

*Original Research Article***Biological Aging and Cox Hazard Analysis of Mortality Trends in a Mennonite Community From South-Central Kansas**PHILLIP E. MELTON,^{1*} M. ZLOJUTRO,¹ K. KIMMINAU,² AND M.H. CRAWFORD¹¹Department of Anthropology, University of Kansas, Lawrence, Kansas 66045²Kansas Health Institute, Topeka, Kansas 66603

ABSTRACT This study investigated mortality in 568 individuals from the Goessel Mennonite community in rural central Kansas. There were three main objectives to this research: 1) characterize mortality trends within a biologically well-defined Mennonite community; 2) determine what biochemical, morphological, and physiological risk factors could be related to all-cause mortality, stratified by age and sex; and 3) compare these results to previously described variables that were associated with both biological age and mortality in this population. Mortality data were obtained from three sources: Kansas Vital Records, the Social Security death index, and church records. In total, 221 (39%) individuals were found to have died in this population between January 1980–June 2002. Analogous to the larger US population, the three leading causes of death in this community were heart disease, cancer, and stroke, accounting for 60% of all deaths. Besides advancing age, the greatest biological risk factor in this population was decreased amounts of albumin in men (relative risk, 2.47), potentially indicating underreported cases of either chronic kidney disease or frailty syndrome for males. Cox proportional hazard models demonstrated that increased amounts of total cholesterol may provide a protective effect for elderly individuals. We conclude, based on the previously described heritability of both albumin ($h^2 = 0.40$) and total cholesterol ($h^2 = 0.50$) in this population, that underlying genetic factors associated with both chronic degenerative diseases and biological aging may have important implications for understanding mortality patterns in this community. *Am. J. Hum. Biol.* 18:387–401, 2006. © 2006 Wiley-Liss, Inc.

Throughout the last century, the average life expectancy at birth rose from approximately 47 years in 1900, to roughly 77 years in 2000 for the United States population (Crews, 2003). This dramatic increase in the human life span has led to an epidemiological transition where chronic degenerative disorders (e.g., cardiovascular disease, cerebrovascular disease, and cancer) have overtaken infectious diseases as the leading causes of mortality in several human populations. Due to the multifactorial complexity of these chronic disorders, a number of epidemiological studies investigated the underlying biological, cultural, environmental, genetic, and nutritional risk factors associated with these diseases (Dawber, 1980; Kagan and Honolulu Heart Program, 1996; Toshima et al., 1994). However, two deficiencies are apparent in a number of these studies: 1) they used biologically and culturally heterogeneous populations rather than homogeneous groups; and 2) the majority of these studies investigated the relative risk of mortality with

chronological age rather than biological age. For chronic degenerative diseases with large genetic components, understanding associated risk factors may be more successful in populations that have experienced relative isolation, due to religious and ethnic differences, and/or geographic barriers. This isolation may serve as a buffer against “heterogenic noise” that masks underlying risk factors (MacCluer, 1993).

Biological age can be defined as the variation in total biological, morphological, and behavioral characteristics observed after the age of reproduction (Crawford, 2000). In this sense, there are individuals who appear to be older

Grant sponsor: National Institute of Aging; Grant number: AG01646; Grant sponsor: Kansas Attorney Generals Settlement Fund; Grant number: KUCR KAN 30471.

*Correspondence to: Phillip E. Melton, Department of Anthropology, University of Kansas, Fraser Hall, Room 622, 1415 Jayhawk Blvd., Lawrence, KS 66045. E-mail: pmelton@ku.edu

Received 12 September 2005; Accepted 17 January 2006

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/ajhb.20514

and persons who appear to be younger than their chronological age implies (Uttley and Crawford, 1994). This individual variation can be viewed as a function of genetic and environmental interactions related to senescence as chronological age advances. Biological age may be measured using either univariate or multivariate regression methods (Uttley and Crawford, 2000). Using chronological age and its predictors for a given individual, predicted age can be estimated using regression, and the residual (the difference between chronological and predicted age) may be considered an estimate of biological age (Bell, 1972). Therefore, individuals with negative residuals are considered older biologically than their peers, whereas individuals with positive residual values are considered biologically younger than their chronological age implies (Uttley and Crawford, 2000). Duggirala et al. (2002) demonstrated that biological aging has strong genetic determinants in the present study's Mennonite community, and that the biochemical markers they used to estimate biological age were independent of genetic and environmental effects associated with their predictors. While biological aging has long been of interest to human biologists, only a handful of studies have investigated its underlying effects on mortality in human populations (Borkan, 1978; Kesteloot, 2001; Uttley, 1991; Uttley and Crawford, 1994, 2000). These studies demonstrated that individuals who biologically age at a faster pace than their peers also have a higher likelihood of mortality.

The Mennonites of Kansas and Nebraska provide an excellent opportunity to investigate mortality and biological aging in a genetically homogenous population. Non-Amish Mennonites are an Anabaptist religious denomination that arose in Central Europe during the 1500s. Persecution, migration, and subsequent fissioning of the three major Anabaptist groups (Mennonites, Hutterites, and Amish) led to a unique history that had a tremendous effect on biological variation within these populations (Crawford 2000). The Mennonite communities of Kansas and Nebraska moved to the US from Russia in 1874, and are an agriculturally oriented population that is considered culturally and genetically distinct, relative to the wider national populace (Crawford, 2005). These Mennonite communities are biologically well-defined, with a unique immigrant history, as described elsewhere (Crawford, 2000). In brief, upon arrival in the US in the 1870s, the Mennonite population fissioned into two major

groups (Fig. 1): one group settled in south-central Nebraska (Henderson), while the other community of Goessel settled in central Kansas. Another Midwestern Mennonite community (Meridian, KS) is considered to be a heterogeneous group composed of Pennsylvanian Dutch and German, mixed with a large Mennonite immigrant population from southern Russia (Crawford et al., 1989).

This study investigated mortality in the rural Goessel Mennonite community of south-central Kansas, in order to determine what biological risk factors may be affecting this population. A number of research studies have investigated mortality within this Mennonite population. Everson et al. (1995) found that life expectancies after age 5 improved dramatically after the move to Gressel from Russia. Arya et al. (2000) examined mortality risk factors associated with nutrition, blood chemistries, and body morphology. Saint George et al. (2000) investigated mortality in birth intervals and survival of offspring to age 5 years from this Mennonite community from two temporal and geographic settings, Russia (1825–1874) and Kansas (1875–1924), to determine the effects of maternal depletion and sibling competition. Stevenson et al. (2004) examined mortality trends in reproduction and survivorship of offspring to age 15 years in Prussian/Russian vs. Kansas Mennonite mothers. Three studies (Uttley, 1991; Uttley and Crawford, 1994, 2000) investigated the efficacy of biological age to predict 10-year survival among Kansas and Nebraska Mennonites. Several previous studies investigated clinical blood chemistries in this population, and were described in detail elsewhere (Crawford, 2000). There were three objectives to this current research project: 1) establish all-cause mortality trends within this community; 2) determine the underlying biochemical, morphological, and physiological risk factors that could be related to all-cause mortality, stratified by chronological age and sex; and 3) compare these results to previously described biological factors that were associated with both biological age and mortality in this population.

MATERIALS AND METHODS

Mennonites of Kansas and Nebraska

Data from Kansas and Nebraska Mennonite communities were collected in 1980 and 1981 for a multidisciplinary research project on biological aging and longevity by researchers from the University of Kansas. This original

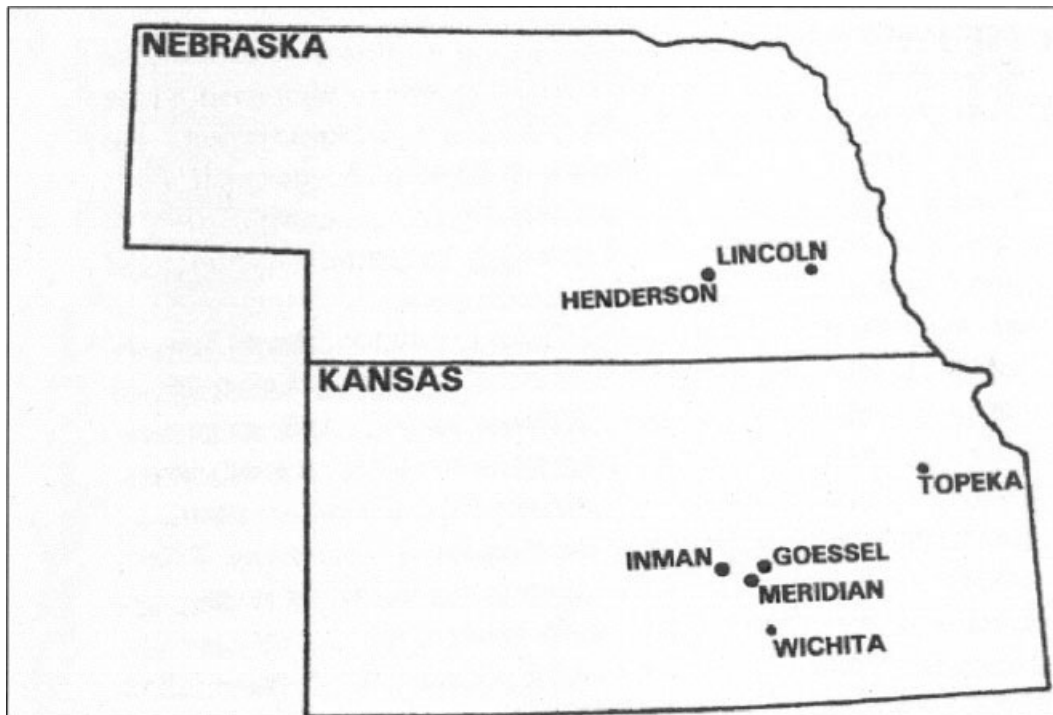


Fig. 1. Geographic locations of Midwest Mennonite communities (after Crawford and Martin, 2000).

1980s Midwest Mennonite research project (MEKAN) compiled a unique data set from approximately 1,200 individuals representing more than 50% of the populations of Goessel, Henderson, and Meridian. These data sets included census questionnaires, hearing assessments, sleep patterns, 24-hr recall nutrition assessment, medical examinations by physicians, medical histories, blood chemistries, anthropometrics, neuromuscular measures, and family histories (Crawford, 2000). This research did not specifically recruit individuals based on disease conditions, signifying that these collected data represent natural biological variation in these communities. The present study investigated measures of mortality in the Goessel Mennonite community, and included 568 subjects (259 males, and 309 females). The Institutional Review Board of the University of Kansas (Lawrence, KS) approved this study, and all participants provided informed consent.

Sources of mortality data

Mortality data were obtained during the summers of 2001 and 2002 by two of the authors (P.E.M. and M.Z.) while working as

summer interns for the Kansas Health Institute. Queries consisting of first name, surname, and date of birth were sent to the Kansas Vital Records Office, in order to obtain death certificates for individuals who participated in the study. Vital Records mortality information was verified through a subsequent search in summer 2002 of the online Social Security death index. In order to further assure the accuracy of these mortality data, additional records of births and deaths were obtained from the church directory in the Goessel Mennonite community in autumn 2002.

Risk factors

In total, 32 biological risk factors were examined for their potential relationship to mortality in this population (Table 1). These data were collected during the 1980–1981 study period, and included blood chemistry profiles, anthropometrics, and physiological data. Fasting and nonfasting blood samples were collected in the field and analyzed for 26 clinical blood-chemistry traits. Blood chemicals were measured using standard

TABLE 1. Risk-factor covariates used in this study

Blood chemistry variables
1. Total cholesterol
2. High-density lipoprotein cholesterol (HDL-C)
3. Triglycerides
4. Low-density lipoprotein cholesterol (LDL-C)
5. Serum glutamic-oxaloacetic transaminase (SGOT)
6. Serum glutamic-pyruvic transaminase (SGPT)
7. Alkaline phosphate
8. γ -glutamyltransferase (GGT)
9. Lactic acid dehydrogenase (LDH)
10. Total bilirubin
11. Blood urea nitrogen (BUN)
12. Creatinine
13. Uric acid
14. BUN creatinine ratio
15. Total protein
16. Albumin
17. Globulin
18. Albumin/globulin ratio
19. Sodium
20. Potassium
21. Chloride
22. Calcium
23. Phosphorus
24. Thyroxine (T4)
25. Glucose
26. Hemoglobin (Hgb)
27. Total iron
Anthropometric variables
28. Height (m)
29. Weight (kg)
30. Body mass index (BMI)
Physiological markers
31. Systolic blood pressure
32. Diastolic blood pressure

automated techniques by Roche Laboratories (Wichita, KS). Low-density lipoprotein cholesterol (LDL-C) was calculated using the Freeman equation. This equation is: Total cholesterol – High-density lipoprotein cholesterol (HDL-C) – (Triglycerides/5). Anthropometric data were measured using standardized techniques (Devor et al., 1986a,b). Body mass index (BMI) was measured as weight (kg) divided by height (m²). Blood pressure was determined by measuring systolic and diastolic pressures, using a sphygmomanometer.

Analytical methods

Descriptive statistics and analysis of variance. Descriptive statistics (mean and standard deviation) were compiled for each of the 32 variables shown in Table 1, along with age at study entry (recorded age of individuals when they enrolled in the original 1980–1981 study). In order to compare the characteristics of survivors vs. the deceased, a series of analyses of variance (ANOVAs) for continuous variables was used.

These data were then further divided to account for sex differences that are known to exist in blood chemistries for this population (Martin and Crawford, 2000). All analyses for descriptive statistics and ANOVAs were completed using the MINITAB 12.1 (1998) statistical package.

Cox proportional hazard model. A series of stepwise multivariate Cox proportional hazard models (Cox, 1972) was constructed to estimate the effects of biochemical and anthropometric covariates on mortality in this population. The equation for this model is:

$$h(t) = h_0 \exp \left(\sum_k \beta_k x_k \right) \quad (1)$$

where $h(t)$ is hazard or risk of mortality at time t , β_k is the set of unknown parameters to be estimated, and X_k are the K covariates measured at time of baseline. $h_0(t)$ is a baseline hazard function, and is defined when all covariates are set to 0.

Data from the three mortality sources described above were used to determine the censoring of observations. During the 22-year span between the 1980s Midwest Mennonite project and the present study (1980–2002, measured in months from January 1980–June 2002, for a total of 270 months), a total of 221 deaths occurred among the original 556 subjects. In estimating the risk of mortality, those who survived (335 subjects) until the end of the search period (270 months) were treated as right-censored individuals. Right-censored observations refer to those cases for which the dependent value (in this case death) is unknown or censored because the individuals survived until the end of the follow-up period. Those individuals who suffered an event, in this case death, are said to be uncensored and are given a value in months for when the event occurred, which is included in the model. For example, if an individual was 62 at the beginning of the study and died in June 1988, he or she was given a value of 102 and treated as an uncensored observation. Parameters in this model were estimated using a multivariate forward and backward stepwise selection model, using the proportional hazard regression (PHREG) procedure in SAS 9.1 (SAS Software, 2003). Relative risks (RR) are presented in terms of estimated hazard ratios, obtained by taking the exponent β_k and the 95% confidence interval. Covariates used in

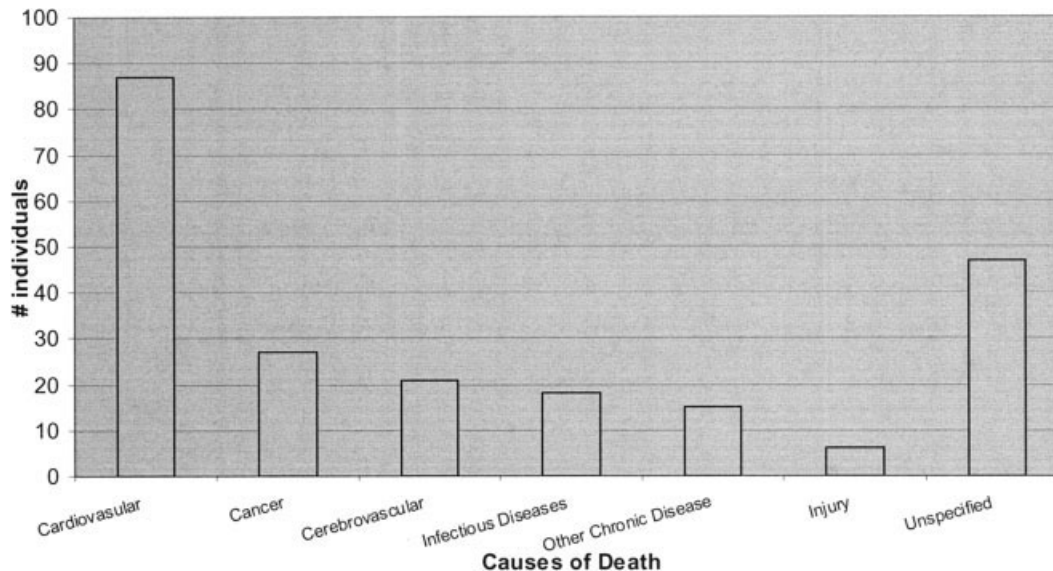


Fig. 2. Leading causes of mortality for Goessel community (1980–2002).

this model included age at entry and the aforementioned blood chemistries and physiological and anthropometric markers listed in Table 1.

RESULTS

Mortality statistics

In total, 221 (39%) individuals from the original study cohort were deceased at the end of the study period in summer 2002. Death certificates were obtained for 174 individuals from the Kansas Vital Records Office. Due to a small number of people born in the 19th century and thus well over 100 years of age, a cross-check of the Vital Records Office data was conducted using the online Social Security death index (SSDI) during summer 2002. This resulted in finding an additional 35 names that were not located in the original Vital Records searches. A subsequent Vital Records query with these 35 individuals located 16 of these individuals, based on first name and surname. The primary reason given for this discrepancy by the Kansas Vital Records Office was due to a Y2K computer error that classified individual born in the mid-19th century as still living by switching their date of birth with the date they died. Other potential explanations provided were that individuals moved out of state, or death certificates were not filed with the state Vital Records Office. An additional search of church records in 2002 resulted in 12 other deceased

individuals who had not been found in either database. The average age of death for individuals in this cohort was 84 (86 for females, and 82 for males).

Leading causes of death

In total, 62 different causes of death (data not shown) were listed on the acquired death certificates, and these were collapsed into seven categories (cardiovascular, cancer, cerebrovascular, infectious disease, other chronic degenerative diseases, injury, and unspecified) relating to common chronic degenerative diseases (Fig. 2). Cause of death for 47 participants were unspecified because they were not found in the Vital Records database (19 SSDI, and 12 church records), or death certificates (16 individuals) were unavailable.

Leading causes of mortality, separated by category for the Goessel Mennonite cohort, are shown in Figure 2. Cardiovascular disease (CVD) was the leading cause of death in this population, totaling 87 individuals (39% of total deaths). The second leading cause of fatality was cancer (e.g., malignant neoplasms), which occurred in 27 individuals (12% of total deaths). Cerebrovascular (e.g., stroke) events were the third leading mortality cause, and occurred in 21 individuals (9% of total deaths). A total of 18 (8% of total death) individuals died of infectious disease, while other chronic

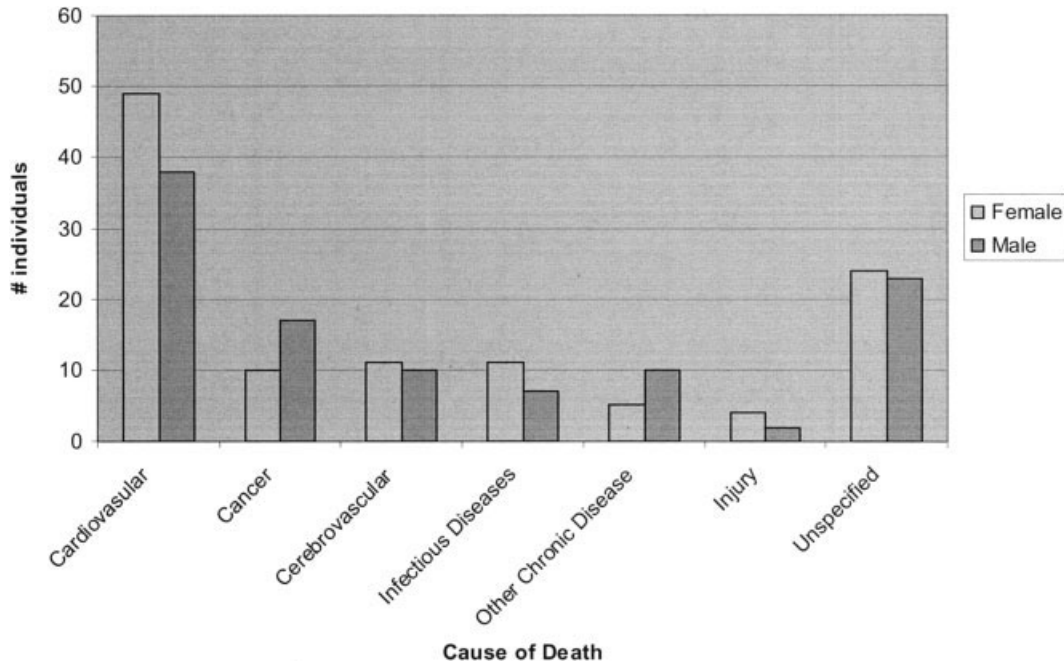


Fig. 3. Leading causes of mortality for Goessel community (1980–2002), separated by sex.

or unclassified diseases affected 15 individuals. Six individuals died due to accidents.

Figure 3 shows the leading causes of mortality for males and females. Heart disease was the leading cause of death for both males and females. Cancer was the second most common cause of death for males, while cerebrovascular disease and infectious diseases were tied for the second most common cause of death for females. Cerebrovascular diseases were the third leading cause of mortality in males, and malignant neoplasms were the third leading cause for females. Other chronic diseases affected 5 females and 10 males. Injury or accidental death occurred in 4 females and 2 males. Relatively equal numbers of female and male causes of mortality were unspecified.

Descriptive statistics and analysis of variance

Table 2 lists descriptive statistics (means and standard deviations) for age at entry, plus all 32 variables listed in Table 1 comparing deceased and surviving individuals. In total, 14 variables were found to be significantly different between surviving and deceased study participants, using ANOVA. Nine variables (age at

entry, blood urea nitrogen (BUN), creatinine, uric acid, BUN/creatinine ratio, albumin/globulin ratio, phosphorus, glucose, and systolic blood pressure) were found to be highly statistically significant ($P < 0.001$). The remaining six variables (total cholesterol, triglycerides, total protein, albumin, globulin, and chloride) were statistically significant at $P < 0.05$.

Descriptive statistics for differences between surviving vs. deceased females and males are shown in Tables 3 and 4. Eleven variables were found to be statistically significant for mortality vs. survivorship in Mennonite females. Eight of these variables (age at entry, alkaline phosphate, BUN, creatinine, uric acid, BUN/creatinine ratio, height, and systolic blood pressure) demonstrated high statistical significance between deceased and survived individuals. Triglycerides, albumin/globulin ratio, and glucose were found to be moderately significant between living and deceased females in this population. In contrast to females, 13 variables were found to be statistically significant for males regarding mortality vs. survivorship. Seven variables (age at entry, BUN, albumin, albumin/globulin ratio, glucose, height, and systolic blood pressure) were found to be highly statistically significant for males, and six variables (serum glutamic-

TABLE 2. Descriptive statistics of survivor vs. deceased biological variables (both sexes)

Variable	Deceased (n = 221)	Survived (n = 347)
Age at entry (mean ± SD)**	72.39 ± 10.53	46.47 ± 16.98
Blood chemistry variables (213 deceased, 305 survivors)		
Total cholesterol (mg/dl)*	233.66 ± 44.98	223.27 ± 46.31
HDL (mg/dl) (ns)	45.44 ± 14.66	46.54 ± 15.24
LDL ¹ (ns)	148.25 ± 50.50	142.74 ± 43.57
Triglycerides (mg/dl)*	189.34 ± 101.56	168.47 ± 112.54
SGOT (IU/l) (ns)	20.85 ± 31.15	17.93 ± 6.17
SGPT (IU/l) (ns)	23.19 ± 25.61	23.11 ± 11.89
GGT (IU/l) (ns)	26.17 ± 30.34	25.56 ± 31.47
Alkaline phosphate (IU/l) (ns)	90.61 ± 25.59	82.38 ± 62.00
LDH (IU/l) (ns)	192.47 ± 50.92	186.26 ± 39.13
Total bilirubin (mg/dl) (ns)	0.42 ± 0.17	0.44 ± 0.24
BUN (mg/dl)**	20.90 ± 5.72	17.17 ± 4.23
Creatinine (mg/dl)**	1.19 ± 0.27	1.09 ± 0.22
Uric acid (mg/dl)**	5.38 ± 1.38	4.86 ± 1.26
BUN/creatinine ratio**	17.80 ± 4.42	16.12 ± 4.23
Total protein (g/dl)*	6.78 ± 0.48	6.88 ± 0.44
Albumin (g/dl)*	4.04 ± 0.25	4.33 ± 2.15
Globulin (g/dl)*	2.79 ± 0.80	2.67 ± 0.35
Albumin/globulin ratio**	1.50 ± 0.24	1.60 ± 0.25
Sodium (meq/l) (ns)	141.21 ± 2.77	140.93 ± 4.66
Potassium (meq/l) (ns)	4.63 ± 0.96	4.72 ± 2.65
Chloride (meq/l)*	102.04 ± 3.98	102.83 ± 3.76
Calcium (mg/dl) (ns)	9.49 ± 0.43	9.54 ± 0.51
Phosphorus (mg/dl)**	3.07 ± 0.43	3.18 ± 0.48
Thyroxine (T-4) (mcgm) (ns)	8.24 ± 1.77	8.33 ± 1.65
Glucose (mg/dl)**	112.44 ± 34.39	99.59 ± 19.30
Hgb (g/dl) (ns)	14.6 ± 1.35	14.42 ± 2.25
Total Iron (µg/dl) (ns)	85.85 ± 34.94	86.83 ± 34.94
Anthropometric variables (157 deceased, 302 survivors)		
Height (m) (ns)	1.68 ± 0.10	1.7 ± 0.10
Weight (kg) (ns)	73.16 ± 14.13	73.92 ± 13.10
BMI (ns)	25.95 ± 3.92	25.54 ± 4.10
Blood-pressure variables (150 deceased, 308 survivors)		
Systolic BP**	139.64 ± 20.67	127.67 ± 16.97
Diastolic BP (ns)	80.66 ± 9.82	79.79 ± 8.97

¹Calculated using Freeman equation. ns, not significant.
 *P < 0.05.
 **P < 0.001.

oxalocetic transaminase (SGOT), creatinine, total protein, globulin, calcium, and phosphorus) were found to be significant at P < 0.05.

Cox proportional hazards model

In order to establish all-cause mortality patterns in this Mennonite community, three Cox proportional hazards models were analyzed. The first model was unadjusted for possible confounding variables such as age and sex, the second model was adjusted for sex, and the third was adjusted for age by dividing age into three variables (younger than 40, 40–64, and over 65). Results of these analyses are presented in Table 5, and include parameter estimates, hazard ratios, 95% confidence intervals, and associated P-values (only significant variables shown). All models demonstrated that age at entry into the study was associated with mortality, and was highly

statistically significant. In the unadjusted model, other predictors included elevated levels of hemoglobin (Hgb), which increased the risk of mortality by 11%, and SGOT and high glucose levels, which increased the probability of mortality by approximately 1%. Two items were associated with a decreased probability of mortality in this model, and these included total cholesterol and γ-glutamyltransferase (GGT), both of which decreased the probability of death by approximately 1%.

In the hazard model that was adjusted for sex, elevated levels of serum glutamic-pyruvic transaminase (SGPT) had a 1% higher likelihood of causing mortality in females, and total cholesterol had a 1% higher likelihood of reducing death. In males, increased levels of serum albumin had a 2.47 times greater likelihood of causing mortality, while increased levels of HDL-C had a 2% greater probability and glucose a 1% greater risk. Increased levels of

TABLE 3. Descriptive statistics of survivor vs. deceased biological variables (females)

Variable (mean \pm SD)	Deceased (n = 114)	Survived (n = 195)
Age at entry***	74.50 \pm 10.06	48.47 \pm 17.02
Blood chemistry variables (109 deceased, 169 survivors)		
Total cholesterol (mg/dl) (ns)	239.94 \pm 41.67	228.45 \pm 50.42
HDL (mg/dl) (ns)	49.15 \pm 14.47	50.38 \pm 16.35
LDL ¹ (ns)	155.00 \pm 40.09	147.20 \pm 44.85
Triglycerides (mg/dl)*	175.43 \pm 88.13	156.37 \pm 108.90
SGOT (IU/l) (ns)	22.73 \pm 43.64	17.40 \pm 6.66
SGPT (IU/l) (ns)	24.79 \pm 35.20	21.44 \pm 11.08
GGT (IU/l) (ns)	26.47 \pm 33.06	21.77 \pm 28.09
Alkaline phosphate (IU/l)***	92.62 \pm 23.53	76.41 \pm 25.79
LDH (IU/l) (ns)	193.94 \pm 59.05	186.64 \pm 41.63
Total bilirubin (mg/dl) (ns)	0.41 \pm 0.16	0.42 \pm 0.25
BUN (mg/dl)***	20.23 \pm 5.84	16.14 \pm 4.16
Creatinine (mg/dl)***	1.10 \pm 0.23	0.99 \pm 0.19
Uric acid (mg/dl)***	5.13 \pm 1.27	4.40 \pm 1.18
BUN/creatinine ratio***	18.61 \pm 4.49	16.40 \pm 3.98
Total protein (g/dl) (ns)	6.83 \pm 0.51	6.92 \pm 0.47
Albumin (g/dl) (ns)	4.02 \pm 0.26	4.39 \pm 3.00
Globulin (g/dl) (ns)	2.81 \pm 0.49	2.77 \pm 0.37
Albumin/globulin ratio*	1.46 \pm 0.26	1.53 \pm 0.21
Sodium (meq/l) (ns)	141.26 \pm 3.00	140.62 \pm 5.30
Potassium (meq/l) (ns)	4.61 \pm 1.05	4.86 \pm 3.67
Chloride (meq/l) (ns)	101.99 \pm 4.25	102.82 \pm 4.24
Calcium (mg/dl) (ns)	9.56 \pm 0.48	9.56 \pm 0.56
Phosphorus (mg/dl) (ns)	3.18 \pm 0.40	3.23 \pm 0.51
Thyroxine (T-4) (mcgm) (ns)	8.37 \pm .157	8.54 \pm .174
Glucose (mg/dl)**	110.75 \pm 31.25	100.09 \pm 19.62
Hgb (g/dl) (ns)	14.10 \pm 1.21	13.82 \pm 1.61
Total iron (μ g/l) (ns)	84.05 \pm 37.95	83.18 \pm 33.78
Anthropometric variables (73 deceased, 175 survivors)		
Height (m)***	1.60 \pm 0.07	1.64 \pm 0.06
Weight (kg) (ns)	65.99 \pm 12.39	68.29 \pm 13.26
BMI (ns)	25.65 \pm 4.20	25.59 \pm 4.86
Blood-pressure variables (73 deceased, 174 survivors)		
Systolic BP***	142.15 \pm 21.64	128.01 \pm 18.67
Diastolic BP (ns)	80.69 \pm 9.14	79.81 \pm 9.36

¹Calculated using Freeman equation. ns, not significant.

*p < 0.05.

**p < 0.01.

***p < 0.001.

GGT had a 14% likelihood of reducing the probability of mortality for males in this population.

In the age-adjusted survival analysis, individuals over 65 years of age had a 4 times greater likelihood of mortality, while increased levels of SGOT had a 4% increased probability of mortality, and increased levels of glucose, a 1% increased probability. In this model, individuals with increased levels of SGPT had a 4% chance of reducing mortality.

Cox hazard analysis and lipid levels

An interesting result demonstrated by the Cox hazard analysis occurred in lipid levels for this community. In a population where cardiovascular disease is the leading cause of mortality, one would expect to see an increased risk associated with various lipids measured in this

population. In the Cox hazard analysis (Table 5), two lipids (total cholesterol and HDL-C) appear, but both results indicate the opposite outcome of what one would expect in a population considered to be at high risk for heart disease. In the unadjusted and the Goessel female hazard models, it appears that higher levels of total cholesterol and low levels of HDL-C provide a beneficial effect in old age. While this reduced risk ratio is negligible compared to overall risk of death for this population, it appears that as chronological age increased, levels of total cholesterol provided a beneficial effect to survivorship in this Mennonite community. In order to determine if this result was in fact real or a statistical anomaly, we averaged lipid levels in 5-year age cohorts from 60–>90 years of age at study entry date for surviving and deceased individuals. Figure 4 illustrates this difference, and shows total cholesterol lev-

TABLE 4. Descriptive statistics of survivor vs. deceased biological variables (males)

Variable (mean ± SD)	Deceased (n = 107)	Survived (n = 152)
Age at entry***	70.13 ± 10.60	43.88 ± 16.62
Blood chemistry variables (104 deceased, 136 survivors)		
Total cholesterol (mg/dl) (ns)	228.95 ± 43.16	218.82 ± 41.21
HDL (mg/dl) (ns)	42.38 ± 13.48	42.80 ± 11.97
LDL ¹ (ns)	146.46 ± 41.17	139.50 ± 39.67
Triglycerides (mg/dl) (ns)	201.7 ± 113.8	182.6 ± 111.00
SGOT (IU/l) (ns)	18.98 ± 5.48	18.73 ± 5.86
SGPT (IU/l)*	21.79 ± 8.30	25.43 ± 12.87
GGT (IU/l) (ns)	27.18 ± 28.02	33.82 ± 35.82
Alkaline phosphate (IU/l) (ns)	88.23 ± 27.40	82.80 ± 22.58
LDH (IU/l) (ns)	189.76 ± 41.70	183.60 ± 36.60
Total bilirubin (mg/dl) (ns)	0.43 ± 0.17	0.47 ± 0.24
BUN (mg/dl)***	21.34 ± 5.62	18.51 ± 4.00
Creatinine (mg/dl)**	1.28 ± 0.25	1.20 ± 0.20
Uric acid (mg/dl) (ns)	5.55 ± 1.41	5.44 ± 1.18
BUN/creatinine ratio (ns)	16.89 ± 4.12	15.80 ± 4.60
Total protein (g/dl)*	6.74 ± 0.43	6.86 ± 0.43
Albumin (g/dl)***	4.06 ± 0.24	4.28 ± 0.32
Globulin (g/dl)*	2.68 ± 0.35	2.58 ± 0.31
Albumin/globulin ratio***	1.54 ± 0.22	1.66 ± 0.28
Sodium (meq/l) (ns)	141.20 ± 2.45	142.25 ± 4.19
Potassium (meq/l) (ns)	4.67 ± 0.85	4.63 ± 0.57
Chloride (meq/l) (ns)	102.42 ± 3.24	102.96 ±
Calcium (mg/dl)*	9.40 ± 0.36	9.54 ± 0.49
Phosphorus (mg/dl)**	2.96 ± 0.43	3.13 ± 0.46
Thyroxine (T-4) (mcgm) (ns)	8.06 ± 1.99	8.00 ± 1.40
Glucose (mg/dl)***	113.87 ± 37.83	98.96 ± 17.68
Hgb (g/dl) (ns)	15.10 ± 1.29	15.31 ± .209
Total iron (µg/dl) (ns)	89.38 ± 31.99	92.74 ± 34.52
Anthropometric variables (84 deceased, 127 survivors)		
Height (m)***	1.74 ± 0.06	1.79 ± 0.06
Weight (kg) (ns)	79.30 ± 13.11	80.47 ± 8.76
BMI (ns)	26.06 ± 3.65	25.29 ± 2.84
Blood-pressure variables (77 deceased, 134 survivors)		
Systolic BP***	137.05 ± 19.96	128.06 ± 14.18
Diastolic BP (ns)	80.79 ± 10.63	80.16 ± 7.76

¹Calculated using Freeman equation. ns, not significant.

*p < 0.05.

**p < 0.01.

***p < 0.001.

els between deceased and surviving individuals for both sexes. In all but two cohorts (71–75 and >90), individuals who survived had higher total cholesterol levels than those who were deceased. The second lipid variable considered a significant risk factor for mortality in this population is HDL-C in males. Figure 5 illustrates the difference between surviving and deceased male individuals in 5-year age cohorts. Individuals surviving into old age have surprisingly lower HDL-C levels than deceased participants. In this Mennonite population, it would appear that males with slightly increased levels of HDL-C have a small advantage of survivorship. However, this advantage does not hold for individuals between ages 66–70 and over age 75.

DISCUSSION

In this study, we investigated three sources of mortality data in order to ensure the accu-

racy of death determination for this population. These included national (SSDI), state (Kansas Vital Records), and local (church records) sources of mortality data. The initial query through the Kansas Vital Records Office, the primary source of mortality data for the state, failed to locate 35 individuals who were deceased, indicating an initial error rate of approximately 15%. These results confirm that even at the dawn of the 21st century, mortality data are still not complete for all deceased individuals. This also confirms the need to examine multiple sources of mortality data, from a variety of levels, in order to assure the accuracy of results. The three leading causes of mortality (cardiovascular, malignant neoplasm, and cerebrovascular diseases) in this community reflect the patterns observed in the larger US population. These three causes of death accounted for approximately 60% of the total deaths in the

TABLE 5. Cox proportional hazard analysis for mortality for Goessel Mennonite community (1980–2002)

Covariate	Parameter est. (β)	Hazard ratio (exp β)	95% Confidence interval hazard ratio	P-value
Unadjusted				
Age at entry	0.09043	1.095	1.081–1.108	<0.0001
Hgb	0.10627	1.112	1.004–1.232	0.0420
GGT	–0.00699	0.993	0.988–0.998	0.0116
SGOT	0.00723	1.007	1.003–1.011	0.0007
Total cholesterol	–0.00485	0.995	0.992–0.999	0.0049
Glucose	0.00513	1.005	1.001–1.009	0.0164
Sex-adjusted				
Males				
Age at entry	0.09137	1.096	1.076–1.116	<0.0001
HDL	0.0250	1.021	1.005–1.037	0.0107
GGT	–0.01437	0.986	0.977–0.994	0.0012
Albumin	0.90572	2.474	1.0256–5.962	0.0436
Glucose	0.00747	1.007	1.002–1.013	0.0093
Females				
Age at entry	0.10622	1.112	1.092–1.133	<0.0001
SGPT	0.00558	1.006	1.001–1.006	0.0274
Total cholesterol	–0.00597	0.994	0.990–0.998	0.0043
Age-adjusted				
Age >65	1.28468	3.614	3.31–3.92	<0.0001
Glucose	0.00614	1.006	1.002–1.006	0.0086
SGOT	0.03890	1.04	1.01–1.07	0.0285
SGPT	–0.03495	0.966	0.964–0.968	0.0017

Goessel cohort, whereas at the national level, they accounted for 57% of the mortality total (Hoyert et al., 2005), indicating little difference between this population and mortality trends at the national level.

ANOVA results reported here are comparable to previous research done on mortality in this Mennonite community. Increase in the age at entry, BUN, creatinine, glucose, and systolic blood pressure, and decrease of albumin/globulin ratio, were found to be statistically significant between survivors and deceased individuals in all three ANOVA models (Tables 3–5). Arya et al. (2000) investigated nutrition, obesity, and mortality in a subset of the Goessel Mennonite population aged 41 years and older, and reported a twofold increase in the probability of mortality between individuals with increased age, BUN levels, and high albumin/globulin ratio. Uttley and Crawford (2000) examined biological aging and survivorship in both the Goessel and Henderson communities using discriminate function analyses, and found that elevated BUN, BUN/creatinine, and albumin levels contributed significant differences between survival and mortality. Height was not found to be significantly different for both sexes combined, but was found to be significant for males and females when considered separately. This latter result among males is concordant with a study by Uttley (1991) which found that shorter Mennonite men had a higher risk of mortality.

A comparison of causes of death between males and females indicates that different blood chemistry variables may be responsible for determining mortality patterns between the sexes. Increased amounts of triglycerides, alkaline phosphate, and uric acid all contributed significantly to mortality in females but not in males. In males, decreased SGPT, total protein, calcium, phosphorus, albumin, and increased globulin levels were significantly different. All of these variables, except calcium and total protein, were previously shown to be significantly different and related to sexual dimorphism in this population (Martin and Crawford, 2000). Uttley and Crawford (2000) established, with discriminant function analysis, that different variables separated out deceased and surviving individuals based on sex, and concluded that physiological data on this population should be analyzed separately by sex. The reason for some of these differences may be due to the aging process and the significant biological changes that accompany menopause in females. Albumin is highly correlated with age in males, while triglyceride and uric acid levels both increase with age in females (Martin and Crawford, 2000).

A statistical difference demonstrated by ANOVA between survivors and deceased individuals in BUN, creatinine, BUN/creatinine ratio, albumin, albumin/globulin ratio, and uric acid indicates a potential underreporting of renal disease in this population. A relationship

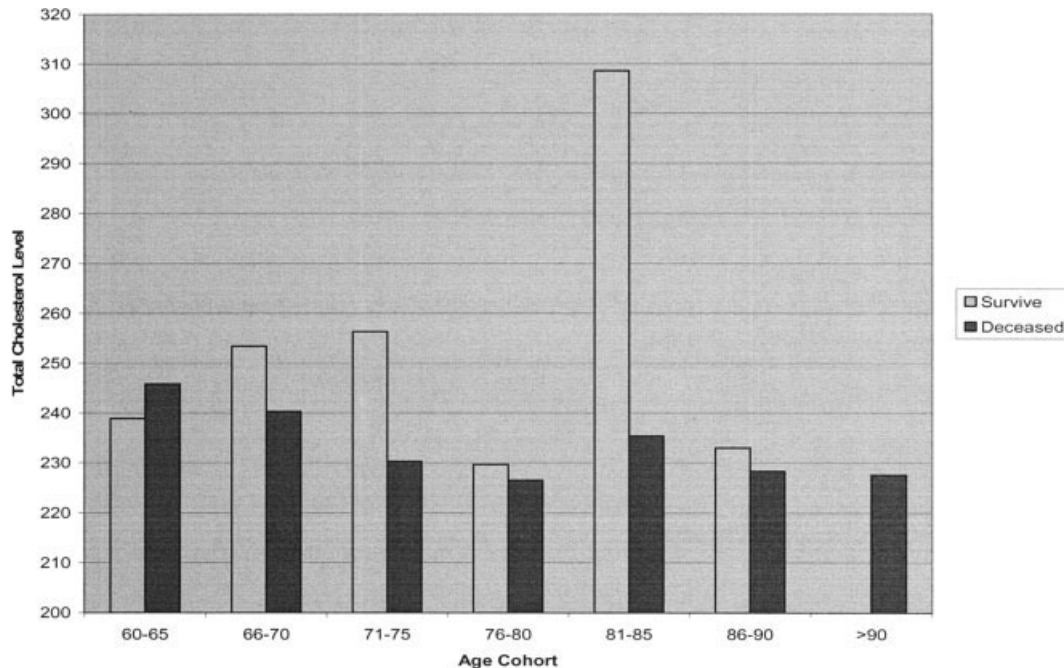


Fig. 4. Total cholesterol of survivors vs. deceased in 5-year age cohorts (both sexes).

was previously described between decreased levels of albumin and cardiovascular disease in end-stage renal disease patients (Foley et al., 1996), and therefore the primary cause of death listed on the death certificate may not be the true underlying cause that resulted in fatality. Another often overlooked biological component is the underlying genetic factors that may impact mortality in a population. Duggirala et al. (2000) determined that three of these variables associated with renal function have significant genetic components in this population (albumin heritability (h^2) = 0.42, creatinine h^2 = 0.40, and BUN h^2 = 0.31). In addition to these findings, they also reported an additive genetic influence for creatinine and a high random environmental variance in BUN that increased with age (Duggirala et al., 2000).

Further evidence for underreported renal disease can also be demonstrated from the results of the Cox proportional hazard analysis (Table 5). Aside from age at entry, the two variables with the highest hazard ratios for mortality were albumin (RR, 2.47) in males, and Hgb (RR, 1.11) in the unadjusted model. While P -values for both these variables are not high (~ 0.04), they remain significant when considered along with all other variables in a

multivariate stepwise regression model. High Hgb levels were associated with an increased risk of arteriovenous fistula thrombosis, hypertension, cardiovascular events, and clinical maintenance of high Hgb levels in cancer. In addition, chronic kidney-disease patients with elevated Hgb levels and known cardiovascular disease demonstrated a higher mortality risk (Strippoli et al., 2004).

Albumin was also found to be highly significant for males in the ANOVA, indicating (in conjunction with its significant heritability) its importance for understanding mortality in this population. Albumin is the primary protein produced by the liver, and has important functions in the transport of various biological substances through the bloodstream, as well as in maintaining osmotic pressure in the body. While levels of albumin are normally reduced with age, chronic diseases (such as renal disease, advanced heart disease, and cancer) also lower albumin levels (Campion et al., 1988; Salive et al., 1992). Albumin is often used as an indicator of nutritional status, and low levels are associated with a higher risk of mortality and morbidity in individuals with end-stage renal disease (Doumas and Peters, 1997). Other investiga-

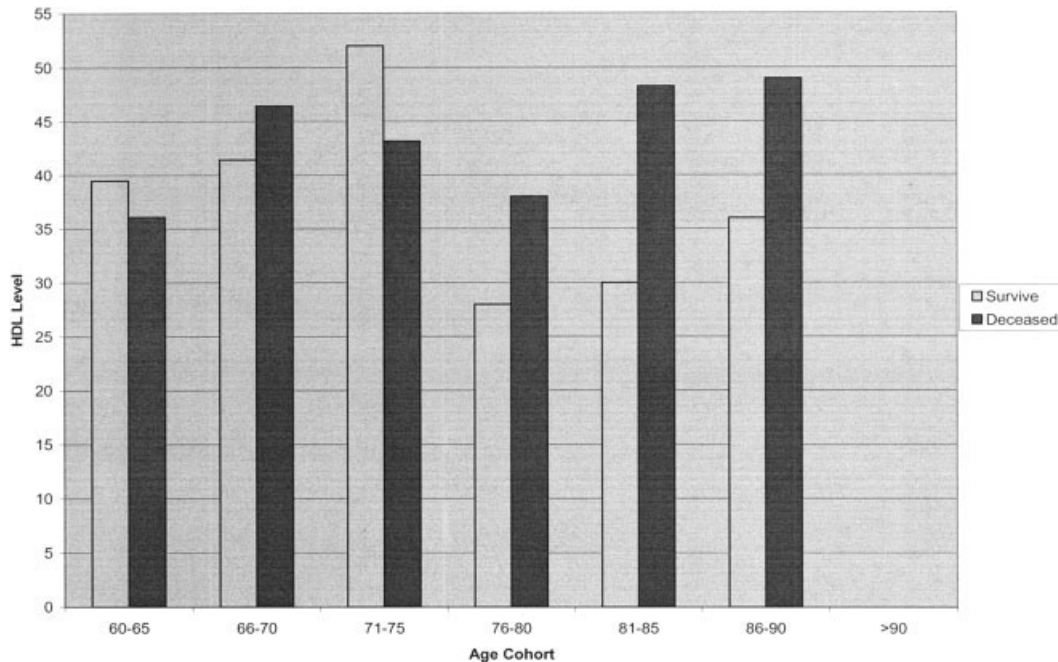


Fig. 5. HDL levels in surviving and deceased males.

tions predicted that serum albumin levels may be a predictor for subclinical diseases in the healthy elderly (Klonoff-Cohen et al., 1992), or that hypoalbuminemia may be associated with “frailty syndrome” or decreased physical performance over time (Corti et al., 1994). However, contradictory results were reported for this latter finding, and indicated that albumin concentration was not associated with decreased physical performance, but that a combination of low albumin with low total cholesterol levels may increase the risk of future functional decline (Schalk et al., 2004).

Two potential explanations for underreported causes of mortality in this Mennonite population are chronic kidney disease and/or “frailty syndrome.” Chronic kidney disease occurs when the kidneys no longer remove waste from the body effectively. Symptoms for chronic kidney disease may appear slowly over a time period of 30–40 years, and include variables such as advancing age, high blood pressure, sex, low levels of albumin, increased levels of total cholesterol, elevated glucose, and metabolic syndrome (Chen et al., 2004). Chronic kidney disease also increases the risk of death from cardiovascular disease, end-

stage renal disease, and all-causes, yet the identification of individuals with renal insufficiency is difficult (Muntner et al., 2002). While the exact underlying relationship between heritability and mortality and renal function in this population is not entirely understood, the presence of several important genetic components that contain both additive and environmental effects offers important epidemiological factors that need to be further elucidated.

Frail individuals are considered to be at risk for morbidity and death due to the degeneration of a number of physiological systems (Schalk et al., 2004). Frailty is clinically characterized by the presence of three or more of the following variables: unintentional weight loss, self-reported exhaustion, weakness (based on grip strength), slow walking speed, and reduced physical activity (Fried et al., 2001). An association between low levels of serum albumin and total cholesterol was also previously investigated, with differential results (Schalk et al., 2004). Previous research on mortality in this Mennonite population suggested that frailty may be an important factor in survivorship (Uttley and Crawford, 1994). This conclusion, based on a number of functional traits

associated with biological age (including forced expiratory volume in 1 sec, grip strength, trunk flexibility, and reaction time) showed that individuals with reduced function died earlier.

Other contributing factors to mortality

Other biological factors that contribute to mortality in this population are largely indicative of liver (SGOT, SGPT, GGT, and glucose) or heart (total cholesterol and HDL-C) function. The relatively small risk for several of these variables is statistically significant and contributes to the mortality trends found in this population. In a trend similar to biological variables associated with renal disease, both total cholesterol ($h^2 = 0.50$) and HDL-C ($h^2 = 0.73$) were shown to have a high heritable component, indicating strong, genetically influenced phenotypic variation in this population (Duggirala et al., 2000). This research also revealed that environmental factors demonstrated an important function in shaping the observed differences for both these variables. Heritable factors for variables associated with hepatic function are unknown for this population. However, a substantial genetic component was reported from twin-studies research. Whitfield et al. (2002) investigated Australian twins, and demonstrated high heritable components for 3 of 4 liver variables. These researchers estimated the heritability for GGT ($h^2 = 0.52$), SGPT ($h^2 = 0.48$), and SGOT ($h^2 = 0.32$). Another study by Bathum et al. (2001) investigated the genetic component of these variables in Danish twins, and found heritability to be between 35–61% for SGPT, GGT, LDH, and bilirubin.

The enzymes GGT, SGPT, and SGOT were previously reported to have an association with cardiovascular risk factors, but the strongest association was shown for GGT (Wannamethee et al., 1995; Arndt et al., 1998; Karlson et al., 2000). GGT is involved in replenishing intracellular glutathione and controlling apoptosis in atheromatous plaques (del Bello et al., 1999; Paolicchi et al., 1999). Other studies showed that increased GGT levels are associated with hypertension, stroke, noninsulin-dependent diabetes, and death from cardiovascular disease (Whitfield et al., 2002). GGT was also associated with BMI and several cardiovascular risk factors including lipid levels, blood pressure, impaired glucose tolerance, and insulin resistance (Whitfield, 2001). GGT is highly correlated with both

SGOT and SGPT, and over half of its genetic component is shared with these variables. All three of these variables are also positively associated with BMI, and may reflect the prevalence of fatty liver in obese individuals. The BMI for this population is borderline-obese for both males and females, with the presence of liver functions contributing to mortality in this Mennonite population.

The finding that higher levels of total cholesterol demonstrate a low risk of mortality in aging populations is concordant with a number of recent epidemiological studies. These studies suggested that there is a significant difference between risk factors and total cholesterol levels in elderly populations compared to middle-aged populations for all-cause mortality (Brescianini et al., 2003; Iribarren et al., 1995; Schatz et al., 2001; Schupf et al., 2005; Volpato et al., 2001; Weverling-Rijnsburger et al., 1997). This research showed an inverse relationship between total cholesterol and risk of either cardiovascular or all-cause mortality in elderly populations (Schupf et al., 2005). These researchers offered several potential hypotheses for this occurrence, including selective mortality (Karlman et al., 2004; Weverling-Rijnsburger et al., 1997) or a relationship between endotoxin and the lipoprotein-binding site, indicating that individuals with higher levels of LDL-C may be better at fighting infectious diseases (Rauchhaus et al., 2000). However, all these researchers concluded that understanding the relationship between mortality and total cholesterol in aging populations needs further clarification.

CONCLUSIONS

Death is the ultimate outcome of the aging process, and therefore it is intricately intertwined with the concept of biological age. However, it is difficult to separate factors of normal biological aging from those factors associated with chronic degenerative diseases and their underlying genetic and environmental factors. In a study of the genetic determinants of biological aging, Duggirala et al. (2002) found a number of factors that are significantly associated with mortality, and that are highly informative predictors of biological aging. These factors include glucose, albumin, BUN, and systolic blood pressure, all of which were found to be significantly associated with biological age for both the Goessel and Henderson Mennonite communities. The presence

of several significant variables associated with biological age (also implicated with mortality risk factors) may indicate that biological age and chronic degenerative diseases are, not surprisingly, related, as both deal with the aging process and demonstrate considerable genetic and environmental influences.

In this study, we reported on mortality trends for a single Mennonite cohort from south-central Kansas. These results indicate a potential underreporting of underlying causes of death, including chronic kidney disease and frailty, that may be associated with an aging population. The present findings, while analogous to the wider US population, should be interpreted with some caution. This study used a small sample size, although the sample does represent 50% of the community from which it was drawn. Another caveat is that we used data from fasting and nonfasting individuals, and this may alter the results. Chittoor and Crawford (2005) found statistically significant differences between a combination of fasting and nonfasting individuals from the Henderson Mennonite cohort, but whether or not these would influence the present results is unknown. However, we are interested in normal biological variation, and since "nonfasting" is the natural condition in this population, it may be more informative in defining biological risk factors. Since this study investigated a single environmentally and genetically homogenous population, any comparisons to the larger heterogeneous general public should be avoided. However, these data also demonstrate the efficacy of adopting an anthropological approach to epidemiology. The investigation of a single homogenous population lacking some risk factors provides a less ambiguous interpretation of the interaction of biology and culture in survivorship and risk.

ACKNOWLEDGMENTS

We thank the Mennonite communities of Kansas and Nebraska for their participation in this project. We also thank Lea Steele for comments on the project analytical design, John Rule for comments on statistical techniques, and Greg Crawford of the Office of Health Care Information at the Kansas Department of Health Environment for help in accessing death certificates. Two of the authors (P.E.M. and M.Z.) were supported as summer interns by the Kansas Health Institute, and we thank their staff for help and guidance.

LITERATURE CITED

- Arndt V, Brenner H, Rothenbacher D, Zschenderlein B, Fraisse E, Fliedner TM. 1998. Elevated liver enzyme activity in construction workers: prevalence and impact on early retirement and all-cause mortality. *71:405–412*.
- Arya R, Singh G, Mosher M, Haas J, Crawford MH. 2000. Nutrition, obesity, and mortality in Kansas Mennonites. In: Crawford M, editor. *Different seasons: biological aging among the Mennonites of the Midwestern United States*. Lawrence, KS: University of Kansas Press.
- Bathum L, Petersen HC, Rosholm JU, Hyltoft Petersen P, Vaupel J, Christensen K. 2001. Evidence for a substantial genetic influence on biochemical liver function tests: results from a population-based Danish twin study. *Clin Chem 47:81–87*.
- Bell B. 1972. Significance of functional age for interdisciplinary and longitudinal research in aging. *Aging Hum Dev 3:145–147*.
- Borkan G. 1978. *The assessment of biological age during adulthood* [Ph.D. dissertation]. Ann Arbor: University of Michigan.
- Brescianini S, Maggi S, Farchi G, Mariotti S, Di Carlo A, Baldereschi M, Inzitari D. 2003. Low total cholesterol and increased risk of dying: are low levels clinical warning signs in the elderly? Results from the Italian Longitudinal Study on Aging. *J Am Geriatr Soc 51: 991–996*.
- Campion EW, deLabry LO, Glynn RJ. 1988. The effect of age on serum albumin in healthy males: report from the Normative Aging Study. *J Gerontol 43:18–20*.
- Chittoor G, Crawford MH. 2005. Effects of fasting vs. nonfasting on serum lipids, lipoproteins, and total cholesterol on Mennonites from Henderson, NE. Unpublished manuscript.
- Chen J, Muntner P, Hamm LL, Jones DW, Batuman V, Fonseca V, Whelton PK, He J. 2004. The metabolic syndrome and chronic kidney disease in U.S. adults. *Ann Intern Med 140:167–174*.
- Corti MC, Guralnik JM, Salive ME, Sorkin JD. 1994. Serum albumin level and physical disability as predictors of mortality in older persons. *JAMA 272:1036–1042*.
- Cox D. 1972. Regression models and life tables. *J R Stat Soc [B] 34:187–220*.
- Crawford MH. 2000. *Different seasons: biological aging among the Mennonites of the Midwestern United States*. Lawrence: University of Kansas Press. 178 p.
- Crawford MH. 2005. Genetics of biological aging in Mennonites of Midwestern United States. *Przegl Anthropol Anthropol Rev 68:3–18*.
- Crawford MH, Dykes DD, Polesky HF. 1989. Genetic structure of Mennonite populations of Kansas and Nebraska. *Hum Biol 61:493–514*.
- Crews D. 2003. *Human senescence: evolutionary and biocultural perspectives*. Cambridge: University of Cambridge Press. 291 p.
- Dawber TR. 1980. *The Framingham Study: the epidemiology of atherosclerotic disease*. Cambridge, MA: Harvard University Press. 257 p.
- del Bello B, Paolicchi A, Comporti M, Pompella A, Maellaro E. 1999. Hydrogen peroxide produced during gamma-glutamyl transpeptidase activity is involved in prevention of apoptosis and maintenance of proliferation in U937 cells. *FASEB J 13:69–79*.
- Devor EJ, McGue M, Crawford MH, Lin PM. 1986a. Transmissible and nontransmissible components of anthropometric variation in the Alexanderwohl Mennonites: I. Description and familial correlations. *Am J Phys Anthropol 69:71–82*.
- Devor EJ, McGue M, Crawford MH, Lin PM. 1986b. Transmissible and nontransmissible components of anthropo-

- metric variation in the Alexanderwohl Mennonites: II. Resolution by path analysis. *Am J Phys Anthropol* 69: 83–92.
- Doumas BT, Peters T Jr. 1997. Serum and urine albumin: a progress report on their measurement and clinical significance. *Clin Chim Acta* 258:3–20.
- Duggirala R, Uttley M, Williams K, Arya R, Blangero J, Crawford MH. 2002. Genetic determination of biological age in the Mennonites of the Midwestern United States. *Genet Epidemiol* 23:97–109.
- Everson PM, Stevenson JC, Rogers L. 1995. Mortality in a migrating Mennonite church congregation. *Hum Biol* 67:69–86.
- Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC, Barre PE. 1996. Hypoalbuminemia, cardiac morbidity, and mortality in end-stage renal disease. *J Am Soc Nephrol* 7:728–736.
- Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, et al. 2001. Frailty in older adults: evidence for a phenotype. *J Gerontol [A]* 56:146–156.
- Hoyert DL, Kung H-C, Smith BL. 2005. Deaths: preliminary data for 2003. Atlanta: Centers for Disease Control. p 48.
- Iribarren C, Reed DM, Chen R, Yano K, Dwyer JH. 1995. Low serum cholesterol and mortality. Which is the cause and which is the effect? *Circulation* 92:2396–2403.
- Kagan A, Honolulu Heart Program. 1996. The Honolulu Heart Program: an epidemiological study of coronary heart disease and stroke. Amsterdam: Harwood Academic. 204 p.
- Karlamañla AS, Singer BH, Reuben DB, Seeman TE. 2004. Increases in serum non-high-density lipoprotein cholesterol may be beneficial in some high-functioning older adults: MacArthur Studies of Successful Aging. *J Am Geriatr Soc* 52:487–494.
- Karlson BW, Wiklund O, Hallgren P, Sjölin M, Lindqvist J, Herlitz J. 2000. Ten-year mortality amongst patients with a very small or unconfirmed acute myocardial infarction in relation to clinical history, metabolic screening and signs of myocardial ischaemia. *J Intern Med* 247:449–456.
- Kesteloot H. 2001. Evolution of all-cause and cause-specific mortality in the age-class 75–84 years during the period 1970–1996. A worldwide overview. *Verh K Acad Geneesk Belg* 63:405–431.
- Klonoff-Cohen H, Barrett-Connor EL, Edelstein SL. 1992. Albumin levels as a predictor of mortality in the healthy elderly. *J Clin Epidemiol* 45:207–212.
- MacCluer JW. 1993. The anthropological perspective in genetic epidemiology. *Hum Biol* 65:1025–1028.
- Martin L, Crawford MH. 2000. Aging and sexual dimorphisms in levels of serum biochemical markers in Mennonites. In: Crawford M, editor. *Different seasons: biological aging among the Mennonites of the Midwestern United States*. Lawrence: University of Kansas Press. p 77–81.
- Muntner P, He J, Hamm L, Loria C, Whelton PK. 2002. Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. *J Am Soc Nephrol* 13:745–753.
- Paolicchi A, Minotti G, Tonarelli P, Tongiani R, De Cesare D, Mezzetti A, Dominici S, Comporti M, Pompella A. 1999. Gamma-glutamyl transpeptidase-dependent iron reduction and LDL oxidation—a potential mechanism in atherosclerosis. *J Invest Med* 47:151–160.
- Rauchhaus M, Coats AJ, Anker SD. 2000. The endotoxin-lipoprotein hypothesis. *Lancet* 356:930–933.
- Saint George D, Everson PM, Stevenson JC, Tedrow L. 2000. Birth intervals and early childhood mortality in a migrating Mennonite community. *Am J Hum Biol* 12:50–63.
- Salive ME, Cornoni-Huntley J, Phillips CL, Guralnik JM, Cohen HJ, Ostfeld AM, Wallace RB. 1992. Serum albumin in older persons: relationship with age and health status. *J Clin Epidemiol* 45:213–221.
- Schalk BW, Visser M, Deeg DJ, Bouter LM. 2004. Lower levels of serum albumin and total cholesterol and future decline in functional performance in older persons: the Longitudinal Aging Study Amsterdam. *Age Ageing* 33: 266–272.
- Schatz IJ, Masaki K, Yano K, Chen R, Rodriguez BL, Curb JD. 2001. Cholesterol and all-cause mortality in elderly people from the Honolulu Heart Program: a cohort study. *Lancet* 358:351–355.
- Schupf N, Costa R, Luchsinger J, Tang MX, Lee JH, Mayeux R. 2005. Relationship between plasma lipids and all-cause mortality in nondemented elderly. *J Am Geriatr Soc* 53:219–226.
- Stevenson JC, Everson PM, Grimes M. 2004. Reproductive measures, fitness, and migrating Mennonites: an evolutionary analysis. *Hum Biol* 76:667–687.
- Strippoli GF, Craig JC, Manno C, Schena FP. 2004. Hemoglobin targets for the anemia of chronic kidney disease: a meta-analysis of randomized, controlled trials. *J Am Soc Nephrol* 15:3154–3165.
- Toshima H, Koga Y, Blackburn HW. 1994. Lessons for science from the Seven Countries Study: a 35-year collaborative experience in cardiovascular disease epidemiology. New York: Springer. 243 p.
- Uttley M. 1991. The relationship of measures of biological age to survivorship among Mennonites [dissertation]. Lawrence: University of Kansas.
- Uttley M, Crawford MH. 1994. Efficacy of a composite biological age score to predict ten-year survival among Kansas and Nebraska Mennonites. *Hum Biol* 66:121–144.
- Uttley M, Crawford MH. 2000. Biological age in Mennonite research. In: Crawford M, editor. *Different seasons: biological aging among the Mennonites of the Midwestern United States*. Lawrence: University of Kansas. p 101–126.
- Volpato S, Leveille SG, Corti MC, Harris TB, Guralnik JM. 2001. The value of serum albumin and high-density lipoprotein cholesterol in defining mortality risk in older persons with low serum cholesterol. *J Am Geriatr Soc* 49: 1142–1147.
- Wannamethee G, Ebrahim S, Shaper AG. 1995. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. *Am J Epidemiol* 142:699–708.
- Weverling-Rijnsburger AW, Blauw GJ, Lagaay AM, Knook DL, Meinders AE, Westendorp RG. 1997. Total cholesterol and risk of mortality in the oldest old. *Lancet* 350: 1119–1123.
- Whitfield JB. 2001. Gamma glutamyl transferase. *CRC Crit Rev Clin Lab Sci* 38:263–355.
- Whitfield JB, Zhu G, Nestler JE, Heath AC, Martin NG. 2002. Genetic covariation between serum gamma-glutamyltransferase activity and cardiovascular risk factors. *Clin Chem* 48:1426–1431.