption; as well as the cumulative CHD event rates at fixed attained ages during follow-up, as a measure of goodness of fit for the additive hazards models (**Supplementary Table S5**).

Analyses were repeated i) by study population; ii) for AL sub-scores; iii) stratifying participants in the pre-dysregulation and dysregulation phases (including elevated blood pressure [>140/90mmHg], type 2 diabetes or obesity). Finally, analyses were conducted to examine the conditional exchangeability assumption, investigating the potential impact of unmeasured confounding of the exposure-mediator relationship on DE and DS estimates (outlined in **Supplementary Methods)**. Analyses were conducted using R and SAS version 9.4 (SAS institute).

RESULTS

3,031 participants were diagnosed with coronary heart disease (CHD), with a greater proportion in the low educational group (**Table 1**). AL score was lowest among those with high educational status. Those with low education were more likely to be current smokers and smokers in the high education category had a lower smoking intensity. The proportion of alcohol abstainers was highest among low education groups, however males in the low education group had higher daily alcohol intake. Obesity, elevated blood pressure and diabetes, was highest among those in the low educational class.

CHD incidence was greater among both men and women in the low education group compared to the high education group (**Table 2**). Overall, AL mediated educational inequalities observed in CHD, with the highest overall proportion mediated observed for AL among women (36%). Of the 142 additional CHD events per 100,000 person years in women, 28% (95%CI 19% to 44%) were attributable to the pure indirect effect (DE) and 8% (95%CI 0% to 16%) to the mediated interaction (*differential susceptibility*). In men the overall proportion mediated by AL was 19% {pure indirect effect (*differential exposure*) 16% (95%CI 11% to 23%); with mediated interaction (*differential susceptibility*) of 3% (95%CI 0% to 6%)}.

The mediating effect of smoking was stronger among men than women, accounting for the highest overall proportion of observed mediation in men (**Table 2**). The total effect, comparing low to high education, was 250 CHD events per 100,000 person-years, of which 28% was mediated via smoking (pure indirect effect= 23% (95%CI 16% to 34%); mediated interaction=5% (95%CI -1% to 10%). In contrast, alcohol use and BMI did not mediate the association between educational class and CHD incidence as strongly as smoking, with no

evidence of a *differential susceptibility* effect. However, the effect of *differential exposure* to alcohol and BMI was larger in women than in men.

The contributions of the AL sub-scores to the educational gradient in CHD are reported in **Table 3.** Inflammation contributes, though modestly, to the educational gradient in CHD both in terms of *differential exposure*, (men 12% [95%CI 9% to 18%]; women 11% [95%CI 7% to 18%]) and *differential susceptibility* (men 5% [95%CI 2% to 8%]; women 9% [95%CI 4% to 14%]). The contribution via the metabolic system was largely attributable to *differential exposure*, with a stronger effect in women. Cardiovascular system markers contributed minimally. Results stratifying by the pre-dysregulation and dysregulation stage were largely similar to the main analyses across groups (**Table 4**). Analyses stratifying by age revealed evidence of *differential susceptibility* to AL among those age 35-60 years (men 5% 95%CI 2% to 9%; women 15% 95%CI 5% to 24%), but not for those aged 60-85 years (**Supplementary Table 6**).

Figure 1 depicts additional CHD events by educational level by gender in each cohort. While largely consistent across sites, a number of negatively mediated interactions were observed for men in Italy-Latina and Germany-MONICA/KORA Augsburg and women in Italy-Brianza and Northern Sweden.

Figures S5 and S6 show that the main findings were generally robust to an unmeasured confounder if it had an effect of comparable magnitude to age or neighbourhood deprivation.

DISCUSSION

In this study we found evidence that AL contributes to the educational inequalities in coronary heart disease incidence. We observed *differential exposure* and *differential susceptibility* to the effects of AL on CHD risk, however the majority of the mediating effects of AL (and other behavioural factors) were through *differential exposure*. *Differential susceptibility* effects were modest, the main pathway for the latter appearing to be through susceptibility to inflammation.

It is fairly well-accepted that education is *causally* associated with cardiovascular outcomes and all-cause mortality [24,25]. For example a recent Mendelian Randomisation (MR) study found that 3.6 years of additional education reduced the "predisposition" to CVD by about one third [24], however, the mechanisms are largely unknown [24]. While a 1-SD longer education was also associated with a 35% lower odds of smoking and 0.17 kg/m^2 lower BMI, we know that polygenes predict only a small proportion of the population variance in such behavioural traits. Indeed, Kaufman calls for caution of MR methods predominance when we know little about the mechanisms by which risk factors trigger disease [26].

The notion of di*fferential susceptibility* aligns with the sufficient-component-cause model [27] and thus may be understood as conditional, or a feature of causal interaction. The possibility that SES inequalities are generated by *differential exposure* has been aligned with mediation, insofar as we pose the counterfactual question of what CVD outcomes would be in the low SES group, had they the same risk factor distribution as the high SES group [1]. The extent to which observed SES differences are driven by *differential exposure* or *susceptibility* is important, as optimum policy responses should vary according to the balance of the two mechanisms.

Previous studies largely used regression methods to investigate the mediating role of behavioural factors in the association between education and CVD [28–31]. Few have aimed to disentangle the contribution of behavioural factors, in terms of *differential exposure* and *susceptibility*, reporting mixed findings, possibly reflecting differing methodologies. Hussein *et al*, applying Oaxaca Blinder Decomposition, observed inequality in CVD incidence between high and low SES was largely attributable to *differential exposure* to diabetes, hypertension, social environment and neighbourhood socioeconomic conditions [7]. Contributions via *differential susceptibility* for smoking and alcohol, were negligible. Nordahl *et al*, adopting comparable methods to ours, found educational inequalities in CVD mortality were mediated considerably through behavioural factors, notably smoking (26% for men, 34% for women), with a significant effect via *differential susceptibility*, particularly for women (20%) [5]. In our study of CHD incidence, the effect mediated by smoking was similar for men but smaller for women (28% and 11%, respectively) and the proportion mediated via *differential susceptibility* was small (5%). However Nordahl *et al* investigated cardiovascular mortality, focused on older people and disparate findings may reflect differences in the effect of smoking on educational inequalities in CVD across European populations [32].

No previous study has investigated the contribution of *differential exposure* and *susceptibility* to AL in educational inequalities in CHD. AL was a mediator of the educational gradient in CHD, with mediation largely via *differential exposure*. In the same populations, we previously found that the educational gradient in AL in men was largely attributable to *differential susceptibility* to behavioural factors [14]. In particular, being a never smoker or having moderate alcohol intake was less protective in terms of AL accumulation in less educated men compared to their more educated counterparts. In women, the educational gradient in AL remained largely un-explained by both *differential exposure* and *susceptibility*

to the same behavioural factors. Taken together, our two companion analyses suggest the need to identify other factors causally linked to differential AL accumulation in lower social classes.

We observed some *differential susceptibility* to the effects of AL on CHD incidence, the main pathway for which was via inflammation. The importance of inflammation in CHD has been reported [33]. Contrastingly, evidence suggests that cortisol, which modulates inflammation, may be associated with CHD [34]. Unfortunately markers of the hypothalamic-pituitary-adrenal axis were unavailable in this study. However, it is *differential exposure* rather than *susceptibility* to AL that explains more of its observed mediation of the educational inequalities in CHD incidence. So if there is a policy implication, it may be that even if certain groups are more vulnerable to the accumulation of AL, in all likelihood we must act early in the life course to mitigate this and Marmot's appeal to proportionate universalism does justice to the joint mechanisms revealed by our analysis [35].

Despite this, we must also be mindful that the contribution of 'traditional risk factors' may vary across populations and may not explain all of the disparities observed [36]. It is possible that in these middle-aged cohorts it is already too late to mitigate the effects of *differential susceptibility* to AL, and the sensible policy response is to focus on minimising exposure – such as taxation, regulations and upstream targeting of the generative factors affecting other harmful behaviours. This coheres with evidence for intergenerational early life transmission of health behaviours and with accumulating evidence for the epigenetic embedding of early life stressors and their effects on adult disease risk [37,38]. It is worthwhile noting that we did not control for certain factors that might moderate the consequences of AL such as marital stability and spousal education level, which can affect the consequence of stressors in adult life [39]. Finally it is salutatory to be reminded in a theoretical exposition by VanderWeele, built upon a sufficient cause framework, that while

statistical mediation implies mechanism, mechanisms may exist which are not tractable by current methods of statistical mediation [20].

Strengths and limitations

This study included a large sample size, from 21 cohorts with long follow-up periods, providing adequate power to conduct mediation analyses, investigating both differential exposure and susceptibility.

However, we considered a number of mediators individually (not adjusting for other mediators) and the assumption that the mediators do not affect each other may be difficult to justify. It is likely they may be related, through direct pathways or common causes, affecting both occurrence and the effect of other mediators (*differential exposure* and *susceptibility*) (**Figure 2).** Methods are only now being developed for multivariate and high dimensional cases [40]. In particular, we recognise the possibility that AL accumulation itself may influence other lifestyle factors such as smoking. However, in this study the median age of smoking initiation was 18 years and 17 years in women and men, respectively (data not shown), while AL accumulation was captured at baseline. Furthermore, a previous study found no evidence of a correlation between AL at age 9 and smoking at age 17, while smoking was a mediator for AL accumulation at age 17.[41] Indeed, the estimation of direct and indirect effects requires strong assumptions, namely, no unmeasured confounding of the exposure-outcome, mediator-outcome and exposure-mediator relations, and no mediator-outcome confounder affected by exposure. While we could adjust for age and study population, there may be residual confounding due to unmeasured confounders or imprecisely measured covariates. Reassuringly, sensitivity analyses investigating unmeasured confounding revealed that an unmeasured confounder would need a strong correlation (stronger than age and neighbourhood deprivation) with both education and AL to affect our

observed estimates (**Figures S5 & S6**). Cholesterol and blood pressure were included in our AL score. However these may not only influence CHD via stress accumulation but also via other pathways. In addition, we were unable to include biomarkers from the hypothalamic–pituitary–adrenal axis or immune system in our AL as these were not captured consistently across all populations. It is of note that when compared to MR approaches, regression based analyses of mediation somewhat underestimate the proportion mediated, reflecting the latter approach being more susceptible to measurement error [20]. When measurement error of a mediator is differential across exposure levels, the interaction (*differential susceptibility*) is considered liable to underestimation [42].

Finally, AL and other mediators were measured at baseline, and for some cohorts this was many years ago (1980's-1990's), thus the distribution of exposures has likely changed over time, particularly in the well educated. As we did not capture changes across time this could lead to an underestimation of the mediated effect [43]. Future studies, including repeated measures over longer follow-up periods, are warranted.

Conclusion

In this prospective cohort study, we found evidence for the effect of *differential exposure* to AL on CHD incidence. As *differential susceptibility* in men was found in our companion paper to have a predominant role in the accumulation of AL in low educational classes [14], further investigation of AL-related risk factors is needed. Meanwhile, any preventive action aiming to control factors linked to the disproportionate exposure to excess AL may help to reduce CHD morbidity, in particular among those with lower education.

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Ethical approval: All participating studies adhered to the Declaration of Helsinki and are responsible for ethical approval and patient consent, according to local rules at the time of study enrolment. For different study populations, the list of approvals is the following: (study name, Ethics Committee name, approval ID):

- Northern Sweden, Research ethic Committee of Umea University, 2012-280-32M;
- FINRISK 1997, Ethics Committee at National Public Health Institute of Finland, 38/96;
- PRIME-Northern Ireland, Office for Research Ethics Committees Northern Ireland, 06/NIR02/107;
- MONICA/KORA Augsburg, Ethik-Kommission Bayerische Landesärztekammer, 05004;
- MONICA-Brianza, Comitato Etico Azienda Ospedaliera San Gerardo - Monza, 192/2005;
- MATISS Study (Latina), Comitato Etico Istituto Superiore di Sanità, PRE/96/06;
- Moli-Sani study, Comitato Etico Università Cattolica del Sacro Cuore – Roma, Prot.Pdc.P99 (A. 931/03-138-04)/CE/2004.
- MONICA-Catalonia: Director/Board of the Institute of Health Studies, ID not assigned.

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Authors' contributions: GV, FK, MMF and BH conceived the research question. BH, FK, GV drafted the manuscript along with MMF and HF. GV conducted the statistical analyses. KK, TZ and SB are guarantor of the MORGAM/BiomarCaRE database. TPH, SuS, VS, TB, FK, MMF, BT, StS, CG, IL, PL are the principal investigators of the cohorts included in the current analyses. WM, DF, KK, TPH, SuS, VS, BT, AP, TB, PA, StS, CG, IL, PL, BM, TZ and SB actively contributed to the interpretation of the results and made critical revision of the manuscript.

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FIGURE LEGENDS

Figure 1. Additional coronary heart disease events per 100,000 person-years due to the mediated interaction of allostatic load, by educational level in men and women by study population and overall estimate.

Figure 2 Directed Acyclic Graph describing the intertwined pathways of education on CHD through allostatic load, smoking and alcohol use.

Table 1. Distribution of Allostatic Load Scores, behavioral and anamnestic CVD risk factors and follow-up endpoints across the educational classes in men and women 35-74 years old, free of cardiovascular disease at baseline

Characteristic [a]	**Men**				**Women**			
	Educational class				**Educational class**			
	Low (N=9415)	**Intermediate (N=6266)**	**High (N=9629)**	**p**	**Low (N=9849)**	**Intermediate (N=6556)**	**High (N=9613)**	**p**
Age, years (SD)	52.4 (9.6)	53.1 (9.2)	51.8 (9.3)	<.0001	51.8 (9.6)	52.7 (9.7)	51.3 (9.7)	<.0001
Allostatic Load Score	0.23	0.11	-0.39	<.0001	0.70	0.06	-0.69	<.0001
Smoking status, %								
Never smokers	32.0	33.8	41.6	<.0001	59.6	62.1	59.5	<.0001
Former smokers	34.8	37.0	36.9		14.1	17.7	21.9	
1-10 cigarettes/day	7.1	7.3	6.3		9.7	9.5	9.7	
11-20 cigarettes/day	17.7	14.7	10.8		13.9	9.3	7.9	
>20 cigarettes/day	8.4	7.2	4.5		2.7	1.4	1.0	
Alcohol intake, drinks/day (%)								
0 (Abstainers)	20.2	18.7	17.7	<.0001	49.2	43.8	39.2	<.0001
1-2 drinks/day	42.9	46.1	52.9		46.0	51.1	55.3	
3-4 drinks/day	30.1	29.8	26.9		4.7	5.0	5.5	
5 or more drinks/day	6.7	5.4	2.6		0.1	0.1	0.1	
Body Mass Index, Kg/m^2 (%)[b]								
Normal weight	28.5	28.4	30.8	<.0001	33.7	37.2	46.2	<.0001
Overweight	49.3	50.6	51.6		37.7	37.5	34.9	
Obese	22.2	21.0	17.6		28.6	25.3	18.9	
Elevated blood pressure[c]**, %**	49.2	48.3	47.7	0.14	44.8	43.9	37.7	<.0001
History of diabetes, %	4.6	4.1	3.8	0.03	3.9	3.6	2.9	0.002
Outcomes, n								
Coronary heart disease	978	532	562	-	522	214	223	-

[a] age-adjusted mean or proportion estimated at age 52
[b] normal weight: BMI was considered 18.5-25 Kg/m2; overweight, BMI 25-30 Kg/m2; obese, BMI >= 30 Kg/m^2
[c] elevated blood pressure was considered >140/90mmHg

Table 2. Rate Difference in additional coronary heart disease events per 100,000 person-years by educational Level (decomposition of Total Effect into direct, Indirect and Mediated Interaction effects) in men and women.

Mediator [a]	Men			Women		
	Educational Level [b]		Proportion mediated in low education (95%CI)	Educational Level [b]		Proportion mediated in low education (95%CI)
	Low	Intermediate		Low	Intermediate	
	RD (95% CI)[c]	RD (95% CI)[c]		RD (95% CI)[c]	RD (95% CI)[c]	
Allostatic Load Score						
Total Effect	246 (173 to 319)	131 (56 to 206)		157 (110 to 204)	64 (14 to 114)	
Pure Direct Effect (PDE)	201 (128 to 273)	100 (26 to 174)		100 (55 to 146)	40 (-10 to 90)	
Pure Indirect Effect (PIE)	39 (33 to 44)	35 (31 to 40)	16 (11 to 23)	44 (35 to 54)	27 (23 to 32)	28 (19 to 44)
Mediated Interaction (MI)	7 (0 to 14)	-4 (-9 to 0)	3 (0 to 6)	12 (0 to 25)	-3 (-7 to 1)	8 (0 to 16)
Smoking [d]						
Total Effect	250 (176 to 325)	134 (59 to 209)		158 (112 to 204)	65 (15 to 114)	
Pure Direct Effect (PDE)	181 (110 to 253)	92 (17 to 166)		133 (88 to 177)	58 (8 to 107)	
Pure Indirect Effect (PIE)	58 (47 to 68)	46 (39 to 52)	23 (16 to 34)	17 (12 to 23)	9 (6 to 12)	11 (7 to 17)
Mediated Interaction (MI)	12 (-2 to 25)	-3 (-10 to 4)	5 (-1 to 10)	8 (-1 to 17)	-2 (-5 to 0)	5 (-1 to 11)
Alcohol[e]						
Total Effect	240 (167 to 313)	126 (52 to 200)		156 (110 to 202)	63 (14 to 113)	
Pure Direct Effect (PDE)	241 (167 to 314)	127 (51 to 202)		144 (98 to 190)	59 (9 to 108)	
Pure Indirect Effect (PIE)	6 (-5 to 18)	-1 (-7 to 5)	3 (-2 to 8)	11 (6 to 16)	5 (3 to 7)	7 (4 to 12)
Mediated Interaction (MI)	-7 (-21 to 7)	0 (-6 to 5)	-3 (-10 to 3)	1 (-7 to 9)	0 (-2 to 2)	1 (-5 to 6)
Body Mass Index						
Total Effect	246 (173 to 319)	131 (57 to 205)		152 (106 to 198)	60 (10 to 109)	
Pure Direct Effect (PDE)	235 (162 to 308)	121 (47 to 195)		135 (89 to 181)	51 (1 to 100)	
Pure Indirect Effect (PIE)	13 (7 to 18)	9 (5 to 14)	5 (3 to 9)	17 (9 to 25)	9 (5 to 14)	11 (6 to 19)
Mediated Interaction (MI)	-1 (-9 to 7)	1 (-4 to 5)	0 (-4 to 3)	0 (-12 to 12)	-1 (-4 to 3)	0 (-9 to 8)

[a] Analysis adjusted for age and population

[b] Reference category was considered high education

[c] RD: Risk difference. This was estimated from the Additive Hazard survival model, with age on the time scale and adjusting for population.

[d] In men smoking categorized as; never smoker, 1-10 cigs/day, 11-20 cigs/day, >20 cigs/day. For women smoking categories included; never smokers,1-10 cigs///day, ≥1 cigs/day.

[e] In men alcohol use categorized as; teetotalers, 1-2 drinks/day, 3-4 drinks/day, ≥5 drinks/day. For women alcohol categories included teetotalers, 1-2 drinks/day, ≥3 drinks/day.

Table 3. Rate Difference in additional coronary heart disease events per 100,000 person-years by educational Level (decomposition of TE into direct, Indirect and Mediated Interaction effects) for allostatic sub-scores as mediators in men and women

Allostatic load sub-score[a]	**Men**			**Women**		
	Educational Level [b]		**Proportion mediated in low education (95%CI)**	**Educational Level** [b]		**Proportion mediated in low education (95%CI)**
	Low	**Intermediate**		**Low**	**Intermediate**	
	RD (95% CI)[c]	**RD (95% CI)**[c]		**RD (95% CI)**[c]	**RD (95% CI)**[c]	
Cardiovascular system						
Total Effect	245 (173 to 318)	130 (56 to 204)		158 (112 to 204)	64 (16 to 113)	
Pure Direct Effect (PDE)	236 (163 to 308)	125 (51 to 199)		146 (101 to 192)	57 (9 to 106)	
Pure Indirect Effect (PIE)	7 (5 to 9)	8 (6 to 10)	3 (2 to 5)	10 (6 to 13)	7 (4 to 9)	6 (4 to 10)
Mediated Interaction (MI)	3 (0 to 5)	-3 (-5 to 0)	1 (0 to 2)	2 (-3 to 7)	0 (-2 to 3)	1 (-2 to 4)
Metabolic system						
Total Effect	247 (174 to 320)	131 (57 to 205)		154 (107 to 201)	61 (11 to 111)	
Pure Direct Effect (PDE)	219 (146 to 291)	109 (36 to 182)		106 (61 to 151)	40 (-10 to 91)	
Pure Indirect Effect (PIE)	26 (22 to 31)	22 (19 to 26)	11 (8 to 16)	41 (31 to 51)	22 (18 to 26)	27 (17 to 42)
Mediated Interaction (MI)	2 (-4 to 7)	0 (-4 to 4)	1 (-2 to 2)	7 (-6 to 21)	-2 (-6 to 2)	5 (-5 to 13)
Inflammation						
Total Effect	246 (173 to 319)	131 (57 to 206)		158 (112 to 204)	64 (15 to 114)	
Pure Direct Effect (PDE)	204 (132 to 276)	110 (36 to 184)		126 (81 to 172)	55 (6 to 105)	
Pure Indirect Effect (PIE)	30 (24 to 36)	30 (25 to 34)	12 (9 to 18)	18 (13 to 23)	13 (11 to 16)	11 (7 to 18)
Mediated Interaction (MI)	12 (5 to 20)	-8 (-13 to -3)	5 (2 to 8)	14 (6 to 22)	-4 (-6 to -2)	9 (4 to 14)

[a] Analysis adjusted for age and centre

[b] Reference category included high education

[c] RD: Risk difference. This was estimated from the Additive Hazard survival model, with age on the time scale and adjusting for population.

Table 4. Rate Difference in additional coronary heart disease events per 100,000 person-years by educational Level (decomposition of TE into direct, Indirect and Mediated Interaction effects) for allostatic load as mediators, separately for individuals in the pre-dysregulation and in the dysregulation phases

Allostatic load score[a]	**Men**			**Women**		
	Educational Level [b]		**Proportion mediated in low education (95%CI)**	**Educational Level** [b]		**Proportion mediated in low education (95%CI)**
	Low	**Intermediate**		**Low**	**Intermediate**	
	RD (95% CI)[c]	**RD (95% CI)**[c]		**RD (95% CI)**[c]	**RD (95% CI)**[c]	
Pre-dysregulation						
Total Effect	213 (128 to 298)	129 (40 to 219)		118 (68 to 168)	50 (-3 to 103)	
Pure Direct Effect (PDE)	186 (103 to 269)	111 (22 to 199)		87 (38 to 135)	40 (-13 to 93)	
Pure Indirect Effect (PIE)	22 (17 to 27)	21 (17 to 26)	10 (6 to 18)	19 (11 to 28)	13 (9 to 18)	16 (8 to 32)
Mediated Interaction (MI)	5 (-2 to 12)	-3 (-8 to 3)	2 (-1 to 5)	12 (0 to 24)	-3 (-7 to 0)	10 (0 to 21)
Dysregulation						
Total Effect	242 (125 to 358)	107 (-9 to 222)		179 (102 to 256)	69 (-14 to 152)	
Pure Direct Effect (PDE)	214 (98 to 330)	88 (-27 to 203)		130 (55 to 204)	54 (-30 to 137)	
Pure Indirect Effect (PIE)	24 (19 to 28)	21 (17 to 25)	10 (6 to 20)	33 (23 to 43)	17 (14 to 21)	19 (11 to 37)
Mediated Interaction (MI)	4 (-2 to 9)	-3 (-7 to 1)	2 (-1 to 4)	16 (1 to 30)	-3 (-7 to 1)	9 (1 to 18)

[a] Analysis adjusted for age and centre

[b] Reference category included high education

[c] RD: Risk difference. This was estimated from the Additive Hazard survival model, with age on the time scale and adjusting for population.