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Effects of long-term developmental patterns of body weight on levels of C-reactive protein and fibrinogen among North-American men and women: The Spokane Heart Study

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Abstract

This study examined heterogeneity in body weight development by identifying distinct developmental trajectories of body weight. These trajectories were further investigated by relating them to markers of low-grade inflammation later in life. Data from approximately 400 healthy volunteers from the Spokane Heart Study were collected in two-year intervals and four waves of data were available for the current analyses. Body weight was measured by Body Mass Index (BMI) and low grade inflammation by high sensitivity C-reactive protein (CRP) and fibrinogen. Contemporary statistical techniques – latent class growth models – were used to analyze heterogeneity in body weight and linear regressions were run to analyze possible associations between trajectories of body weight and CRP/fibrinogen levels. Six trajectories of BMI were identified (three stable trajectories, two increasing trajectories and a decreasing trajectory), which differed significantly on CRP- and fibrinogen levels, highlighting the importance of weight trajectories. The differences were only partly explained by variations in lifestyle habits.

Introduction

Worldwide, the prevalence of being overweight (i.e. a body mass index (BMI) of 25-29.9) or obese (BMI ≥ 30) is rapidly increasing across all age, gender and racial groups [101]. Ample studies have shown that being overweight or obese is associated with many chronic diseases such as arthritis [102], certain cancers [103] and sleep apnoea [104]. Further, it has been shown that these weight categories are associated with cardiovascular diseases (CVD). High levels of BMI are consistently found to be strong risk factors for hypertension [105] and stroke [106], amongst others and markers of low-grade inflammation might in part explain these relationships [107, 108]. Well-known examples of such markers of low-grade inflammation include C-reactive protein (CRP) and fibrinogen.

CRP is a protein produced in the liver in response to inflammation or due to increased adipocyte activity [109, 110]. Extensively studied, especially cross-sectionally, CRP has been shown to be elevated in obese individuals from various study populations [109–111]. Moreover, CRP has also been found to be an independent marker of elevated cardiovascular disease-risk [109, 112]. Fibrinogen, also produced in the liver, is a marker of both inflammation and haemostasis and has been shown to predict future CVD as well [113]. Studies investigating fibrinogen and adiposity demonstrate elevated fibrinogen values in overweight and obese individuals [114].

Many longitudinal studies have shown that weight gain [115, 116] and greater weight variability over time [117, 118] are associated with an increase in levels of several low-grade inflammation markers, whereas others did not find an association at all [119]. These inconsistent findings allow for weight variability research to remain of high interest, partly driven by the rapid progress that has been made in the statistical analysis of longitudinal data. Contemporary techniques, i.e. latent class growth models (LCGM), make possible the analysis of *distinct trajectories* of BMI and subsequent associations with markers of low-grade inflammation. It has recently been shown [92, 95, 120] that the development of BMI is heterogeneous over time, where not only weight loss or weight gain in itself, but also distinct trajectories of BMI, may be of importance for the quantification of various health risks. For example, Mustillo et al. [91] identified four distinct obesity trajectories in a cohort of 9-16 year old children, each with distinct consequences for psychopathology. Further, in a cohort of elderly participants, Kuchibhatla [121] identified three distinct trajectories of BMI whereas Botoseneanu and Liang [122] identified five distinct trajectories

in a cohort of subjects of similar age. In all studies, distinct trajectories of BMI coincided with differential health- or disease consequences. In the context of the increasing obesity epidemic, researching the subsequent health- and disease consequences of distinct BMI trajectories in a range of cohorts is, therefore, critical. Particularly the possibility to identify high-risk individuals, who may need supplementary treatment or preventive measures in addition to the standard care or prevention, is important. In this light, the evaluation of (health- and disease consequences of) distinct trajectories might also allow for the identification of low-risk individuals who need less treatment. Using "conventional" statistical approaches (e.g. calculating BMI change scores) will probably masked this heterogeneity in development.

The aim of the current study is twofold; first, we will analyze heterogeneity of development of BMI over time, thereby determining the number and characteristics of distinct developmental patterns of body weight in an adult study population. Second, we will relate these distinct subgroups to late-life CRP and -fibrinogen levels, revealing subgroups specifically at risk for future CVD.

Materials and Methods

The Spokane Heart Study

The Spokane Heart Study is a longitudinal study of healthy, community dwelling participants investigating the development of pre-clinical atherosclerosis in order to the predict risk for coronary artery disease from traditional and non-traditional factors [9, 10, 123, 124]. Beginning in 1994, participants were enrolled every 2 years until 2006, and data was collected at 2-year intervals. Visits therefore, take place at different years for different participants and not all participants were followed up an equal number of times. In total, 1,147 participants in the Spokane Heart Study have at least one visit available.

The study was reviewed and approved by the Institutional Review Board of the participating university and community hospitals, and written informed consent was given by all participants at all measurement occasions. For consistency in sample sizes over time and to avoid possible period-effects, only visits 1-4 were analyzed. For the current study, participants who had multiple (three or more) measurements [120] of body weight over time (N=441) and from whom valid blood samples were available (N=407) were included. The characteristics of this selection were compared to the 1,147 participants of the Spokane Heart Study and showed no relevant differences on BMI at the first

visit, CRP nor fibrinogen (BMI; 26.66 (4.42) kg/m^2 versus 27.24 (4.39) kg/m^2, CRP; 2.5 (3.80) mg/dL versus 2.87 (3.60) mg/dL and fibrinogen; 319.73 (207.29) mg/dL versus 321.78 (124.24) mg/dL respectively).
In total, 203 (49.9%) women and 204 men were included in the study. Participants in the Spokane Heart Study are predominately Caucasian (>98%) and the obesity rates at visit 1 (conducted around 1994 for this sample were comparable to the nationwide obesity rates (16.2% versus 14.4% nationwide [125]).

Body weight
Body weight was quantified by means of the body mass index (BMI). The BMI was calculated by dividing body mass (kilograms) by height squared (meters). Body weight was measured using a calibrated scale and height was measured using a wall-mounted stadiometer, generally available at community hospitals throughout the USA.

C-reactive protein and fibrinogen
Fasting blood samples were collected in the morning of visit 1 and visit 4 and separated by centrifugation into serum and plasma. High-sensitivity CRP in plasma was analyzed using nephelometric assays at the Centre for Disease Control and Prevention (CDC), Atlanta, GA, USA. As recommended by the CDC and the American Heart Association, levels >10 mg/dL were not included in the analyses as they may indicate active infection or trauma [126]. Fibrinogen was analyzed by Pathology Associates Medical Laboratory, a nationally certified laboratory in Spokane, Washington and also expressed in mg/dL.

Covariates
Baseline lifestyle covariates were utilized. Smoking status was asked in a questionnaire and stated whether the participant was a smoker or a non-smoker. Physical exercise levels were determined by a self-reported questionnaire measuring the extent of engagement in several sports activities. The answers on all questions were combined to form a binary variable indicating whether or not a participant engages regularly (approximately five hours per week according to the 2008 physical activity guidelines for adults developed by the Centres of Disease Control) in sportive activities. Dietary information was determined by a self-reported questionnaire asking whether the participant

was on a restricted (i.e. low-fat and/or low-salt and/or low-cholesterol and/or vegetarian) and a binary variable (restricted diet yes/no) was included in the analyses. Information on current oral contraceptive usage (females only) was obtained by a self-reported questionnaire and scored as a binary variable (users versus non-users). Information on other relevant medication usage known to influence low-grade inflammation (e.g. blood pressure medication, cholesterol medication and diabetes medication) was available and obtained from a self-reported questionnaire. This variable was binary coded and included as such in the analyses.
Possible effect modification by gender was assessed by creating interaction terms (class membership dummies*gender) and evaluating the *P*-values of these interaction terms.

Statistical Methods
Analyses were conducted using the Mplus 7.0 [127, 128] and the PASW 20.0 software packages.
Analyses were conducted in two steps, described in detail below. All continuous outcome variables were checked for normality and log transformed where necessary, and all assumptions underlying the analyses were checked. Significance results are given as two-sided *P*-values unless stated otherwise, where a *P*-value <0.05 is regarded as statistically significant.

Step 1 – Latent class growth modelling
In step one, distinct developmental patterns of BMI were identified by means of latent class growth modelling, LCGM [18, 33, 42, 62]. LCGM is a contemporary statistical technique in obesity research. The underlying aim of the technique is to capture the heterogeneity of development of BMI over time in *k* number of subgroups (or classes) with comparable BMI development. Each subgroup has its own growth parameters (intercept, slope) which are unobserved (or latent) continuous variables.

Class identification
To determine the optimal number of latent classes, a forward procedure [18, 120] was performed, starting with a one class solution (i.e. there are no subgroups in the study sample; all individuals follow the same trajectory over time), then adding more classes one at a time to investigate whether or not model fit improves due to the additional class. To compare fit between subsequent

models, we used several model fit indices and other criteria, as suggested in the literature [17]. First, we used two model fit indices; the Bayesian Information Criterion (BIC), and the bootstrap Likelihood Ratio Test (BLRT). The BIC [67] is commonly used within latent variable modelling analyses, considering both the likelihood of the model as well as the number of parameters in the model; a lower BIC value shows a better model fit (a decrease of at least 10 points is suggested as sufficient improvement [67]). The BLRT [65] provides a *P*-value, indicating that a model with one less class (*k*-1 class model) has to be rejected in favour of the *k* class model. Both fit indices have shown to be consistent indicators of the optimal number of classes [17]. To further determine the optimal number of classes, we secondly looked at the posterior probabilities of belonging to each of the *k* classes [17]. Study participants were assigned to their most-likely class based on these probabilities and only solutions with posterior probabilities of 0.8-0.9 or higher were accepted [17, 18]. The probability for the class to which the individual was assigned to should be considerably higher than the other probability/probabilities. In this way, the classes clearly distinguish from each other. Additionally, we considered the usefulness of the separate classes in practice. This was done in two ways: by examining the shape of the trajectories and the number of individuals in each class. Clinically uninterpretable solutions were rejected in favour of interpretable solutions [18].

Step 2 – Linear Regression Analyses

The first step of the analyses provided us with a classification of the study population into distinct subgroups, based on the development of BMI. The classification was coded as a categorical variable with *k* number of categories, or classes. To study the possible association between the distinct subgroups and CRP and fibrinogen, several linear regression analyses were performed with the markers of low-grade inflammation as the outcome variables. First, "crude" analyses were conducted, in which class membership was related to CRP or fibrinogen, correcting only for age, gender, relevant medication use and oral contraceptive usage. Second, adjusted analyses were carried out, adding the lifestyle covariates to the regression model.

Results will be reported as unstandardised regression coefficients, indicating the mean difference in the markers of low-grade inflammation between the distinct trajectories of BMI. Corresponding 95% confidence intervals and two-sided *P*-values are presented alongside.

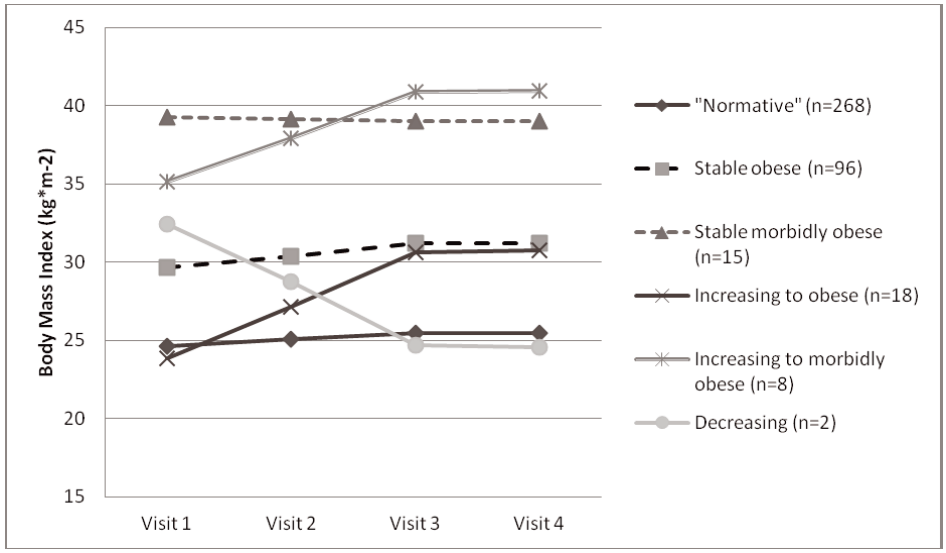

Figure 4 Six developmental trajectories of BMI

Table 5 Model fit indices

Number of classes	Bayesian Information Criterion	Bootstrapped Likelihood Ratio Test	Average posterior probability (min-max)	Number of subjects per class
1	7604.838	Not available	1.00	407
2	7561.913	*P*<0.001	0.916 (0.843-0.988)	385/22
3	7532.887	*P*<0.001	0.933 (0.873-0.986)	8/377/22
4	7524.385	*P*<0.001	0.922 (0.824-1.00)	15/2/27/363
5	7521.011	*P*<0.001	0.897 (0.821-1.00)	2/13/16/364/12
6	7527.809	*P*=0.08	0.869 (0.790-1.00)	2/15/268/8/18/96

P-values of the interaction terms investigating effect modification by gender were all non-significant (*P*=0.05, 0.429, 0.616, 0.649 and 0.670) and therefore no stratification by gender was made.

Additional analyses
In order to determine if conventional statistical approaches provided any further information and/or stronger, or weaker, prediction of CRP and fibrinogen by BMI, we calculated change scores of BMI (BMI at visit 4 minus BMI at visit 1) and related this continuous variable to CRP and fibrinogen using the same regression analysis steps described in Step 2 above.

Results
Figure 4 shows the "best" model of the LCGM. Based on the model fit indices and additional information available (see **table 5**), selecting the "best" model was relatively difficult, because of some inconsistency in model fit indices. Based on the BIC, a 5-class solution could be considered as being the best model, but the BLRT pointed towards a 6-class model. After taking into account the clinical interpretation of the 4-, 5- and 6-class models in particular, a final decision for the 6-class model was made, also because the BLRT has proved to be a very consistent model fit index [17]. Therefore, the best LCGM solution was a six class model, where most people could be classified in a "normative" class, characterized by stable BMI values around 25 kg/m^2. Subsequently, two more stable subgroups emerged; a stable obese- (N=96) and a stable morbidly obese (N=15) subgroup. The stable obese subgroup was characterized by stable BMI values around 30 kg/m^2, although slight increases are apparent after visit 2. The stable morbidly obese is characterized by clearly stable BMI values of just under 40 kg/m^2. Further, two groups started out with an increase and levelled, starting at different baseline values (normal-weight and obese; N=18 and N=8, respectively). The first subgroup showed a steep increase between visit 1 and -2 (approximately 7 BMI-points) and an increase of similar extent was visible for the latter subgroup.
Finally, a small group (N=2) decreased from an obese baseline state (BMI values between 30-35 kg/m^2) towards a "normative" state (BMI values similar to the normative subgroup). Because of the small sample size, this class was not analyzed further. The fit information of the BIC, BLRT, posterior probabilities and number of subjects per class is visualized in **table 5**.

Table 6 Characteristics of the distinct developmental patterns of Body Mass Index identified in the Spokane Heart Study

	Normative	Increasing to obese	Stable obese	Increasing to morbidly obese	Stable morbidly obese
Sample size	268	18	96	8	15
Age at baseline	48.63 (9.82)	44.11 (8.55)	48.30 (8.00)	51.38 (3.85)	49.67 (5.43)
Body mass index (kg/m^2) at visit 1[1]	24.55 (2.55)	23.94 (2.49)	30.29 (1.88)	35.00 (2.67)	39.00 (3.53)
Body mass index (kg/m^2) at visit 2[1]	24.90 (2.84)	27.56 (2.90)	31.00 (2.71)	38.00 (3.12)	39.07 (3.73)
Body mass index (kg/m^2) at visit 3[1]	25.25 (2.51)	30.50 (2.72)	32.04 (2.04)	40.62 (3.20)	39.36 (3.27)
Body mass index (kg/m^2) at visit 4[1]	25.54 2.55)	31.00 (3.63)	31.87 (1.99)	41.88 (3.40)	37.87 (5.89)
C-reactive protein (mg/dL) at visit 1[1]	1.65 (1.71)	1.85 (2.05)	2.46 (1.95)	4.43 (2.30)	4.30 (2.53)
C-reactive protein (mg/dL) at visit 4[1]	1.92 (2.07)	3.75 (2.45)	3.10 (2.33)	4.41 (3.17)	3.95 (2.74)
Difference in CRP (visit 4-visit 1)[1]	0.26 (2.09)	1.90 (2.14)	0.57 (2.34)	-0.029 (4.15)	-0.41 (2.38)
Fibrinogen (mg/dL) at visit 1[1]	316.10 (239.33)	317.81 (100.73)	310.52 (120.11)	420.34 (151.01)	397.68 (91.27)
Fibrinogen (mg/dL) at visit 4[1]	346.10 (124.10)	390.56 (94.09)	360.39 (157.39)	490.10 (201.60)	433.99 (105.61)
Difference in fibrinogen (visit 4-visit 1)[1]	30.27 (249.73)	72.75 (116.62)	47.65 (127.85)	69.76 (161.75)	36.31 (98.43)
% Female[2]	50.7	61.1	40.6	37.5	86.7
% current smokers at visit 1[2]	8.60	11.0	9.50	0.00	6.70
% regular engagement in sports at visit 1[2]	38.60	27.80	22.80	37.5	13.30
% Restricted diet at visit 1[3]	51.9	55.6	40.6	75.0	53.3
% medication use at visit 1[3]	12.3	0.00	13.5	62.5	33.3

[1] Continuous variables are presented as means (SD)

[2] Categorical variables are presented as %

[3] Relevant medication include medication for cholesterol, blood pressure and/or diabetes

Table 6 shows characteristics of the five classes. It can be seen that the different trajectories do not differ a great deal on lifestyle variables, although the increasing to morbidly obese trajectory is the only class with no smokers and the subject classified in the increasing to morbidly obese trajectory have the highest numbers of subjects on a restricted diet (i.e. low-salt, low-fat, low-cholesterol or vegetarian) and an almost equal number of subjects participating in sports activities compared to subjects classified into the normative trajectory.

Tables 7 and 8 show the results of the associations between the BMI-trajectories and CRP/fibrinogen at visit 4. In all models, the normative subgroup was used as the reference category. For CRP, compared to the normative subgroup, all subgroups showed significantly higher (ranging from 1.83 times to 2.89 times higher) values in the first model. Correcting for relevant lifestyle variables did not affect these associations much. For fibrinogen, the results show a slightly different picture. Although all subgroups show higher levels of fibrinogen compared to the normative subgroup (ranging from 13.44 to 139.57 mg/dL higher in model 1), only the difference between the increasing to morbidly obese subgroup and the normative subgroup was statistically significant and the difference between the normative- and the stable morbidly obese was borderline significant.

To evaluate the importance of the BMI-*trajectories* relative to each other, the above analyses were also run with different reference categories. Interestingly, the unfavourable trajectories only differed slightly with each other, on both CRP- and fibrinogen levels, where differences in fibrinogen levels were more profound. When comparing the stable obese trajectory and the increasing towards obesity (both showing similar BMI-values at the last time point (see **table 6**) large differences in both CRP- and fibrinogen were apparent, although these differences are only borderline significant. Further comparisons are not presented here, but can be partly drawn from the results presented in **tables 7 and 8**.

Table 7 Unstandardised regression coefficients (b), 95% confidence intervals and *P*-values for the associations between BMI-trajectory membership and C-reactive protein

	Model 1[a]			**Model 2[b]**		
	b[d]	**95% confidence interval**	***P*-value**	**b[d]**	**95% confidence interval**	***P*-value**
Increasing to obese[c]	2.24	1.39, 3.60	*P*=0.001	2.12	1.32, 3.38	*P*=0.002
Stable obese[c]	1.83	1.45, 2.33	*P*<0.001	1.73	1.37, 2.19	*P*<0.001
Increasing to morbidly obese[c]	2.89	1.44, 5.81	*P*=0.003	3.01	1.52, 5.97	*P*=0.002
Stable morbidly obese[c]	2.30	1.36, 3.87	*P*=0.002	2.17	1.30, 3.62	*P*=0.003

[a] Model 1 correcting only for age, gender, relevant medication use and oral contraceptive use
[b] Model 2 correcting further for lifestyle variables
[c] "Normative" trajectory is set as the reference category
[d] Regression coefficients represented as ratio's because of log-transformed CRP

Table 8 Unstandardised regression coefficients (b), 95% confidence intervals and *P*-values for the associations between BMI-trajectory membership and fibrinogen

	Model 1[a]			**Model 2[b]**		
	b	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
Increasing to obese[c]	49.64	-1.39, 3.60	*P*=0.122	49.05	-14.54, 112.65	*P*=0.130
Stable obese[c]	13.44	-17.24, 44.13	*P*=0.389	13.10	-18.08, 44.29	*P*=0.409
Increasing to morbidly obese[c]	139.57	49.14, 230.00	*P*=0.003	140.39	49.65, 231.14	*P*=0.003
Stable morbidly obese[c]	63.45	-4.78, 131.68	*P*=0.068	63.30	-5.50, 132.10	*P*=0.071

[a] Model 1 correcting only for age, gender, relevant medication use and oral contraceptive use
[b] Model 2 correcting further for lifestyle variables (diet, physical activity, smoking behaviour)
[c] "Normative" trajectory is set as the reference category

Table 9 presents the same analyses as **table 7 and 8** do, but with the calculated change scores of CRP and fibrinogen (visit 4 minus visit 1) as the outcome variable. Again, the normative trajectory was set as the reference category. For CRP, much smaller regression coefficients appeared and only the difference in change scores between the increasing to obese- and normative trajectory was statistically significant. This regression coefficient is, however, difficult to interpret; 1.41 can be interpreted as the difference in CRP change score between the increasing to obese trajectory and the normative trajectory, indicating that the former subgroup increased more in CRP over time (which is also reflected in **table 6**). For fibrinogen, smaller regression coefficients were also observed, in particular the regression coefficient comparing the change scores of the stable morbidly obese and normative subgroups (reflected in **table 6** too).

Table 9 Unstandardised regression coefficients (b), 95% confidence intervals and *P*-values for the associations between BMI-trajectory and change scores of C-reactive protein and fibrinogen

	C-reactive protein			Fibrinogen		
Model 1[a]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
Increasing to obese[c]	1.41	0.37, 2.46	0.008	44.64	-69.40, 158.69	0.442
Stable obese[c]	0.26	-0.27, 0.80	0.336	16.83	-38.70, 71.70	0.547
Increasing to morbidly obese[c]	0.24	-1.32, 1.80	0.760	79.28	-87.08, 245.64	0.349
Stable morbidly obese[c]	-0.22	-1.45, 1.01	0.727	9.98	-113.38, 133.34	0.874
Model 2[b]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
Increasing to obese[c]	1.36	0.32, 2.41	0.011	49.02	-66.57, 164.60	0.405
Stable obese[c]	0.17	-0.37, 0.72	0.532	17.04	-38.84, 72.92	0.549
Increasing to morbidly obese[c]	0.34	-1.23, 0.90	0.673	84.89	-82.43, 252.22	0.319
Stable morbidly obese[c]	-0.29	-1.52, 0.95	0.650	10.78	-113.87, 135.43	0.865

[a] Model 1 correcting only for age, gender, relevant medication use and oral contraceptive use
[b] Model 2 correcting further for lifestyle variables (diet, physical activity, smoking behaviour)
[c] "Normative" trajectory is set as the reference category

Table 10 Unstandardised regression coefficients (b), 95% confidence intervals and P-values for the associations between change scores of BMI and C-reactive protein and fibrinogen levels at visit 4

		C-reactive protein			**Fibrinogen**	
Model 1[a]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
BMI change score	1.10	1.06, 1.15	*P*<0.001	3.73	-1.69, 9.15	0.177
Model 2[b]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
BMI change score	1.10	1.06, 1.15	*P*<0.001	3.65	-1.81, 9.12	0.189

[a] Model 1 correcting only for age, gender, relevant medication use and oral contraceptive use
[b] Model 2 correcting further for lifestyle variables (diet, physical activity, smoking behaviour)

Table 11 Unstandardised regression coefficients (b), 95% confidence intervals and P-values for the associations between change scores of BMI and change scores of C-reactive protein and fibrinogen levels

		C-reactive protein			**Fibrinogen**	
Model 1[a]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
BMI change score	0.18	0.09, 0.27	*P*<0.001	5.01	-3.92, 13.94	0.271
Model 2[b]	**b**	**95% confidence interval**	***P*-value**	**b**	**95% confidence interval**	***P*-value**
BMI change score	0.19	0.10, 0.27	*P*<0.001	5.48	-3.51, 14.47	0.232

[a] Model 1 correcting only for age, gender, relevant medication use and oral contraceptive use
[b] Model 2 correcting further for lifestyle variables (diet, physical activity, smoking behaviour)

Tables 10 and 11 present the additional analyses, or the results of "conventional" analyses. Change scores of BMI were calculated and related to CRP- and fibrinogen levels at visit 4 in **table 10**. This table shows that BMI change scores are positively associated with CRP- and fibrinogen levels at visit 4, although regression coefficients are only statistically significant for the analyses with CRP. This (adjusted; model 2) regression coefficient can be interpreted as follows: an increase in BMI change score of 1 point *more* between visits 1 and 4 results in 1.11 times higher CRP-levels at visit 4, whereas for fibrinogen this results in 3.65 mg/dL more fibrinogen.
Small effects are also shown in **table 11**, where the outcome variable is the change score of CRP/fibrinogen. An increase in BMI change score of 1 unit *more* is associated with an increase of 0.19 mg/dL more in CRP change score and 5.48 mg/dL more in fibrinogen change score (again, the analyses with CRP are statistically significant and the analyses with fibrinogen are not).

Discussion

The current study investigated the heterogeneity of BMI development over six years. We identified six distinct developmental trajectories of BMI (see **figure 4**). Subsequently, we assessed the associations between these trajectories and CRP- and fibrinogen levels assessed at visit 4 as well as change scores of these levels. "Unfavourable" BMI trajectories differed to a great extent from the normative trajectory with regards to CRP- and fibrinogen levels at visit 4, but to a much lesser extent with regards to their CRP- and fibrinogen change scores. When "conventional" analyses were conducted (i.e. replacing the BMI-trajectories with BMI change scores), much smaller regression coefficients were observed as well.

Heterogeneity in body weight development and low-grade inflammation

To our knowledge, this is the first population-based study that investigates the effects of distinct *trajectory shapes* of body weight on markers of low-grade inflammation. There are some studies looking into the relationship between the development of body weight and markers of low-grade inflammation, such as CRP and fibrinogen, over time. However, although a number of studies are available, comparison with these studies is difficult because of two important reasons. First, definitions of development of body weight are inconsistent across studies. For example, some researchers categorize study participants in arbitrary subgroups, where some pull participants who lose weight throughout

the follow-up period together with stable-weight participants [129], whereas others categorize according to established criteria [116]. Other researchers calculate only change scores [130] when examining the development of body weight over time. Second, many terms for weight instability, such as weight variability, weight fluctuation or weight cycling are used but it is unclear if, and how these definitions vary. Although the statistical methodology and/or definition differs between these studies, they mostly point consistently to negative health consequences, including death, when increased weight variability, -cycling or -fluctuation or weight increase is apparent [130, 131]. Fluctuations in body weight over time are not uncommon; there is increasing evidence that both initial weight gain, particularly into an overweight- or obese status, in combination with subsequent (intentional) weight loss, have undesirable health consequences too. As worldwide, the obesity epidemic is spreading [101, 105, 125], and attempts for (sustained) weight loss are often unsuccessful, these natural weight developmental patterns are of concern.

While these studies allow for interesting and valuable results, they do not give insight in the *trajectory* of body weight between beginning and the end of follow up and its effects on, in this case, CRP or fibrinogen levels. Importance of these trajectories for traditional cardiovascular disease markers [97, 132] and psychological health [91, 92, 133] has been highlighted previously and should not remain unnoticed in this setting either. In our study, we found six distinct trajectories of body weight development, each with a distinct trajectory. Comparable trajectories were found in previous cohorts consisting of children and older adults in particular. For example, Kuchibhatla et al. [121] analyzed a community based sample aged 65-105 years old and identified a normal weight-, an overweight- and an obese trajectory in their sample, but failed to detect increasing trajectories. Stable trajectories comparable to our trajectories were identified by Botoseneanu and Liang [122], Rosenberger et al. [134] and Mustillo et al. [91, 133], where trajectories showing more variation over time were revealed in other studies [91, 95, 133, 135]. Generally, trajectories of similar shape are found across different cohorts and decreasing trajectories are scarcely detected in observational studies. Consistent across studies too [111, 116, 136], the unfavourable trajectories (i.e. those with increasing trajectories, or stable overweight/obese trajectories) in our study showed higher levels of markers of low-grade inflammation compared to the normative trajectory. Interestingly, however, the unfavourable trajectories did not differ significantly from each other. Moreover, although the BMI-trajectories clearly

differ with respect to their visit 1 and visit 4 values of CRP and fibrinogen, the increase in these markers over time differed only slightly; it appeared only the two increasing trajectories (although not to the same extent) differ with the normative trajectory with regards to their CRP- and fibrinogen change scores. As BMI and CRP in particular were hypothesized to be associated longitudinally, it follows that stable trajectories would not necessarily have increased change scores. This can also be seen in **table 6**, where descriptive information by BMI-trajectory is represented. It should be noted that although the BMI-trajectories did not differ with regard to their change scores, they differed significantly and to a great extent with regards to their CRP- and fibrinogen levels at visit 1 and 4 cross sectionally. These findings highlight the fact that the sole analysis of change scores can be misleading if no actual change over time is apparent in the sample, even though baseline differences are clear.

Also, even though we identified six distinct trajectories of body weight development, where some showed some degree of variation in trajectory, these trajectories were all still relatively stable regarding their development of BMI. Therefore, we should be aware of the fact that the lack of variation in longitudinal development in this sample may mask even larger differences in CRP- or fibrinogen levels, although trajectories with greater variation are generally found in younger cohorts [91, 133, 137] and cohorts with a very long follow-up period [138].

By the classification of subjects in six distinct trajectory classes, information on the course of BMI during visits 1, 2, 3 and 4 is taken into account, which is ignored when analyzing BMI development using change scores. Therefore, the elevated CRP-values in the stable trajectories (i.e. including subjects that do not change over time) will only have been detected by this approach and would have partly been obscured, would we have solely performed an analysis with BMI change scores. However, categorisation of subjects in six distinct subgroups does cause some information loss, highlighting the importance of both approaches.

Mechanisms

Although the mechanisms relating obesity and CVD are not fully understood, it is thought that long-term overweight and -obesity and prolonged weight fluctuation increase atherogenesis [117]. Atherogenesis is the process of forming plaques in the inner lining of the arteries that in part can lead to atherosclerosis or preclinical CVD. It might be that increased levels of CRP or fibrinogen

mediate the formation of plaques in part together with other factors such as endothelial dysfunction [110, 111]. Clearly, in our study, the trajectories with prolonged overweight/obesity showed increased levels of both markers of low-grade inflammation compared to the normative trajectory, although we were unable to assess a fluctuating trajectory. Importance of distinct trajectories, including fluctuating trajectories [91, 92, 120, 133, 139, 140] have been shown to be of particular interest in this setting. It would, therefore, be interesting for future research to investigate the degree of atherosclerosis in our subgroups, either cross-sectionally or longitudinally to explore the abovementioned hypotheses, or in a cohort with more heterogeneity in weight development (where fluctuating trajectories might be revealed). Unfortunately, information on the degree of atherosclerosis is currently not available within the Spokane Heart Study, but we will strongly consider the inclusion of these measurements in future rounds of measurement.

Some research suggests that weight fluctuation, including weight loss, in overweight/obese individuals may actually have adverse health consequences [141]; accordingly some question the overall risk/benefit value of promoting weight loss interventions and dietary modification in this population (see for example [142]). Our findings, however, suggest that there are longitudinal adverse inflammatory consequences for individuals with both stable overweight/obese BMI trajectories, and with increasing BMI trajectories, relative to non-overweight/obese individuals with stable BMI trajectories.

The results from the analyses regressing class membership on the markers of low-grade inflammation pointed towards a slightly different picture for CRP and fibrinogen; clear differences in CRP and only small (non-significant) differences in fibrinogen were observed. This may lead towards the idea of a threshold effect. CRP, for example is regulated by levels of Interleukin-6 levels, which are directly and extensively secreted by adipose tissue, and therefore, CRP-values will be directly influenced by changes in adipose tissue. Another plausible mechanism linking low-grade inflammation to body weight includes the role of Adiponectin [143, 144]. Adiponectin opposes the role of Interleukin-6 and as Adiponectin levels decrease with increasing levels of overweight and obesity, CRP-levels will increase. Fibrinogen, on the other hand, is less directly tied to adipose tissue [107]. Besides a marker of low-grade inflammation, fibrinogen is also a marker of haemostasis, (or blood clotting) suggesting a possible less direct influence of adipose tissue on its levels.

Methodological issues: strengths and limitations

We used observational data over multiple time points to investigate our research questions as thoroughly as possible. We had information on important confounders known (e.g. oral contraceptive use, lifestyle factors) and were able to correct for those in our models, although we only have information on these factors available at baseline and no continuity in the follow-up period. Further, we used contemporary statistical techniques to unravel heterogeneity in body weight development. LCGM categorize participants on the basis of their developmental pattern, instead of on a-priori classification in predefined subgroups [6, 18]. These techniques have shown to be very promising for applications within obesity research [91, 92, 120, 133, 139, 140] and do provide researchers and clinicians with new insights for further research, in particular due to their *data-driven* nature. The unfavourable trajectories that were identified with LCGM in our study consisted mostly of relatively few participants (8, 15 and 18 participants, respectively). Although these are positive findings in terms of public health, small sample sizes do challenge elaborate statistical analyses. These small sizes might further complicate the generalisability of the BMI-trajectories. Surprisingly, for example, subjects classified into the increasing to morbidly obese trajectory (N=8) reported a relatively healthy lifestyle (non-smoking, high sports participation, often restricting their diets), but still increased approximately 8 BMI points over 8 years. These findings could either suggest socially desirable answering [145], or might point towards a non-representative subgroup.

The importance of body fat distribution, as opposed to body weight for the quantification of cardiovascular disease risk especially has been highlighted in many studies [146, 147]. Unfortunately, the Spokane Heart Study does not have information on body fat distribution available at the visits we used in the current study. Although some studies report that associations between measures of fat distribution such as waist circumference and measures of CVD are stronger compared to associations between BMI and measures of CVD [148], others find associations of similar strength [149], especially for Caucasian subjects. Nevertheless, the utilization of BMI as a measure of body weight is very common in existing scientific research as well as within the World Health Organization, allowing for a broad comparison of the results. This is especially relevant for weight trajectory research, where BMI is the most common utilized weight measure. The lack of body fat distribution measures in our analyses is, however still a limitation that should be taken into account when drawing conclusion from our results.

Conclusions and implications

In conclusion, the results of this study show that in a community-based sample of males and females, the development of body weight is heterogeneous; six distinct trajectory shapes could be identified. Statistically significant differences in CRP- and fibrinogen levels were apparent between the trajectories, where the unfavourable trajectories differed from the normative trajectory and differences were more profound for CRP than for fibrinogen. Our identified BMI-trajectories partly confirm previous findings from studies with varying subject characteristics and many studies find negative health consequences in unfavourable trajectories [91, 95, 133, 150]. Clinical research could, therefore, explore the clinical utility of these distinct trajectories, relative to each other, in disease tracking- and management systems. Overall, more longitudinal research is needed to understand the short- and long-term benefits and risks associated with sustained weight loss, weight gain and weight fluctuation.

Acknowledgement, competing interests

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