Clustering of Lifestyle CVD Risk Factors and Its Relationship With Biological CVD Risk Factors

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The purpose of this study was (a) to investigate whether lifestyle risk factors cluster and (b) to investigate the influence of this clustering on biological CVD risk factors. This study was part of the Amsterdam Growth and Health Study (AGHS), an observational longitudinal study in which 6 repeated measurements were carried out on 181 13-year-old subjects over a period of 15 years. A longitudinal analysis (carried out with generalized estimating equations) showed no significant clustering of lifestyle risk factors at the population level. For each subject at each separate measurement period, lifestyle risk factors were summed to form a cluster score. A longitudinal linear regression analysis showed no significant relationship between the cluster score and biological CVD risk factors, except for a significant inverse relationship with cardiopulmonary fitness. In general, however, the results did not support the assumption that clustering of unhealthy lifestyle is related to biological CVD risk factors.

Cardiovascular disease (CVD) is one of the most serious problems in public health. The term *cardiovascular disease* refers to all affections of the blood circulation system. The most common forms of CVD are coronary heart diseases and cerebrovascular accidents. These affections are the direct or indirect result of atherosclerotic changes of the blood vessels. These changes will ultimately lead to an insufficient oxygen supply of the heart muscle or the brain, which can lead to a heart attack or a stroke (3, 23). An important primary prevention strategy for CVD is to induce favorable changes in lifestyle risk factors like physical inactivity, smoking behavior, and particular dietary patterns. Not only are these lifestyle risk factors directly related to CVD but they are also indirectly related to CVD through their relationship with biological risk factors like hypercholesterolemia, hypertension, and obesity (10).

Studies on lifestyle risk factors and their associations with biological risk factors have often been conducted in adult populations (17), from a preventive point of view, it is more important to study these relationships in young adults. Not only does the origin of CVD lie in early childhood, but it is also believed that

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patterns of unhealthy behavior are formed in adolescence and young adulthood. Although lifestyle variables independently affect health status, interdependencies among these lifestyle variables are frequently observed (8). This clustering of lifestyle risk factors (i.e., a combination of two or more lifestyle risk factors exhibited by one person) may introduce a health risk that is greater than one would expect from the sum of the individual risk factors (2, 11). It is therefore of interest to investigate the relationship between this and biological CVD risk factors. One of the problems of studies addressing the relationship between lifestyle and biological CVD risk factors is their cross-sectional design. Due to a lack of a time course, this means that it is difficult to distinguish between cause and effect (24). The Amsterdam Growth and Health Study (AGHS) is an observational longitudinal study in which, from the age of 13 years onwards, over a period of 15 years, six repeated measurements were carried out (14). Given this design and the fact that both lifestyle and biological CVD risk factors were measured, this study creates the unique possibility to investigate the relationships mentioned above.

The purpose of our study was (a) to investigate whether lifestyle risk factors (physical inactivity, smoking behavior, a lack of alcohol consumption, and unfavorable dietary intake) cluster and (b) to investigate the influence of this clustering on biological CVD risk factors (body fatness, body fat distribution, cardiopulmonary fitness, blood pressure, total serum cholesterol, and the ratio total serum cholesterol:high density lipoprotein cholesterol).

Methods

Design and Subjects

This study is part of the AGHS, an observational longitudinal study that started in 1977 with healthy subjects from a secondary school in Amsterdam. The socioeconomic status of the subjects, derived from questions to their parents about profession, income, and educational level, was just above the average for Dutch families in general (14). At the start of the study, 307 subjects (148 male and 159 female) were recruited. The mean age of the subjects at the first measurement was 13 years. During the first 4 years of the study, annual measurements were carried out. In total, 233 subjects (102 male and 131 female) completed these four annual measurements. In 1985, at the age of 21 years, 200 subjects (93 male and 107 female) were measured for the fifth time, and in 1991 at the age of 27 years, a sixth measurement was carried out on 181 subjects (83 male and 98 female). For the variables of interest in this study, no drop-out effects were observed. The total number of missing observations (for this population of 181 subjects used in the present study) during the measurement period was about 2%.

Lifestyle Risk Factors

Smoking behavior was examined by questionnaire. Subjects were asked if and how much they smoked tobacco, expressed in grams per week. Dietary intake was measured by a modification of a cross-check dietary history. In this dietary history, which was specially developed for the AGHS, all subjects were asked to recall their usual dietary intake by reporting frequency, amounts, and methods of preparation of the foods consumed (21). The dietary history method has been compared to

a 24-hour recall and was found to be the most reliable method to assess dietary intake in the AGHS (22). The method was used to assess the usual food intake during the previous month. All food items consumed were transformed into nutrients by the Dutch Food and Nutrition Table (6).

For the purpose of this study the Keys score was calculated. In this score the intakes of saturated fatty acids, polyunsaturated fatty acids, and cholesterol are combined into one weighted score by applying the following formula: $1.35 \times (2S - P) + 1.5 \times Z$, where S is the intake of saturated fatty acids, P is the intake of polyunsaturated fatty acids (both expressed as percentage of total energy intake), and Z is the intake of cholesterol (mg/100 kcal/day) (16). Alcohol consumption was asked for during the dietary history and expressed in grams per week. The amount of daily physical activity was measured with a structured interview covering 3 months prior to the interview. This method was validated with a heart rate integrator and a pedometer and was shown to be the most appropriate method for measuring daily physical activity in this particular population (28). Daily physical activity was expressed in a total weighted activity score (METs/week), which combines both duration and intensity of daily physical activities during work, school, sport, transportation, and leisure time (29).

Biological Risk Factors

Body fatness was assessed by calculating the sum of four skinfolds thicknesses (biceps, triceps, subscapular, and suprailiacic), according to the method of Weiner and Lourie (30), measured with a Harpenden skinfold caliper (Holtain, UK; van Rietschoten and Houwens, Rotterdam, The Netherlands). Body fat distribution was operationalized by calculating the ratio of the subscapular skinfold thickness and the triceps skinfold thickness. This ratio is probably the best indicator of a central pattern of body fat in the population under study (18).

Cardiopulmonary fitness was measured using a standard running test on a treadmill with a constant speed of 8 kph. In this maximal test the slope was increased every 2 min by 2.5% or 5% depending on the heart rate of the subject (15). The test was continued until complete exhaustion. Maximum slope (%) reached during the running test was used as a measure of cardiopulmonary fitness. Blood pressure was measured by an indirect method using a sphygmomanometer (Speidl-Keller No. 2010, Franken and Itallie, The Netherlands). While the subject was seated in a chair, a standard pressure cuff was placed around the left upper arm. Both systolic and diastolic blood pressure of the arteria brachialis were measured twice, and the lowest value was recorded. Blood pressure was expressed in mmHg. For the determination of serum cholesterol from each subject, approximately 10 ml of venous blood was taken from the vena antecubitus with a vacutainer. Blood sampling and serum preparations were done between 8:30 a.m. and 9:30 a.m. with subjects in a non-fasting state. Analyses were carried out in the Department of Human Nutrition of the Agriculture University of Wageningen.

External quality control took place with samples from a World Health Organization reference laboratory (Lipid Standardization Laboratory, U.S.). Total serum cholesterol (TC) was analyzed according to Huang and colleagues (9) and Abell and colleagues (1), and high density lipoprotein cholesterol (HDL) was analyzed according to Burnstein and Samaille (5). Both were expressed in mmol/L, and in this study both TC and the TC:HDL ratio were used as biological CVD risk factors.

Statistical Analysis

To assess the number of lifestyle risk factors present in each subject at each of the six repeated measurements, the lifestyle risk factors were defined as follows: (a) positive smoking status; (b) being in the highest gender specific quartile of the Keys-scores; and (c) being in the lowest gender specific quartile of the weighted physical activity score. A longitudinal logistic regression analysis was done to investigate if clustering of lifestyle risk factors exists in this population. Therefore, all three lifestyle risk factors were related to each other. This longitudinal analysis with dichotomous variables (high risk vs. low risk), led to an odds ratio that indicates the overall relationship between the lifestyle risk factors along the whole longitudinal period. The longitudinal analysis was done by generalized estimating equations (GEE) (31).

Secondly, for each of the subjects at each of the repeated measurements, the lifestyle risk factors were combined to form a cluster score. A cluster score of zero means that the subject had no lifestyle risk factors present at the time of measurement; a cluster score of three means the subject had all three lifestyle risk factors present. To investigate the longitudinal relationship between the clustering of lifestyle and biological CVD risk factors, GEE was used. To do so, the cluster score was dichotomized (subjects with two and three risk factors vs. the other subjects). The statistical model used in this study has been described extensively by Twisk and colleagues (26). To make the magnitudes of the longitudinal linear regression coefficients comparable to each other, the continuous outcome variables (i.e., the biological CVD risk factors) were transformed into z-scores. The outcome of this longitudinal linear regression analysis is a regression coefficient that indicates the relationship between the dichotomized cluster variable and the biological risk factors along the entire longitudinal period. All statistical analysis were carried out correcting for gender and time. The analysis were carried out using the Statistical Package of the Social Science (SPSS) (25) and the Statistical Package of Interactive Data Analysis (SPIDA) (7). In all analyses a 5% significance level was used.

Results

To analyze whether lifestyle risk factors tend to cluster in the population of the AGHS, a GEE analysis was carried out in which each lifestyle risk factor was related to the other lifestyle risk factors. For physical activity and unfavorable dietary intake (i.e., high Keys-score) the population was divided into quartiles (meaning that the number of subjects in the risk group was about 45). The number of smokers increased during adolescence from 5 at the age of 13 years to 37 at the age of 16 years. At the age of 21 years, 58 subjects were smokers, and at the age of 27 years the number of smokers was 51. The results of the GEE analysis (Table 1) showed no significant relationships between the various lifestyle risk factors. Table 2 shows the number of subjects in the different cluster score groups. Although Table 1 shows that there is no significant clustering of lifestyle risk factors in the population, Table 2 shows that clustering does exist at the individual level (i.e., there are subjects with two or more lifestyle risk factors). Because of this, a linear longitudinal GEE analysis was carried out to investigate whether this clustering of lifestyle risk factors at the individual level does have an influence on biological

Table 1   Odds Ratio (OR), 95% Confidence Interval (CI), and *p* Values for the Longitudinal Relationships Between Lifestyle CVD Risk Factors

	Smoking	Low physical activity score	High Keys score
Smoking			
OR	—	0.75	1.01
CI	—	(0.50–1.12)	0.77–1.44
p	—	0.16	0.74
Low physical activity score			
OR	0.78	—	0.84
CI	0.52–1.17	—	0.58–1.22
p	.23	—	.36
High Keys score			
OR	1.01	0.85	—
CI	0.69–1.48	0.58–1.26	—
p	.96	.42	—

Table 2   Number of Subjects With Different Numbers of Lifestyle Risk Factors at Different Ages

Number of risk factors	Age (years)					
	13	14	15	16	21	27
0	101	92	83	71	68	73
1	58	67	78	82	69	77
2	17	16	20	20	30	28
3	0	1	0	0	4	2

Note. The cluster score was based on: smoking, low physical activity, and unfavorable dietary intake.

CVD risk factors. The results of this analysis (Table 3) showed a significant relationship between the high risk group and the maximum slope ($\beta = -0.13$, $p < .01$). The clustering of lifestyle CVD risk factors was not significantly related to any of the other biological CVD risk factors.

Discussion

In this study no significant clustering of lifestyle CVD risk factors was found, which is in contrast with the results of Raitakari and colleagues (23). In their Cardiovascular Risk in Young Finns study, clustering was based on nonprudent diet (defined as being in the highest percentile of a diet score distribution, having a diet

Table 3 Standardized Regression Coefficients, 95% Confidence Interval (CI) and *p* Values for the Longitudinal Relationships Between the Cluster Score and Biological Risk Factors for CVD

Risk factor	β	95% CI	*p*
Sum skinfold	0.10	−0.02–0.22	.11
Ratio skinfold	0.07	−0.02–0.17	.14
SBP	−0.05	−0.19–0.08	.43
DBP	−0.04	−0.21–0.12	.60
Slope	−0.13	−0.21–(−0.04)	<.01
TC	0.10	−0.05–0.25	.17
TC:HDL	0.07	−0.08–0.23	.35

Note. Sum skinfold = sum of four skinfolds (mm): biceps, triceps, subscapular, and suprailiacal; Ratio skinfold = ratio subscapular skinfold: tripceps skinfold; SBP = systolic blood pressure (mmHg); DBP = diastolic blood pressure (mmHg); Slope = maximum slope (%); TC = serum cholesterol (mmol/L); TC:HDL = ratio total serum cholesterol:high density lipoprotein cholesterol.

with an excess of fat), daily smoking, physical inactivity (only being physically active once a month or less), and frequent inebriation (over 10 occasions during one's lifetime). Their results showed significant clustering of all unhealthy lifestyles. There are some possible explanations for the difference between the present study and the study of Raitakari and colleagues (23). First of all the number of subjects in the AGHS (181) is relatively small compared with the 484 subjects used in the Young Finns study, which makes it more difficult to detect significant relationships.

Another possible explanation can be the fact that the ages of the subjects do not correspond. In the AGHS, the subjects were followed from 13 to 27 years, while the subjects of Young Finns study were followed from 18 to 24 years of age. It is possible that young subjects are able to influence their lifestyle to a lesser extent than somewhat older subjects because of a stronger influence of their parents. To verify this possible explanation for the difference between the two studies, a statistical analysis was done to investigate if AGHS clustering does exist at the ages of 21 and 27 years. The results of this additional analysis showed no significant clustering of lifestyle risk factors, however.

A third possible explanation can be the socioeconomic and educational status of the subjects, which is assumed to be related to clustering of lifestyle CVD risk factors. Kok and colleagues (17) showed twice as much clustering of lifestyle risk factors in subjects with a lower education compared to subjects with a university education. Raitakari and colleagues (23) also found that higher education was an independent protective factor against lifestyle risk factor clustering. The subjects in the AGHS were all students from the same secondary school in Amsterdam, with a socioeconomic status just above the average for Dutch families in general (14). So maybe the absence of significant clustering in the AGHS is due to the relatively high socioeconomic and educational status of the subjects.

In the second part of this study, the association between individual lifestyle risk factor clustering and biological CVD risk factors was investigated. The results of the GEE analysis showed that clustering of lifestyle risk factors was significantly inversely related to cardiopulmonary fitness, which was operationalized as the maximum slope reached during a standard maximal running test on a treadmill. No significant relationships were found between clustering and the sum of four skinfolds, and the ratio of the subscapular and triceps skinfolds.

The relationship between clustering of lifestyle risk factors and these biological risk factors can possibly be covered by the opposite influences lifestyle may have on these biological risk factors. Smoking, for example, is assumed to be inversely related to skinfold thickness, while physical inactivity is assumed to be positively related (12). So a combination of these lifestyle risk factors can cause that the effect of low physical activity is reduced by the opposite effect of smoking. However, in the Young Finns study (23), a positive relationship was found between clustering of lifestyle risk factors and body fatness (operationalized as body mass index). In the present study no significant relationships were found between clustering of lifestyle CVD risk factors and systolic and diastolic blood pressure, which can be due to the same mechanism as described above. Smoking is inversely related to blood pressure (19, 27), while physical inactivity is positively related to blood pressure (13). In the Young Finns study (23) on the other hand, clustering of lifestyle risk factors was positively related to systolic blood pressure; however, this was only observed in women. The results of the present longitudinal analysis, furthermore, did not show any significant relationships between clustering of the lifestyle and lipoprotein levels. It is not clear why no relationships were found; all lifestyle risk factors used in this study are known to have an increasing effect on both TC and the TC:HDL ratio (4, 27).

There are several possible explanations for this more or less unexpected finding. In the present study, low physical activity and unfavorable dietary intake were based on the lower quartile of the weighted activity score and the upper quartile of the Keys-scores. In other words, the distinction between a "high risk" group and a "low risk" group was based on arbitrary relative values, instead of objective absolute values. For example, a physical activity score below 2000 METs/week may be considered to be a lifestyle CVD risk factor (20). It is possible that the subjects in the lowest quartile of the weighted activity score have a physical activity score of more than 2000 METs/week. To verify this, the percentage of subjects was calculated with a physical activity score below 2000 METs/week in the high risk quartiles at all repeated measurements. Only at the age of 21 years do all subjects in the high risk quartile have a physical activity score below 2000 METs/week. During the first 4 years of measurement, only 15% or less had a weighted activity score below 2000 METs/week, while at the age of 27 years, 46% of the high risk quartile had an activity score below 2000 METs/week.

Furthermore, in the present study, all lifestyle risk factors were equally valued. Since differences in potential impact on health exists, it would probably be correct to introduce a weighing factor for each lifestyle risk factor. Smoking, for example, has a much greater impact on the development of CVD than an unfavorable dietary intake. The results of this study could possibly be more realistic when differences in potential impact on health were taken into account. Hulshof and colleagues (11) used a weighing system to assess a cluster score of dietary parameters, which was based on a somewhat subjective and arbitrary interpretation of the authors. When a weighing system is used, it should best be based on solid numbers like odds ratios, relative risks,

or population attributable risks. Because the outcome variables in this study were biological CVD risk factors and because in the literature there are no criterion figures present for the strength of the relationships between lifestyle parameters and biological CVD risk factors, a solid weighing system could not be used.

The purpose of this study was to examine the association between clustering of lifestyle CVD risk factors and the biological CVD risk factors. The best way to do this would have been a comparison between subjects with zero lifestyle risk factors and subjects with all three lifestyle risk factors present. In this way there would have been a good distinction between the two groups. However, the number of subjects with a cluster score of three was practically zero (Table 2). Therefore, we had to include subjects with two lifestyle risk factors in the "high risk" group. As a result of this, the relationship between lifestyle risk factors and biological CVD risk factors may have been less pronounced than it would have been when two extreme lifestyle risk groups were compared.

In conclusion, it can be stated that no significant clustering of CVD lifestyle risk factors was found. There was an inverse relationship found between "individual" clustering of CVD lifestyle risk factors and the maximum slope reached during a standard running test. The results of this study did not support the assumption that clustering of unhealthy lifestyle is related to biological CVD risk factors, and therefore, no recommendations can be given for primary prevention strategies regarding CVD.

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