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

Blánaid Hicks ,[1] Giovanni Veronesi,[2] Marco M Ferrario ,[2] Hannah Forrest,[2] Margaret Whitehead,[3] Finn Diderichsen ,[4] Hugh Tunstall-Pedoe,[5] Kari Kuulasmaa,[6] Susana Sans,[7] Veikko Salomaa,[6] Barbara Thorand,[8] Annette Peters,[8,9] Stefan Soderberg,[10] Giancarlo Cesana,[11] Martin Bobak,[12] Licia Iacoviello,[2] Luigi Palmieri,[13] Tanja Zeller,[14] Stefan Blankenberg,[14] Frank Kee,[1] On behalf of the MORGAM/BiomarCaRE consortium

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/jech-2020-215394).

For numbered affiliations see end of article.

Correspondence to
Dr Blánaid Hicks, Centre for Public Health, Queen's University Belfast, Belfast, Belfast, UK; B.Hicks@qub.ac.uk

Received 21 August 2020
Accepted 13 May 2021

To cite: Hicks B, Veronesi G, Ferrario MM, *et al*. *J Epidemiol Community Health* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/jech-2020-215394

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 with 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 the accumulation of AL in low educational classes, the investigation of AL-related risk factors is warranted.

INTRODUCTION

The degree to which social, biological and behavioural risk factors explain socioeconomic inequalities in cardiovascular disease (CVD) remains uncertain. Two (nonmutually exclusive) mechanisms are hypothesised to play a role.[1] First, 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 the 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 socioeconomic 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 into 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 CHD through a *differential susceptibility* mechanism.[1 10 11] Specifically, it has been proposed that the relationship between 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 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 used data from the BiomarCaRE project, which includes population-based cohort studies harmonised in the MORGAM (MONICA Risk Genetics Archiving and Monograph) Project with stored serum/plasma.[15 16] 21 European cohorts (from six countries as outlined in online supplemental 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 49–60) and Brianza and Catalonia (individuals aged up to 66 and 67 at baseline, respectively)) with no history of acute coronary events or stroke 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-specific, sex-specific 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 (eg, hypertension, diabetes and obesity).[18 19] AL score was computed using eight selected biomarkers corresponding to three different physiological systems: inflammation (C reactive protein), 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 online supplemental table S2. Markers of the neuroendocrine system (eg, epinephrine and cortisol) were not available. AL score was calculated based on the sum of an individual's Z-score for each of the eight individual biomarker components. Before standardisation, 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-specific, sex-specific and fasting status-specific mean and SD values.

Secondary analyses calculated AL subscores 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 five-class variable as nonsmokers (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 12.5 g 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). BMI was categorised as normal (<24.9 kg/m^2), overweight (25–29.9 kg/m^2) and obese (>30 kg/m^2).

Outcome

Hospitalisations for CHD 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 nonfatal 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. 8737 were excluded because of missing data for AL score markers; no follow-up information or missing data on lifestyle factors. Of 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 online supplemental 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 three-way decomposition. This method decomposes the difference in CHD outcome between two educational classes (ie, 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 with the reference group (high education). The estimated average PDE can be interpreted as the additional number of CHD events in the low versus 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 online supplemental methods. We report coefficients (online supplemental tables S3 and S4) and weight distribution (online supplemental 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 hazard models (online supplemental table S5).

Analyses were repeated (1) by study population, (2) for AL subscores, (3) stratifying participants in the predysregulation and dysregulation phases (including elevated blood pressure (>140/90 mm Hg), 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 differential exposure and differential susceptibility estimates (outlined in online supplemental methods). Analyses were conducted using R and SAS V.9.4 (SAS Institute).

Table 1 Distribution of allostatic load scores, behavioural 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

	Men				Women			
	Educational class				Educational class			
Characteristic*	Low (N=9415)	Intermediate (N=6266)	High (N=9629)	P value	Low (N=9849)	Intermediate (N=6556)	High (N=9613)	P value
Age, years (SD)	52.4 (9.6)	53.1 (9.2)	51.8 (9.3)	<0.0001	51.8 (9.6)	52.7 (9.7)	51.3 (9.7)	<0.0001
Allostatic load score	0.23	0.11	−0.39	<0.0001	0.70	0.06	−0.69	<0.0001
Smoking status, %								
Never smokers	32.0	33.8	41.6	<0.0001	59.6	62.1	59.5	<0.0001
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	<0.0001	49.2	43.8	39.2	<0.0001
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 (%)†								
Normal weight	28.5	28.4	30.8	<0.0001	33.7	37.2	46.2	<0.0001
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‡, %	49.2	48.3	47.7	0.14	44.8	43.9	37.7	<0.0001
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	–

*Age-adjusted mean or proportion estimated at age 52.
†Normal weight: BMI was considered 18.5–25 Kg/m^2; overweight, BMI 25–30 Kg/m^2; obese, BMI ≥30 Kg/m^2.
‡Elevated blood pressure was considered >140/90 mm Hg.
BMI, body mass index; CVD, cardiovascular disease.

RESULTS

3031 participants were diagnosed with 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, men 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 with 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 PIE and 8% (95% CI 0% to 16%) to the MI (*differential susceptibility*). In men, the overall proportion mediated by AL was 19% (PIE (*differential exposure*) 16% (95% CI 11% to 23%); with MI (*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 (PIE=23% (95% CI 16% to 34%); MI=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 subscores 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 predysregulation 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 aged 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 (online supplemental table S6).

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

Online supplemental 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.

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

	Men			Women		
	Educational Level†			Educational level †		
	Low	Intermediate	Proportion mediated in low education (95% CI)	Low	Intermediate	Proportion mediated in low education (95% CI)
Mediator*	RD (95% CI)‡	RD (95% CI)‡		RD (95% CI)‡	RD (95% CI)‡	
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 §						
Total effect	250 (176 to 325)	134 (59 to 209)		158 (112 to 204)	65 (15 to 114)	
PDE	181 (110 to 253)	92 (17 to 166)		133 (88 to 177)	58 (8 to 107)	
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)
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¶						
Total effect	240 (167 to 313)	126 (52 to 200)		156 (110 to 202)	63 (14 to 113)	
PDE	241 (167 to 314)	127 (51 to 202)		144 (98 to 190)	59 (9 to 108)	
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)
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)	
PDE	235 (162 to 308)	121 (47 to 195)		135 (89 to 181)	51 (1 to 100)	
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)
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)

*Analysis adjusted for age and population.
†Reference category was considered high education.
‡This was estimated from the additive hazard survival model, with age on the time scale and adjusting for population.
§In men smoking categorised 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.
¶In men alcohol use categorised 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.
RD, risk difference.

DISCUSSION

In this study, we found evidence that AL contributes to the educational inequalities in CHD incidence. We observed *differential exposure* and *differential susceptibility* to the effects of AL on CHD risk, however, a 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 d*ifferential 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 that 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 and 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

Table 3 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) for allostatic sub-scores as mediators in men and women

	Men			Women		
	Educational level †			Educational level †		
	Low	Intermediate	Proportion mediated in low education (95% CI)	Low	Intermediate	Proportion mediated in low education (95% CI)
Allostatic load sub-score*	RD (95% CI)‡	RD (95% CI)‡		RD (95% CI)‡	RD (95% CI)‡	
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)	
PDE	219 (146 to 291)	109 (36 to 182)		106 (61 to 151)	40 (–10 to 91)	
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)
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)	
PDE	204 (132 to 276)	110 (36 to 184)		126 (81 to 172)	55 (6 to 105)	
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)
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)

*Analysis adjusted for age and centre.
†Reference category included high education.
‡This was estimated from the additive hazard survival model, with age on the time scale and adjusting for population.
RD, Risk difference.

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 with their more educated counterparts. In women, the educational gradient in AL remained largely unexplained by both *differential exposure* and *susceptibility* to the same behavioural factors.

Table 4 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) for allostatic load as mediators, separately for individuals in the pre-dysregulation and in the dysregulation phases

	Men			Women		
	Educational level †			Educational level †		
	Low	Intermediate	Proportion mediated in low education (95% CI)	Low	Intermediate	Proportion mediated in low education (95% CI)
Allostatic load score*	RD (95% CI)‡	RD (95% CI)‡		RD (95% CI)‡	RD (95% CI)‡	
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)	
PDE	214 (98 to 330)	88 (-27 to 203)		130 (55 to 204)	54 (-30 to 137)	
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)
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)

*Analysis adjusted for age and centre.
†Reference category included high education.
‡This was estimated from the additive hazard survival model, with age on the time scale and adjusting for population.
RD, risk difference.

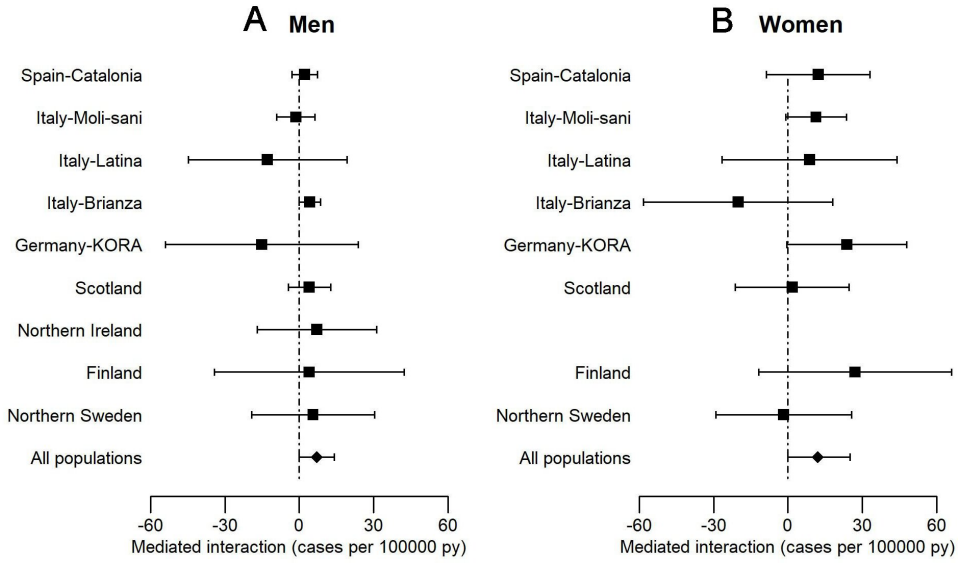

Figure 1 Additional coronary heart disease events per 100 000 person-years due to the mediated interaction of allostatic load, by educational level in men (A) and women (B) by study population and overall estimate.

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 on 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

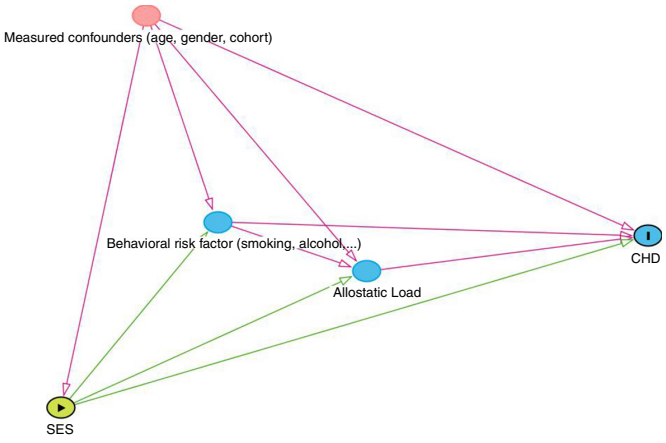

Figure 2 Directed acyclic graph describing the intertwined pathways of education on CHD through allostatic load, smoking and alcohol use. CHD,coronary heart disease.

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).

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 (online supplemental figures S5 and 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 with 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 (1980s–1990s), 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.

Author affiliations
[1]Centre for Public Health, Queen's University Belfast, Belfast, UK
[2]Research Center in Epidemiology and Preventive Medicine, Department of Medicine and Surgery, University of Insubria, Varese, Italy
[3]Department of Public Health and Policy, Institute of Population Health and Sciences, University of Liverpool, Liverpool, UK
[4]Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[5]Cardiovascular Epidemiology Unit, Institute of Cardiovascular Research, University of Dundee, Dundee, UK
[6]Department of Public Health and Welfare, Finnish Institute for Health and Welfare, Helsinki, Finland
[7]Catalan Department of Health, Barcelona, Spain
[8]Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany
[9]German Centre for Cardiovascular Research (DZHK), Partner site Munich Heart Alliance, Munich, Germany
[10]Department of Public Health and Clinical Medicine, Umea Universitet, Umea, Sweden
[11]Research Centre on Public Health, Department of Medicine and Surgery, University of Milan–Bicocca, Monza, Italy
[12]Department of Epidemiology and Public Health, University College London, London, UK
[13]Department of Cardiovascular, Endocrine-Metabolic Diseases, and Ageing, National Institute of Health, Roma, Lazio, Italy
[14]Department of Cardiology, University Heart and Vascular Centre, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

Contributors 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. HT-P, SSa, VS, BT, FK, MMF, BT, SSo, GC, LI, LP are the principal investigators of the cohorts included in the current analyses. MW, FD, KK, HT-P, SSa, VS, BT, AP, TB, AP, SSo, GC, LI, LP, MB, TZ and SB actively contributed to the interpretation of the results and made critical revision of the manuscript.

Funding The BiomarCaRE Project is funded by the EU Seventh Framework Programme (FP7/2007– 2013) under grant agreement HEALTH-F2–2011–278913. The activities of the MORGAM Data Centre have also been sustained by recent funding from EU FP7 project CHANCES (HEALTH-F3–2010–242244). A part of the biomarker determinations in the population cohorts was funded by the Medical Research Council London (G0601463, identification No.80983: Biomarkers in the MORGAM Populations). The MONICA/KORA Augsburg study was initiated and financed by the Helmholtz Zentrum München—German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria.

Competing interests None declared.

Patient consent for publication Not required.

Ethics 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. Scottish Heart Health Extended Study (SHHEC): Tayside Health Board Dundee District, DM/CL/207.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No additional data are available from each of the cohorts. Data may be made available to researchers upon reasonable request.

ORCID iDs
Blánaid Hicks http://orcid.org/0000-0002-5730-9469
Marco M Ferrario http://orcid.org/0000-0003-2741-7124
Finn Diderichsen http://orcid.org/0000-0002-9998-4972

REFERENCES

1 Diderichsen F, Hallqvist J, Whitehead M. Differential vulnerability and susceptibility: how to make use of recent development in our understanding of mediation and interaction to tackle health inequalities. *Int J Epidemiol* 2019;48:268–74.
2 Ferrario MM, Veronesi G, Kee F, *et al*. Determinants of social inequalities in stroke incidence across Europe: a collaborative analysis of 126 635 individuals from 48 cohort studies. *J Epidemiol Community Health* 2017;71:jech-2017-209728.
3 Veronesi G, Ferrario MM, Kuulasmaa K, *et al*. Educational class inequalities in the incidence of coronary heart disease in Europe. *Heart* 2016;102:958–65.
4 Hallqvist J, Diderichsen F, Theorell T, *et al*. Is the effect of job strain on myocardial infarction risk due to interaction between high psychological demands and low decision latitude? Results from Stockholm heart epidemiology program (sheep). *Soc Sci Med* 1998;46:1405–15.
5 Nordahl H, Lange T, Osler M, *et al*. Education and cause-specific mortality. *Epidemiology* 2014;25:389–96.
6 Nordahl H, Osler M, Frederiksen BL, *et al*. Combined effects of socioeconomic position, smoking, and hypertension on risk of ischemic and hemorrhagic stroke. *Stroke* 2014;45:2582–7.
7 Hussein M, Diez Roux AV, Mujahid MS, *et al*. Unequal exposure or unequal vulnerability? contributions of neighborhood conditions and cardiovascular risk factors to socioeconomic inequality in incident cardiovascular disease in the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2018;187:1424–37.
8 Mauss D, Li J, Schmidt B, *et al*. Measuring allostatic load in the workforce: a systematic review. *Ind Health* 2015;53:5–20.
9 Cadar D, Abell J, Hackett R, *et al*. Associations of allostatic load with coronary heart disease and dementia in the English longitudinal study of ageing. *Innov Aging* 2018;2:271.
10 Saban KL, Mathews HL, DeVon HA. Epigenetics and social context: implications for disparity in cardiovascular disease. *aging Dis* 2014;5:346–55.
11 McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev* 2007;87:873–904.
12 Robertson T, Benzeval M, Whitley E, *et al*. The role of material, psychosocial and behavioral factors in mediating the association between socioeconomic position and allostatic load (measured by cardiovascular, metabolic and inflammatory markers). *Brain Behav Immun* 2015;45:41–9.
13 Johnson SC, Cavallaro FL, Leon DA. A systematic review of allostatic load in relation to socioeconomic position: poor fidelity and major inconsistencies in biomarkers employed. *Soc Sci Med* 2017;192:66–73.
14 Veronesi G, Kee F, Hicks B, *et al*. Decomposing the educational gradient in allostatic load across European populations. What matters the most: differentials in exposure or in susceptibility? *J Epidemiol Community Heal* 2020.
15 Zeller T, Hughes M, Tuovinen T, *et al*. BiomarCaRE: rationale and design of the European BiomarCaRE project including 300,000 participants from 13 European countries. *Eur J Epidemiol* 2014;29:777–90.
16 Evans A*et al*. MORGAM (an international pooling of cardiovascular cohorts). *Int J Epidemiol* 2004;34:21–7.
17 Karvanen J, Veronesi G, Kuulasmaa K. Defining thirds of schooling years in population studies. *Eur J Epidemiol* 2007;22:487–92.
18 Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev* 2010;35:2–16.
19 McEwen BS, Stellar E. Stress and the individual. mechanisms leading to disease. *Arch Intern Med* 1993;153:2093–101.
20 VanderWeele TJ. Mediation and mechanism. *Eur J Epidemiol* 2009;24:217–24.
21 VanderWeele TJ. A three-way decomposition of a total effect into direct, indirect, and interactive effects. *Epidemiology* 2013;24:224–32.
22 Lange T, Vansteelandt S, Bekaert M. A simple unified approach for estimating natural direct and indirect effects. *Am J Epidemiol* 2012;176:190–5.
23 Lange T, Hansen JV. Direct and indirect effects in a survival context. *Epidemiology* 2011;22:575–81.
24 Tillmann T, Vaucher J, Okbay A, *et al*. Education and coronary heart disease: mendelian randomisation study. *BMJ* 2017;358:j3542.
25 Davies NM, Dickson M, Davey Smith G, *et al*. The causal effects of education on health outcomes in the UK Biobank. *Nat Hum Behav* 2018;2:117–25.
26 Kaufman JS. Commentary: causal inference for social exposures. *Annu Rev Public Health* 2019;40:7–21.
27 Rothman KJ. Reviews and commentary causes, 1976. Available: https://academic.oup.com/aje/article-abstract/104/6/587/139202 [Accessed 21 Sep 2018].
28 Diderichsen F, Andersen I, Lange T, *et al*. Re: testing differential associations between smoking and chronic disease across socioeconomic groups. *Epidemiology* 2019;30:e38–9.
29 Dégano IR, Marrugat J, Grau M, *et al*. The association between education and cardiovascular disease incidence is mediated by hypertension, diabetes, and body mass index. *Sci Rep* 2017;7:12370.
30 Kershaw KN, Droomers M, Robinson WR, *et al*. Quantifying the contributions of behavioral and biological risk factors to socioeconomic disparities in coronary heart disease incidence: the MORGEN study. *Eur J Epidemiol* 2013;28:807–14.
31 de Mestral C, Bell S, Stamatakis E, *et al*. Testing differential associations between smoking and chronic disease across socioeconomic groups: pooled data from 15 prospective studies. *Epidemiology* 2019;30:48–51.
32 Veronesi G, Tunstall-Pedoe H, Ferrario MM, *et al*. Combined effect of educational status and cardiovascular risk factors on the incidence of coronary heart disease and stroke in European cohorts: implications for prevention. *Eur J Prev Cardiol* 2017;24:437–45.
33 Liberale L, Montecucco F, Tardif J-C, *et al*. Inflamm-ageing: the role of inflammation in age-dependent cardiovascular disease. *Eur Heart J* 2020;41:2974–82.
34 Crawford AA, Soderberg S, Kirschbaum C, *et al*. Morning plasma cortisol as a cardiovascular risk factor: findings from prospective cohort and Mendelian randomization studies. *Eur J Endocrinol* 2019;181:429–38.
35 Caan W. Fair society, healthy lives. timing is everything. *BMJ* 2010;340:c1191.
36 Petrovic D, de Mestral C, Bochud M, *et al*. The contribution of health behaviors to socioeconomic inequalities in health: a systematic review. *Prev Med* 2018;113:15–31.
37 Komulainen K, Mittleman MA, Jokela M, *et al*. Socioeconomic position and intergenerational associations of ideal health behaviors. *Eur J Prev Cardiol* 2019;26:1605–12.
38 Cunliffe VT. The epigenetic impacts of social stress: how does social adversity become biologically embedded? *Epigenomics* 2016;8:1653–69.
39 Ding X, Barban N, Mills MC. Educational attainment and allostatic load in later life: evidence using genetic markers. *Prev Med* 2019;129:105866.
40 Song Y, Zhou X, Zhang M, *et al*. Bayesian shrinkage estimation of high dimensional causal mediation effects in omics studies. *Biometrics*2020;76:biom.13189
41 Doan SN, Dich N, Evans GW. Childhood cumulative risk and later allostatic load: mediating role of substance use. *Health Psychology* 2014;33:1402–9.
42 Lundberg M, Hallqvist J, Diderichsen F. Exposure-dependent misclassification of exposure in interaction analyses. *Epidemiology* 1999;10:545–9.
43 Blakely T, McKenzie S, Carter K. Misclassification of the mediator matters when estimating indirect effects. *J Epidemiol Community Health* 2013;67:458–66.