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Estimation of seasonal variations in risk factor profiles and mortality from coronary heart disease

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Summary. *Objective:* Seasonal variations in coronary heart disease (CHD) and related risk factors have been reported previously. However, no studies to date quantify the contribution of seasonal variations in risk factors to actual mortality in both men and women using a single database of sufficient size and follow-up.

Methods: We investigated the database from the Western Austrian Vorarlberg Health Monitoring and Promotion Programme (VHM&PP) including over 450.000 repeated measurements of 149.650 individuals between 1985 and 1999.

Results: Of a total of 1266 deaths from CHD (ICD-9 410–414), 353 deaths occurred between December and February (27.9%), in contrast to 275 (21.7%) between June and August. While the frequency of deaths through acute myocardial infarction (ICD-9 410) was similar over the seasons, chronic forms of CHD (ICD-9 414) occurred significantly ($p < 0.001$) more frequently in winter. Total cholesterol, blood pressure and body mass index showed pronounced seasonal variations with average levels significantly higher during the winter months in all age groups and both sexes, giving an estimated increase in SCORE risk of 6.8% in men and 3.6% in women. However by contrast, use of single time point risk factor data tended to over-estimate subsequent 10 year mortality if measured in winter and the converse in summer.

Conclusion: For the first time, this study quantifies the contribution of seasonal risk factor variation to CHD mortality. The consistent effect across demographic groups suggests that this is a real physiological phenomenon and not an artefact of living conditions. Interpretation of standard risk scores should take account of this seasonal fluctuation in subsequent investigation and follow-up.

Key words: Coronary heart disease, mortality, risk factors, seasonal variations, SCORE.

Introduction

Seasonal variation in mortality, particularly from cardiovascular and respiratory conditions, has been repeatedly observed in epidemiological studies [1] and is known to contribute to excess morbidity and mortality in winter [2]. Few studies have been large enough to examine systematically the question of seasonal variation on coronary heart disease events and on risk factors in both men and women, across the age spectrum and with repeated measures in the same standardised database. While mortality rates from coronary heart disease (CHD) have declined substantially in many Western countries [3], seasonal fluctuations remain a significant issue and it is possible that at least some of the between country variations in patterns are attributable to this phenomenon [4]. The role of cardinal risk factors such as smoking, blood pressure, body weight, and cholesterol has been investigated [2]. It has been observed that total cholesterol and lipoprotein profiles show seasonal variation [5–7], that winter blood pressures exceed those in summer [8–9] and that obesity is more common in winter [10]. A number of studies have attempted to investigate underlying mechanisms and it is not clear whether this is an intrinsic physiological phenomenon or related to external factors associated with lifestyle and living conditions, such as increased activity and dietary changes during summer or variations in housing standards, including availability of central heating, in winter. Direct effects of environmental factors such as ambient temperature [11] and ultraviolet radiation [12], have been proposed. Indirect mechanisms have been suggested also, such as the association between respiratory infections and CHD mortality [13].

No studies published to date explain the contribution of these seasonal variations in risk factors to actual mortality, nor has the practical clinical significance of this phenomenon been explored. We therefore examined the database from the Vorarlberg Health Monitoring and Promotion Programme (VHM&PP) [14] in order (1) to estimate the magnitude of seasonal differences in CHD mortality and risk factors and (2) to establish a direct relation of an individual's risk profile according to the season of examination with actual mortality.

Methods

Study population and measurements

In Vorarlberg, the westernmost province of Austria, population-based documentation of cardiovascular risk factors has

been performed routinely since 1970 by the Agency for Social- and Preventive Medicine. From the outset, this ongoing extensive risk factor surveillance and treatment referral programme has comprised medical examinations of more than two thirds of the entire population of this province.

A total of 149,650 individuals, 67,413 men (44.9%) and 82,237 women (55.1%), participated in the VHM&PP between 1985 and 1999. During this period, men underwent 191,629 (42.2%) and women 262,819 examinations (57.8%); a total of 454,448 in all. The participants underwent unequal numbers of repeated examinations, ranging from 1 to 14 examination visits. Characteristics of the study population have been described previously [14–16]. The examinations were performed in a standardized way by trained general practitioners and internists and included a physical examination and the recording of socio-demographic information. The physical examination included a fasting blood sample and measurement of height, weight, blood pressure, total cholesterol, triglyceride, and blood glucose. The methodology has been described previously [17]. Informed consent to store and process the data was obtained from all participants at each examination time-point. A total of 5,393 persons died in the course of follow-up and cause of death was linked to the database using a validated record-linkage procedure.

Statistical analysis

Estimates of risk factor values were given as means ± standard errors and tabulated according to season, gender and age-groups. Seasons were categorized according to metereologic standards into spring (March–May), summer (June–August), autumn (September–November) and winter (December–February). Regression models using the General Estimation Equation (GEE) [18] method were used to adjust risk factor values for confounding effects of age, body mass index, smoking, work status, country of origin, year of visit, days between visits and interactions, as appropriate. GEE-regression models were selected in order to make full use of all repeated measurements. Mortality through CHD (ICD-9 410–414) during follow-up was recorded and analyzed with regard to seasonality and age of death. A subgroup of the participants, 23,526 men and 31,861 women (55,387 participants in total) were followed-up for at least 10 years. For this subgroup, based on the measurements of the first examination, the risk of death from CHD was calculated in a stratified manner according to seasonality, applying the recently published SCORE [19] risk function for low risk regions. The SCORE risk assessment is derived from a large dataset of prospective European studies and is recommended by the European Society of Cardiology for use in the clinical management of cardiovascular risk in clinical practice. Separate risk functions were developed for high- and low risk regions of Europe. The SCORE risk function used in this paper predicts 10-year risk of fatal CHD calculating the absolute risk of an individual based on the person's age, gender, total cholesterol, systolic blood pressure and smoking status. We compared the predicted fatal CHD events by the SCORE function with the estimated 95% confidence intervals of the actual observed CHD events within 10-year follow-up. Statistical analysis was performed using Stata [20] statistical software.

Results

Health examinations were performed most frequently in spring (125,958 visits, 27.8%) followed by autumn (124,257 visits, 27.3%), winter (105,905 visits, 23.3%) and summer (98,328 visits, 21.6%). There were only small differences in seasonal patterns of participation regarding age, sex, work status, nationality and year of examination.

Table 1 shows adjusted estimates of risk factors according to seasonal pattern (summer vs. winter). It can be seen that in all age groups and for both men and women there are clear and significant effects, with average risk factor levels lower in the summer months. The phenomenon was most marked in the case of total cholesterol, systolic blood pressure and body mass index with a very slight trend in the opposite direction in the case of glucose. While the trend for triglyceride is similar for women and men, it is not significant in the youngest age category of men. In men 50 years and older there is a significant difference ($z = 6.14$, $p < 0.001$). The estimated 10 year risk SCORE for fatal CHD, also shown, was accordingly significantly lower in summer for all demographic groups. While the absolute risk was considerably higher in men than women and was strongly age related, the relative risk for winter compared with summer was very similar in all male groups, and the two younger female groups, averag-

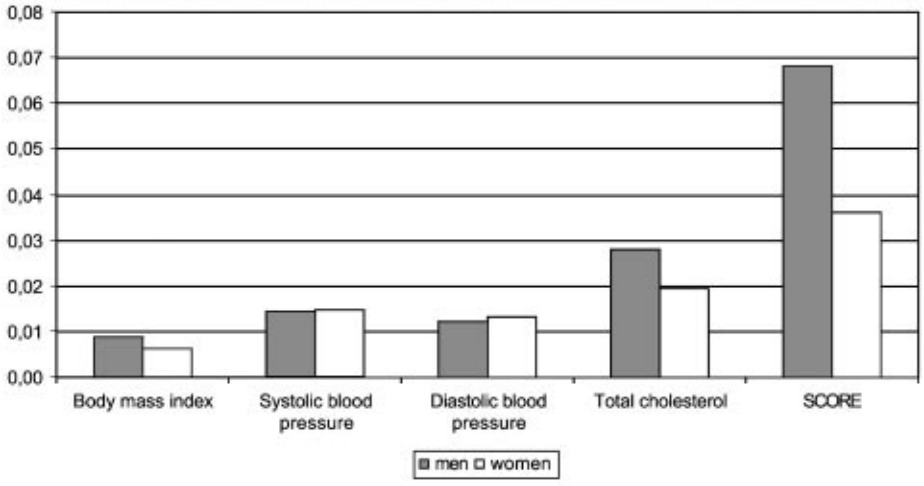

Fig. 1. Excess percentage of SCORE and risk factor values in winter versus summer

Table 1. Comparison of adjusted estimates of risk factors between visits in summer and winter by sex and age-groups, VHM&PP 1985–1999

Men	19–49 years		50–64 years		65 years and older		All age-groups
	Summer[a] (n=17610, 23299 visits)[b]	Winter (n=20226, 27518 visits)	Summer (n=8232, 11423 visits)	Winter (n=9588, 13852 visits)	Summer (n=3717, 5454 visits)	Winter (n=4151, 6141 visits)	Effect of winter vs. summer (Sig.)
Body mass index (kg/m^2)	25.03±0.02[c]	25.26±0.18	26.33±0.24	26.55±0.02	26.09±0.04	26.29±0.04	z=14.17, p<0.001
Systolic blood pressure (mmHg)	126.97±0.10	128.67±0.09	137.76±0.17	139.97±0.16	146.52±0.28	148.89±0.26	z=9.47, p<0.001
Diastolic blood pressure (mmHg)	80.36±0.07	81.25±0.61	84.35±0.10	85.39±0.09	83.75±0.14	85.01±0.13	z=7.06, p<0.001
Total cholesterol (mg/dl)	213.17±0.25	219.16±0.25	232.91±0.36	239.38±0.35	226.89±0.50	233.95±0.53	z=15.77, p<0.001
Triglycerides (mg/dl)[d]	126.57±0.43	127.26±0.4	137.5±0.63	143.25±0.6	126.63±0.78	130.81±0.77	z=–0.09, p=0.928[e]
Fasting blood glucose (mg/dl)	83.71±0.14	83.07±0.14	90.01±0.17	89.64±0.16	92.12±0.25	91.72±0.24	z=–4.85, p<0.001
SCORE: 10 year risk for fatal CHD (%)	0.14±0.001	0.15±0.001	1.58±0.013	1.72±0.014	5.00±0.054	5.36±0.055	z=10.35, p<0.001
Women	19–49 yrs		50–64 yrs		65 yrs and older		All age-groups
	Summer (n=23217, 32684 visits)	Winter (n=23828, 32985 visits)	Summer (n=11079, 16222 visits)	Winter (n=11584, 16655 visits)	Summer (n=6166, 9246 visits)	Winter (n=6084, 8753 visits)	Effect of winter vs. summer (Sig.)
Body mass index (kg/m^2)	23.55±0.02	23.74±0.02	26.17±0.027	26.33±0.02	26.51±0.04	26.59±0.04	z=8.81, p<0.001
Systolic blood pressure (mm Hg)	119.79±0.09	121.79±0.09	137.93±0.15	140.29±0.15	150.27±0.23	151.98±0.23	z=14.32, p<0.001
Diastolic blood pressure (mm Hg)	76.53±0.05	77.66±0.05	83.69±0.08	84.69±0.08	84.72±0.11	85.76±0.11	z=12.93, p<0.001
Total cholesterol (mg/dl)	202.15±0.20	205.52±0.21	239.64±0.31	244.93±0.33	244.22±0.44	250.57±0.47	z=12.99, p<0.001
Triglycerides (mg/dl)[d]	91.06±0.22	90.24±0.22	115.50±0.39	116.21±0.38	127.67±0.57	128.78±0.56	z=–5.67, p<0.001
Fasting blood glucose (mg/dl)	84.04±0.12	83.56±0.12	90.60±0.15	90.09±0.15	94.21±0.21	93.60±0.21	z=–4.41, p<0.001
SCORE: 10 year risk for fatal CHD (%)	0.01±0.001	0.011±0.001	0.505±0.005	0.54±0.005	2.02±0.025	2.09±0.026	z=8.86, p<0.001

[a] Summer (June–August), winter (December–February); [b] excluding 1,4% missing values within body mass index and blood pressure and 3,1% missing values within cholesterol, triglygerides, fasting blood glucose and risk score (values missing partly due to missing consent of the participants), excluding measurements before introduction of fasting glucose measurements; [c] estimated means ± standard errors by GEE population-averaged models, adjusted for age, body mass index, smoking, work status, country of origin, year of visits, days between visits and interactions as appropriate; [d] Triglycerides, glucose and risk score were transformed logarithmically for calculating the GEE models, means thus obtained represent geometric means; [e] $z = 6.14$, $p < 0.001$ in men over the age of 50 years.

ing 6.8% higher in men and 3.6% higher in women (Fig. 1). During follow-up, observed mortality from coronary heart disease (ICD-9 410–414) was highest in winter, and was significantly different from mortality in summer ($p < 0.001$). Mortality and risk patterns by age are presented in Table 2. From a total of 1266 deaths, 353 deaths occurred between December and February (27.9%, 95%CI 25.4–30.4%), in contrast to 275/1266 (21.7%, 95%CI 19.5–24.1%) between June and August. However, there were no significant differences according to season in the acute and sub-acute types of CHD (ICD-9 410–411), a statistically significant summer/winter difference ($p < 0.001$) was only observed in chronic types of CHD (ICD-9 414). There were 194/642 (30.2%) ICD-9 414

Table 2. Excess SCORE and CHD mortality in summer versus winter by sex and age-groups, VHM&PP 1985–1999

	19–49 years		50–64 years		65 years and older		All age-groups	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Men								
SCORE (%)	0.14	0.15 (+7.14%)[c]	1.58	1.72 (+8.86%)[c]	5	5.36 (+7.20%)[c]	0.458	0.489 (+6.8%)[c]
Mortality	10[a]	11 (+10%)	34	40 (+17.65%)	97	144 (+48.45%)[c]	141	195 (+38.30%)[c]
Women								
SCORE (%)	0.01	0.011 (+10%)	0.505	0.54 (+6.93%)[c]	2	2.09 (+3.47%)	0.072	0.074 (+3.6%)[c]
Mortality	4	1 (–75%)	9	7 (–22.22%)	121	150 (+23.97%)	134	158 (+17.91%)[b]

[a] CHD deaths (ICD-9 410–414) in 149,650 persons between 1985 and 1998; [b] 628 deaths in summer and winter, 638 deaths in spring and autumn; [c] significantly different ($p < 0.05$).

Table 3. All cause mortality of a subgroup of persons who were followed-up for at least 10 years by season of the first examination, VHM&PP 1985–1999

	Spring		Summer		Autumn		Winter		All seasons	
	n[a]	deaths	n	deaths	n	deaths	n	deaths	n	deaths
Men	7003	530 (7.57%)	5017	400 (7.97%)	6097	470 (7.71%)	6409	442 (6.90%)	24526	1842 (7.51%)
Women	9830	516 (5.25%)	7371	411 (5.58%)	8712	433 (4.97%)	7904	340 (4.30%)[b]	33817	1700 (5.03%)

[a] Number of persons at visit 1 examined between 1985 and 1987, in total 58,343 persons; [b] significantly lower versus summer ($p < 0.05$).

events in winter, whereas only 118/642 (18.4%) deaths were recorded in summer (Fig. 2). Men who died from chronic types of CHD-events were on average 7.3 years older than men who died from acute types, women were 2.8 years older. However, after adjusting for age and gender, there was still a significant effect of seasonality on this type of CHD mortality.

A subgroup of the participants, 23,526 men and 31,861 women (55,387 participants in total) were followed-up for at least 10 years. For this subgroup, based on the measurements of the first examination, the 10-year risk of death from CHD was predicted, using the SCORE risk function. There were 637 (1.15%; 95%CI 1.06–1.24) fatal CHD events, 357 (1.52% 95%CI 1.36–1.68) in men and 280 (0.88% 95%CI 0.78–0.99) in women. This compared to 699 events (1.26%) predicted (relative overestimation overall 9.57%, $p < 0.05$), 413 (1.76%) in men and 286 (0.90%) in women (15.8% relative overestimation in men, $p < 0.05$, 2.3% in women). Figure 3 shows the comparison of actually observed versus predicted fatal CHD events based on this single baseline measure, stratified according to the season in which the measurements had

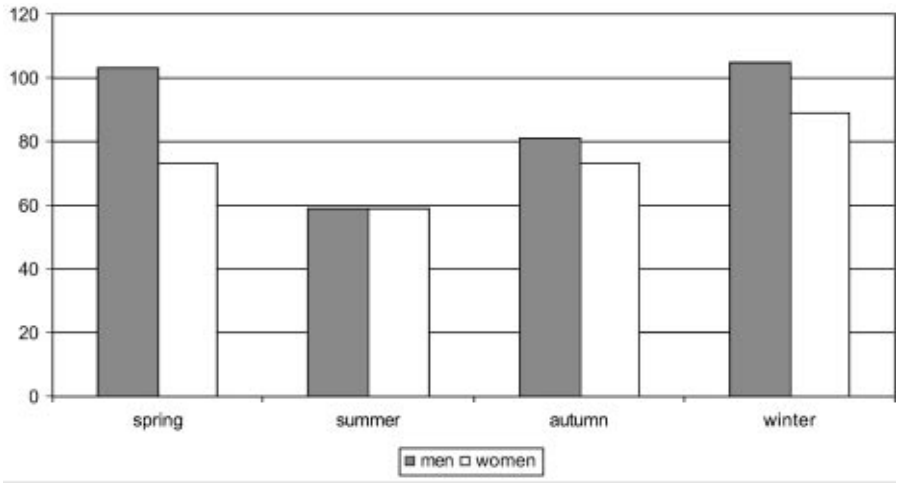

Fig. 2. Observed number of fatal chronic CHD events (ICD-9 414) in 67,413 men and 82,237 women between 1985 and 1999, tabulated by seasons

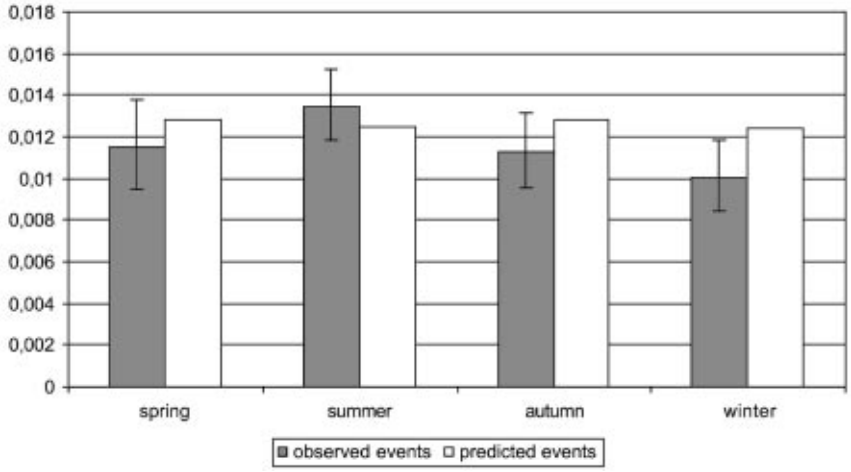

Fig. 3. Percentages and 95% confidence intervals of actual observed fatal CHD events versus predicted CHD events by the SCORE function, tabulated by seasons. SCORE predicted events were based on measurements at visit 1 (1985–1987) for 55,387 persons. Observed events were actual deaths from CHD death within 10 years follow-up

been taken. While the predicted rate of individuals examined in summer was lower than the actual rate, the opposite can be observed for spring, autumn and winter. The overestimation of subsequent mortality for those measured in winter was statistically significant ($p < 0.05$). A gender-specific comparison revealed that this pattern was only observable in men. There were no significant effects of season on the rate of overestimation in women. In men, however the difference was striking. 106 deaths from CHD within 10 years were predicted for men who had their examinations during winter months, but only 73 deaths actually happened (relative overestimation 45.2%, $p < 0.001$). 58.9% of the events that were overestimated by the SCORE function were based on winter measurements. In Table 3, deaths from all causes are presented, categorised according to season of initial examination. This shows that subsequent deaths of individuals examined in winter are lower than in summer in both males and females.

Discussion

We confirm in this very large data set sizeable and significant seasonal variation in both coronary heart disease mortality and in related risk factor levels. For the first time, this study examined seasonal variations of CHD mortality and risk factors using a single database with long-term follow-up and repeated measurements including relatively young respondents. In agreement with the literature [1, 4], we observed a pronounced winter excess mortality of fatal CHD events. While seasonal effects in individual risk factors were modest in magnitude, there were significant and consistent differences of mean risk factor levels between summer and winter. The effects are especially pronounced for total cholesterol levels, blood pressure and body-mass-index. Using the SCORE estimates the relative difference in risk factor profiles for all demographic groups was consistently higher in winter.

The fact that the seasonal effects across gender and age-groups on risk factor profiles are very consistent suggests an intrinsic physiological phenomenon, rather than related to socio-behavioural factors. In keeping with this is the fact that a seasonal effect has also been observed in animal populations [21]. The estimated relative incidence risk is higher overall in men than women but this is weighted by the higher likelihood of caseness in the older groups. In the oldest group of women the phenomenon is least marked but this may relate to differing patterns of risk in that group, observed previously in epidemiological studies, rather than being related to the seasonal effect [15].While we cannot rule out other suggested explanations, including associated reduction in dietary fat intake and increased activity levels in the summer months [22–24], it seems unlikely to be the primary explanation since both of these are likely to differ considerably according to age and sex, a pattern not seen here, especially among the men. Previous studies have focused on the impact of season on older people but our findings demonstrate that the effect is life-long, although its clinical impact on mortality is likely to be most pronounced at higher levels of absolute risk, in older people, as we show here. However the change in risk factor pattern does not account for more than a quarter of the observed mortality in the older groups. This means therefore that standards of living conditions and access to services must be playing a role.

Differences in blood pressure were slightly smaller than previously reported. Wilmshurst et al. [8] found a summer/winter difference of 5 mmHg in for systolic blood pressure. We found differences of less than 3 mmHg, depending on age-group and gender. Differences of this order seem may appear negligible at the individual level but on a population attributable basis are highly significant in contributing to excess morbidity and mortality [25]. In the case of cholesterol there may be a direct metabolic influence due to exposure to sunlight [26]. Previous conflicting reports for cholesterol may have been due to inadequate sample sizes as the effect at individual level is modest in magnitude [22]. A recently established prospective study will investigate physiological mechanisms in more depth [27].

The consistency of this seasonal observation over the lifetime and in different countries suggests that it is not for instance an artefact of screening patterns. However we do show important evidence for the impact of screening too on this phenomenon. Here we present a striking contrast of practical relevance in interpreting risk scores and counselling individual patients. We examined the relationship between mortality and seasonal variation in two ways. Conventionally, risk estimates tend to be based on a single visit, using this information to project long-term outcome. Taking this approach we demonstrate in men who had their risk factor measurements performed during winter months a striking effect of overestimation of predicted events by an internationally validated risk score [19] versus actually observed events. This can be explained in three possible ways. First, men are more likely to receive intervention than women and those classified as high risk in this programme may have benefited from preventive measures and/or medical treatment (mainly for hypertension since statins were not available in general practice at that time). However, since we do not have access to such clinical data in the VHM&PP, we are unable to answer this question definitively. It is true also however that mortality generally is higher in those screened in winter. This is likely to mean that those conscientious individuals who present for screening in winter are also more likely to take other protective measures as well. A more fundamental explanation is that the baseline risk score does not take account of the seasonal within-individual fluctuation and hence overestimates risk for those seen initially in winter. The strength of our study is that we have taken account as well of repeated measures and with this approach have quantified accurately the relative impact on the risk score of the seasonality phenomenon, with findings by this method much closer to the observed difference in mortality in winter or summer. In this sense it is the actual time of death not the time of measurement that is important.

In conclusion, this study has confirmed the consistent phenomenon of seasonal variation in both risk factor and mortality patterns and is large enough to quantify it accurately with an established score. The change in risk factor pattern however explains only partially the excess risk of mortality in older people. This means that socio-environmental factors are also important, supported by the observation that those who present for screening in winter actually have a better prognosis than those who do not, so that protective measures are important in preventing mortality. We urge caution however in predicting mortality with single measures in individuals and suggest that clinicians take explicit account in treatment regimens of season in interpreting likely intra-individual fluctuations in risk factor profile across the calendar year.

References

1. Healy JD (2003) Excess winter mortality in Europe: a cross country analysis identifying key risk factors. J Epidemiol Community Health 57: 784–789
2. Pell JP, Cobbe SM (1999) Seasonal variations in coronary heart disease. Q J Med 92: 689–696
3. Kelleher C (2001) Evolution of cardio-vascular risk factors – light at the end of the tunnel? Wien Klin Wochenschr 113: 552–557
4. Crawford VLS, McCann M, Stout RW (2003) Changes in seasonal deaths from myocardial infarction. QJM 96: 45–52
5. Fyfe T, Dunnigan MG, Hamilton E, Rae RJ (1968) Seasonal variation in serum lipids, and incidence and mortality of ischaemic heart disease. J Atheroscler Res 8: 591–596
6. Gordon DJ, Hyde J, Trost DC, Whaley FS, Hannan PJ, Jacobs DR, Ekelund LG (1988) Cyclic seasonal variation in plasma lipid and lipoprotein levels: the Lipid Research Clinics Primary Prevention Trial Placebo Group. J Clin Epidemiol 41: 679–689
7. Rastam L, Hannan PJ, Luepker RV, Mittelmark MB, Murray DM, Slater JS (1992) Seasonal variation in plasma cholesterol: Implications for screening and referral. Am J Prev Med 8: 360–366
8. Wilmshurst P (1994) Temperature and cardiovascular mortality. Br Med J 309: 1029–1030
9. Kunes J, Tremblay J, Bellavance F, Harnet P (1991) Influence of environmental temperature on the blood pressure of hypertensive patients in Montreal. Am J Hypertens 4: 422–426
10. Van Staveren WA, Deurrenberg PD, Burema J, De Groot LCPGM, Hautvast JGA (1986) Seasonal variation in food intake, pattern of physical activity and change in body weight in a group of young adult Dutch women consuming self selected diets. Int J Obes 10: 133–145
11. Enquselassie F, Dobson AJ, Alexander HM, Steele PL (1993) Seasons, temperature and coronary heart disease. Int J Epidemiol 22: 632–636
12. Scragg R (1982) Seasonal variations of mortality in Queensland. Community Health Stud 6: 120–129
13. Danesh J, Collins R, Peto R (1997) Chronic infections and coronary heart disease: is there are link? Lancet 350: 430–436
14. Ulmer H, Kelleher C, Diem G, Concin H (2003) Long-term tracking of cardiovascular risk factors among men and women in a large population-based health system: the Vorarlberg Health Monitoring & Promotion Programme. Eur Heart J 24: 1004–1013
15. Ulmer H, Kelleher C, Diem G, Concin H (2004) Why Eve is not Adam. Prospective follow-up in 149,650 women and men of cholesterol and other risk factors related to cardiovascular and all-cause mortality. J Womens Health 13: 41–54
16. Ulmer H, Diem G, Bischof HP, Ruttmann E, Concin H (2001) Recent trends and sociodemographic distribution of cardiovascular risk factors: results from two population surveys in the Austrian WHO CINDI demonstration area. Wien Klin Wochenschr 113: 573–579
17. Ulmer H, Bachmann J, Huber K, Concin H, Bischof HP (1997) Follow-up studies of routine health screening in Vorarlberg 1986–1994. Wien Klin Wochenschr 109: 160–164
18. Zeger SL, Liang KY (1986) Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42: 121–130
19. Conroy RM, Pyorala K, Fitzgerald AP, Sans S, Menotti A, De Backer G, De Bacquer D, Ducimetiere P, Jousilahti P, Keil U, Njolstad I, Oganov RG, Thomsen T, Tunstall-Pedoe H, Tverdal A, Wedel H, Whincup P, Wilhelmsen L, Graham IM on behalf of the SCORE project group (2003) Estimation of ten-year risk of fatal cardiovascular disease in Europe: the SCORE project. Eur Heart J 24: 987–1003

20. Stata Corporation (2001) Stata Statistical Software: Release 7, Special edn. College Station, TX
21. Shirai N, Terayama M, Takeda H (2002) Effect of season on the fatty acid composition and free amino acid content of the sardine Sardinops melanostictus. Comp Biochem Physiol B Biochem Mol Biol 131: 387–393
22. Matthews CE, Hebert JR, Freedson PS, Stanek EJ, Merriam PA, Ebbeling CB, Ockene IS (2001) Sources of variance in daily physical activity levels in the seasonal variation of Blood Cholesterol Study. Am J Epidemiol 153: 987–995
23. Bluher M, Hentschel B, Rassoul F, Richter V (2001) Influence of dietary intake and physical activity on annual rhythm of human blood cholesterol concentrations. Chronobiol Int 18: 541–557
24. Donald EA, Basu TK, Overton TR, Hargreaves JA, Thompson GW, Chao E, Peterson RD (1994) Dietary-lipid intake and serum-lipids in older men and women – a longitudinal study. J Clin Biochem Nutr 16: 77–83
25. McMahon S, Peto R, Cutler J, et al (1990) Blood pressure, stroke and coronary heart disease. I: prolonged differences in blood pressures: prospective observational studies corrected for the regression dilatation bias. Lancet 335: 765–774
26. Grimes D, Hindle E, Dyer T (1996) Sunlight, cholesterol and coronary heart disease. QJM 89: 579–589
27. Merriam PA, Ockene I, Hebert JR, Rosal MC, Matthews CE (1999) Seasonal variation of blood cholesterol levels: study methodology. J Biol Rhythms 14: 330–339

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