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## Characteristics of Participants and Nonparticipants in the NHLBI Twin Study

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**Abstract.** The NHLBI Twin Study is a longitudinal study of cardiovascular disease risk factors in 514 pairs of white, middle aged, male, veteran twins. The initial examination took place between 1969-1973. Ten years later, 81% of the living cohort returned for a second examination. Data collected up to 30 years prior to recruitment for the initial examination were used to characterize participants and nonparticipants; data from the initial examination were used to characterize returnees and nonreturnees to the second examination. Participants had significantly lower diastolic blood pressure and higher socioeconomic status than nonparticipants as measured thirty years earlier. Between the first and second examinations, the mortality of participants was less than 50% of the mortality of nonparticipants. Returnees to the second examination had a better health profile at the initial examination than nonreturnees, with significantly lower levels of cigarette smoking, glucose intolerance, hypertension, and diabetes and higher levels of pulmonary function. However, returnees were more obese than nonreturnees. Thus, this study of cardiovascular disease risk factors in twins appears to be affected by response bias in a way similar to studies of individuals. Additional analyses of biases that may affect the genetic component of the study indicated that factors related to classical twin analyses were relatively unaffected by selection.

**Key words:** Bias, Longitudinal studies, Response rates, Selection, Twins

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### INTRODUCTION

The National Heart, Lung, and Blood Institute (NHLBI) Twin Study is a multi-center study of white middle aged male twins. It is among the largest longitudinal

studies of adult male twins in which the twins were physically examined. The cohort of 514 pairs of twins was first examined in 1969-1973 with the objective of estimating the genetic and environmental contributions to the variability of cardiovascular disease risk factors. The second examination was completed approximately ten years later with the goals of reexamining the initial estimates of heritability, assessing the changes in cardiovascular disease risk factors, and relating these changes to changes in environmental factors. This longitudinal study of twins provides the opportunity to analyze subjects, both as individuals and in pairs, and to assess genetic or environmental effects while controlling for confounding factors that may change with time. Underlying all analytic approaches, however, is the need to assess the potential biases created by the selection and participation of subjects in the different phases of the study. The method of defining this study cohort made it possible to compare the characteristics of participants and nonparticipants in the initial and followup examinations using data collected prior to each exam.

Twins for this study were ascertained through the National Academy of Sciences-National Research Council (NAS-NRC) Veteran Twin Registry. For every potential study participant, data were available from the induction physical examination and military service record as well as subsequent mortality followup through the Veterans Administration (VA). These data were used to compare characteristics of participants and nonparticipants in examination one. In turn, data from the first examination were used to compare participants (returnees) and nonparticipants (nonreturnees) in the second examination. Using combined data sets from the NAS-NRC Twin Registry and from examinations one and two of the NHLBI Twin Study, two questions were addressed: 1) What were the differences in induction characteristics and military service data of participants and nonparticipants in the initial examination of the NHLBI Twin Study? 2) What were the differences in examination one characteristics of returnees and nonreturnees to examination two?

## METHODS

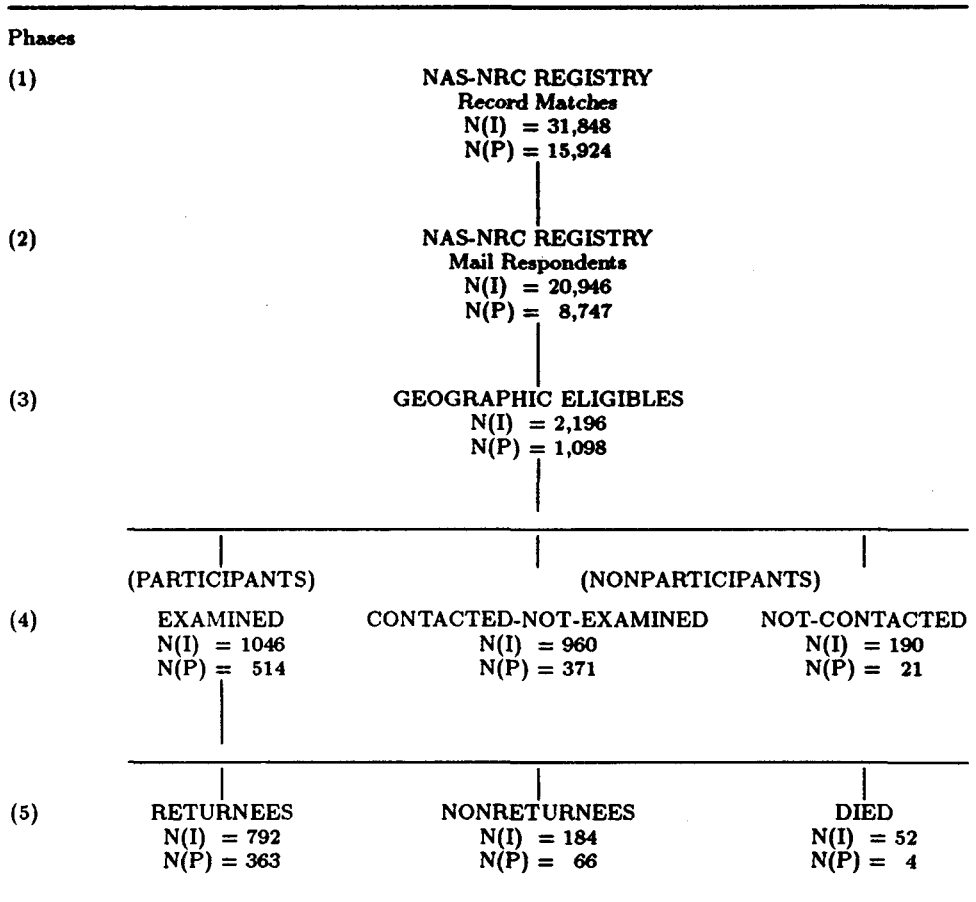
### Phases of the Sample Selection

Table 1 presents a flow chart of the five phases of the selection process. For analysis subgroups within each phase, the number of individuals [N(I)] and the number of complete pairs [N(P)] are shown. Since pairs were the primary unit of study, the recruitment goal was to obtain a sample of complete pairs for the initial examination.

In phase 1, the NAS-NRC created a twin registry by matching state birth records with VA records for 31,848 twins (15,924 pairs) who were born in 39 states during the period 1917-1927. Both brothers must have served in the U.S. Armed Forces during either World War II or the Korean Conflict. Nonwhites were excluded from this match due to small numbers. Details of the design and methodology can be found elsewhere [4]. This approach provided several advantages: the registry was

population-based since state birth records were used to ascertain twins, it provided baseline data since all subjects had military records, it provided an opportunity for contact since the VA could provide last known address, and it provided mortality followup through VA death benefits.

**Table 1 - Phases of sample selection for the NHLBI Twin Study**



N(I) = number of individual subjects  
 N(P) = number of twin pairs

In phase 2, 20,946 individuals (8,747 complete pairs) of the 31,848 twins ascertained by record matching were successfully contacted and responded to a mailed questionnaire. In phase 3, 1,098 pairs were determined to be geographically suitable for the study (they lived within 200 miles of one of the examination centers)

and were invited to participate in the NHLBI Twin Study. Examinations were completed during the period 1969-1973 on 1,046 subjects including 514 pairs of twins (phase 4). The target sample of the 2,196 individuals (1,098 pairs) who were eligible for recruitment was divided into three subgroups for analysis: 1) subjects who could not be contacted (not-contacted); 2) subjects who were contacted but not examined (contacted-not-examined); 3) subjects who were examined (examined). Nonparticipants in the initial examination consisted of 190 subjects (21 complete pairs) in the not-contacted group and 960 subjects (371 complete pairs) in the contacted-not-examined group. Feinleib et al [2] have described further details of the sample selection and response elsewhere. (Since that publication coding corrections have slightly modified the tables of responses to the examination invitation.)

Of the original 1,028 subjects (514 complete pairs) seen at exam one, a total of 792 subjects (363 complete pairs) were returnees for the second examination. Nonreturnees consisted of 184 subjects (66 complete pairs). In addition there were 52 subjects (4 complete pairs) who had died by the time of contact to request participation in the second examination. Eighteen individuals were dropped from the examined group in phase 4 because their cotwins did not participate in the first exam.

## Variables

The underlying design of this study required the consideration of two variables in all analyses: zygosity and examination center. Twins were classified as either monozygotic (MZ) or dizygotic (DZ) on the basis of 22 red blood cell antigens. Pairs were further classified according to the five initial examination centers: Indianapolis, Indiana; Framingham, Massachusetts; or Davis, San Francisco, or Los Angeles, California. When the distributions of the variables did not differ by center, data were combined without adjustment.

Data were collected on sociodemographic, health behavior, psychological, risk factor and disease outcome variables. Analyses of phase 4 subgroups were based on data from military service including the induction physical examination, military rank and discharge data, and morbidity and mortality as determined by requests for VA benefits. Comparisons of participants and nonparticipants for mortality as coded on the death certificates were made for the interval from the start of exam one (1969) to the start of exam two (1980).

Analyses comparing the subgroups in phase 5 were based on the first examination that was done at all five centers. The examination included a physical examination with emphasis on cardiovascular disease; laboratory measurements of lipids and lipoproteins, clinical chemistries, red blood cell antigens and a complete blood count; and questionnaires on health behaviors, family history, twin relationships and occupation and residence history. Body mass index was calculated as weight in kilograms divided by height in meters squared.

### Statistical methods

Analyses were directed at comparisons in two phases of selection, phase 4 and phase 5. Subjects were evaluated as individuals and in pairs. In phase 4 comparisons of induction data for individuals were made by analysis of variance (ANOVA) among the three subgroups. Analyses of pairs compared the absolute value of the within pair differences using ANOVA on these three subgroups. When both members of a pair were not in the same participation subgroup, the entire pair was dropped from the paired analyses. This process substantially reduced the number of observations in the not-contacted and contacted-not-examined subgroups.

In phase 5, comparisons of mean values of the two groups (returnees and nonreturnees) were done using t-tests. In all analyses the group of nonreturnees excluded individuals who had died in the interval between examinations since they were not at risk for second examination.

### RESULTS

Of the 2,196 geographically eligible twins, 8.7% could not be contacted. Of the twin pairs both receiving an invitation to participate in the study (examined and contacted-not-examined), 55.3% of the pairs participated in the initial examination.

**Table 2 - Distribution of twins (and complete pairs) by center and status at examinations 1 and 2**

Center	Examination 1		Examination 2		
	Examined	:	Examined <sup>a</sup>	Not examined	Died <sup>b</sup>
Framingham, MA	210 (105)	:	165 ( 76)	33 (12)	12 (2)
San Francisco, CA	108 ( 54)	:	78 ( 36)	25 (11)	5
Davis, CA	156 ( 78)	:	130 ( 60)	20 ( 6)	6
Los Angeles, CA	272 (136)	:	184 ( 82)	72 (28)	16 (2)
Indianapolis, IN	282 (141)	:	235 (109)	34 ( 9)	13
Total	1028 (514)	:	792 (363)	184 (66)	52 (4)

<sup>a</sup> Exam 2 participation is noted by exam 1 center even though some twins (N=8) were examined at a different center at exam 2.

<sup>b</sup> Includes 2 deaths occurring after March 81 start of exam 2 but before contact with subject to schedule exam was attempted.

Table 2 shows the distribution of twins and twin pairs by the initial examination center and status at the second examination. The five examination centers vary in size, with about half the subjects examined in the three California centers. Of the original cohort of 1,028, 792 (77%) returned for the second exam. By the start of the second exam, 52 individual twins or 5.1% had died, including four pairs.

Among the five centers, death rates ranged from 3.9% to 5.9% and were not significantly different. Among surviving twins, the response rate to examination two was 81.2%. Participation rates were significantly different by center and ranged from 72% to 87% of surviving twins. Considered as pairs, 71% of the original cohort, or 78% of available living pairs, were seen at the second examination.

**Table 3 - Distribution of twins (and concordant pairs) by zygosity and status at examination 2**

Zygosity	Exam 2 status		
	Returnee	Nonreturnee	Died
Monozygotic	376 (177)	103 (41)	21 (2)
Dizygotic	416 (186)	81 (25)	31 (2)
Total	792 (363)	184 (66)	52 (4)

As can be calculated from Table 3, participation rates of at risk MZ and DZ individuals were higher for DZ's (78.5% vs 83.7%). Of available pairs, the proportions of MZ and DZ pairs that were concordant returnees were similar (76.6% vs 79.1%). Death rates were 4.2% and 5.9% for MZ and DZ twins, respectively, and were not significantly different. Four pairs of twins had died: two MZ pairs and two DZ pairs.

**Table 4 - Characteristics at induction of individuals by response status to exam 1**

Characteristic	Examined <sup>a</sup> (N=1046)	Contacted-not-examined (N=960)	Not contacted (N=190)	P
Year of birth	1922.86	1922.52	1923.07	0.008
Age at induction	20.27	20.56	20.23	0.011
Weight (kg)	66.43	65.34	64.93	0.008
Height (cm)	173	172	173	<0.001
Body mass index (kg/m <sup>2</sup> )	22.08	22.06	21.80	0.39
Systolic BP (mm)	126.5	126.7	127.3	NS
Diastolic BP (mm)	74.7	75.7	76.6	0.006
Entry to active duty (yr)	1943.1	1943.1	1943.3	NS
Yr of separation (yr)	1946.7	1946.5	1946.8	NS
Unique diagnoses before 1970	4.91	5.07	4.67	NS
Percent officer rank	9.8	7.1	5.2	0.02

<sup>a</sup> Of these, 1028 (514 pairs) make up the NHLBI Twin Study Cohort.

In Table 4, data from military service for the 1,028 subjects comprising the 514 pairs in the NHLBI Twin Study cohort, along with an additional 18 singletons

whose cotwins did not participate, are compared with the not-contacted and the contacted-not-examined. Significant differences among the group means were seen for age at induction, year of birth, weight, height, diastolic blood pressure and percent who had attained the rank of officer. Those who were examined were younger than the contacted-not-examined, weighed the most, were the tallest, had lowest diastolic blood pressure and highest percentage who had attained the rank of officer. No significant differences were seen for systolic blood pressure, body mass index, year of entry on active duty, year of separation from active duty, or number of unique diagnoses in the military or VA hospitals. Results did not differ when done separately by zygosity.

Data for the variables in Table 4 were also analyzed (not part of Table 4) for absolute within pair differences by zygosity for each of the categories: examined, contacted-not-examined, and not-contacted with 514, 391, and 21 observations, respectively. Analyses of the within pair differences for the examination one variables revealed few significant findings. Only year of entry to active duty differed significantly among the groups for MZ twins. The not-contacted group had significantly greater within pair differences in year of entry to active duty than the groups that were contacted. When comparisons were made between zygositys (data not shown) MZ twins had smaller (though not significantly) within pair differences than DZ twins within each participation category for all of the variables in Table 4 except number of unique diagnoses.

**Table 5 - Standardized mortality ratios of subjects recruited for the NHLBI Twin Study by exam 1 participation status (Mortality from recruitment, 1969, to 2nd exam, 1980)**

Participation status	Cause of death		
	Cardiovascular diseases	Trauma	All causes
Examined	0.39	0.54	0.46
Contacted-not-examined	0.89	1.00	1.07
Not-contacted	1.09	1.94	0.93

Table 5 compares the mortality rates from cardiovascular diseases, trauma and all causes by participation status for the interval between the first and second examinations. The examined group had significantly lower mortality from cardiovascular, trauma and all causes. Standardized mortality ratios were approximately as expected for the contacted-not-examined group and highest for the not-contacted group for cardiovascular diseases and trauma. Mortality from trauma appears to be almost twice the expected rate in the not-contacted group.

Table 6 - Characteristics at exam 1 of individuals by response status to exam 2

Characteristic	Returnees (N=792)	Nonreturnees (N=184)	P
Age	47.8	48.2	NS
Weight (kg)	79.5	75.7	<0.001
Height (cm)	175.0	173.9	0.06
Body mass index (kg/m <sup>2</sup> )	25.9	25.0	<0.001
Systolic BP (mm Hg)	127.8	129.6	NS
Diastolic BP (mm Hg)	81.7	81.7	NS
Forced Vital Capacity	4.20	4.16	NS
Forced Expiratory Volume/1 sec	3.31	3.16	<0.02
Hematocrit (%)	46.8	46.7	NS
One hour glucose (mg/dl) <sup>a</sup>	154.3	164.0	0.03
Total cholesterol (mg/dl)	220.2	221.4	NS
HDL cholesterol (mg/dl)	45.2	45.9	NS
LDL cholesterol (mg/dl)	143.3	144.7	NS
Triglyceride (mEq/l)	449.4	446.0	NS
Cigarettes/day (of smokers)	26.1	27.4	<0.001
Smoke cigar (%)	13.8	8.2	0.01
Smoke pipe (%)	10.1	7.1	0.10
Smoke cigarettes (%)	34.9	58.7	<0.001
Uses filter (%)	29.9	45.6	<0.001
Inhales (%)	32.4	52.2	<0.001
On low calorie diet (%)	5.0	2.2	0.06
Yrs of education	13.1	12.9	NS
Yrs lived with twin	20.4	20.0	NS
Yrs worked with twin	4.76	4.97	NS
Freq see each other	3.00	2.98	NS

<sup>a</sup> After 50 g load of glucose on 12-hour fasting subjects.

Table 6 describes the exam one characteristics from the physical examination and medical history by participation status at exam two. Returnees tended to be younger and more educated. They were slightly taller, an average of 3.8 kg heavier, and had a significantly higher body mass index. Returnees were also more likely to be on a low calorie diet. Among the physiologic measurements, returnees had slightly, though not significantly, lower systolic blood pressure. They also had significantly better pulmonary function, as measured by forced expiratory volume in one second (FEV1), and significantly lower one hour serum glucose following a 50 g glucose challenge. Lipids and lipoproteins showed no differences between returnees and nonreturnees.

Returnees and nonreturnees to exam two differed substantially on smoking habits at exam one. Fifty-nine percent of nonreturnees smoked cigarettes at exam one compared to 35% of returnees. Nonreturnees smoked filter cigarettes more frequently but also inhaled more frequently. On the other hand, cigar and pipe smoking appeared to be more prevalent in those who returned for exam two.



Comparison of sociodemographic characteristics of the twin pairs indicated there were no significant differences in the number of years the twins lived together, worked together, or in the frequency with which they saw each other. There was a significant difference ( $P < 0.03$ ) in the distribution of marital status by exam two participation. Nonreturnees had fewer never married and more currently married and previously married individuals than returnees.

Table 7 - Clinical disease at exam 1 of individuals by response status to exam 2 (prevalence in percent)

	Returnees	Nonreturnees	P
Ischemic heart disease	0.4	0.0	NS
Hypertension	8.9	12.5	0.11
Diabetes	6.3	13.2	0.004
Pulmonary disease	3.5	10.9	<0.001
Clinical obesity	15.1	6.7	0.03

Table 7 presents the prevalence of disease at exam one, as diagnosed by the examining physician, for returnees and nonreturnees to exam two. Ischemic heart disease at exam one was almost nonexistent in the twins alive at the start of the second examination regardless of participation status. However, comparing nonreturnees to returnees, the prevalence was three times higher for pulmonary disease and two times higher for diabetes. Estimates of the prevalence of hypertension were 12.5% for nonreturnees and 8.9% for returnees, but this difference did not reach statistical significance. In contrast, the prevalence of obesity, a clinical assessment on the part of the examining physician, was significantly higher (15% vs 6.7%) in the returnees. These results were not different in MZ and DZ twins when analyzed separately. Additional analyses of the frequency of drug use revealed a significant difference only for tranquilizers which were more likely to be taken by nonreturnees.

## DISCUSSION

This study offered an unusual opportunity to assess the selection biases associated with a longitudinal study of white, middle-aged veteran twins. Comparisons of individuals for biases related to study entry and continuing participation were possible, as well as comparisons of pairs for biases related to the genetic component of this twin study. In addition, the comparisons were based on data collected prior to each examination, and collected in a comparable way, for participants and nonparticipants.

Younger individuals and higher SES individuals participated in the initial examination of this study. In fact, higher SES individuals (as determined by percent achieving the rank of officer while in military service) were easier to contact as well as more likely to participate when contacted. These results are consistent with general findings for volunteers [8]. More surprising is the gradient of lower blood pressures as individuals are easier to contact and, when contacted, easier to examine. In spite of a thirty year interval between the induction blood pressure and recruitment for this study, level of blood pressure appears to be related to participation in this study. While weight shows a similar gradient, much of that can be explained by height since body mass index was not statistically significant.

While morbidity, as determined by number of unique discharge diagnoses from military and VA hospitalizations, showed no significant differences among the three groups, mortality during the interval from recruitment for the first examination to recruitment for the second examination showed dramatic differences by participation. The standardized mortality ratio for the examined twins was approximately half that of the twins who were not examined and half the rates that would have been expected in the general population. This is similar to the difference between examined and not-examined men in the Framingham Heart Study [3]. For both cardiovascular disease and trauma there were clear gradients of decreasing risk over the not contacted, contacted-not-examined, and examined subgroups. This is a clear indication that individuals who fail to respond to a request for participation are different from participants, and that those who cannot be contacted may represent an extreme group of subjects within the population. Participation in the study is associated with improved mortality. While morbidity at the time of recruitment might explain part of these differences, it is worth noting that very little clinically evident morbidity was detected at exam one. Additional possibilities are that participation is an indication of general health seeking behavior or that the exam itself created an intervention which reduce a subsequent mortality. While direct intervention was not part of the exam, individuals with significant clinical findings were referred to their personal physicians.

Back extrapolation of many of the differences in exam one characteristics of returnees and nonreturnees to exam two would also be compatible with the greater mortality of nonparticipants versus participants. Current cigarette smoking was substantially more likely among the nonreturnees, as was the practice of inhaling. In contrast, the percentage of cigar smokers was significantly higher among the returnees and the percentage of pipe smokers was also higher, though not significantly. These data most likely indicate a greater switch away from cigarettes among the returnees. Other significant characteristics which may be related to greater cigarette smoking among the nonreturnees include lower FEV1 and lower body mass index. In addition, nonreturnees had significantly higher one hour post load glucose values. With the possible exception of obesity, all of these findings are compatible with higher mortality among the nonreturnees. Further analyses indicated the differences in obesity were related to the greater prevalence of smoking in the nonreturnees.

Clearly participants in the NHLBI Twin Study are a select group. The fact

that they were drawn from the NAS-NRC Twin Registry required that they pass the induction physical and survive military service as well as survive to middle age. Seltzer and Jablon [9] have shown that selection for military service greatly reduces mortality following separation. Additional selection of the study cohort is apparent for socioeconomic status (per cent officers) and general health (mortality gradient), and possibly cardiovascular disease risk (DBP and cardiovascular mortality gradient) and lifestyle (trauma mortality gradient).

Further selection occurred for the subjects who returned for the second examination. The opportunity for selection for specific objectives of this study were greater at exam two because the subject was aware of the sponsor of the study and of the items included in the previous data collection. As has been shown elsewhere [1], participants have a better health profile.

Of greater importance to this study is the question of the representativeness of the pairs. Lykken et al [6] have described a rule of thumb for assessing the bias of selection from the population by the ratio of the MZ to DZ twins. The bias of twin studies normally results in a higher rate of participation of MZ twins. In the registry, the percentages of MZ and DZ pairs have been estimated to be 44% and 56% , respectively. In this study there were 250 MZ (48.6%) and 264 DZ (51.4%) pairs which indicates a statistically significant, though not extreme, bias favoring participation of MZ pairs. However, it also appears that the pairs were not significantly different for characteristics by participation groups. Few significant within pair differences were found when pairs were grouped by participation status.

Martin and Wilson [7] and Kendler and Holm [5] have warned of the effects of sampling from a twin registry based on the requirement that both members of a pair pass an induction medical examination. It must be acknowledged that the distribution from which this sample is drawn was truncated at induction for many important characteristics including height, weight, blood pressure, glucose, functional status and disease [10]. However, this early selection process also proved to be the strength of this study by providing the data resource to characterize the differences in sample participants and nonparticipants on comparably collected data. These data provide insight into the types of bias that may effect twin studies generally. More specific to this study, knowledge of these data will allow adjustments to statistical methods and to interpretations of future analyses that will maximize the value of this longitudinal twin study.

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