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Total Mortality by Transferrin Saturation Levels: Two General Population Studies and a Metaanalysis

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BACKGROUND: There is evidence for increased mortality in patients with clinically overt hereditary hemochromatosis. Whether increased transferrin saturation (TS), as a proxy for iron overload is associated with increased mortality in the general population is largely unknown.

METHODS: We examined mortality according to baseline TS in 2 Danish population–based follow-up studies (the Copenhagen General Population Study and the Copenhagen City Heart Study) comprising a total of 45 159 individuals, of whom 4568 died during up to 18 years of follow-up, and in a metaanalysis comprising the present studies and an additional general population study.

RESULTS: In combined studies, the cumulative survival was reduced in individuals with TS ≥50% vs <50% (log-rank $P < 0.0001$). Multifactorially adjusted hazard ratios for total mortality for TS ≥50% vs <50% were 1.4 (95% CI 1.2–1.6; $P < 0.001$) overall, 1.3 (1.1–1.6; $P = 0.003$) in men, and 1.5 (1.1–2.0; $P = 0.005$) in women. Results were similar if the 2 studies were considered separately. A stepwise increased risk of total mortality was observed for stepwise increasing levels of TS (log-rank $P < 0.0001$), with the highest risk conferred by TS ≥80% vs TS <20% with a hazard ratio of 2.2 (1.4–3.3; $P < 0.001$). The population-attributable risk for total mortality in the combined studies in individuals with TS ≥50% vs <50% was 0.8%. In metaanalysis, the odds ratio for total mortality for TS ≥50% vs <50% was 1.3 (1.2–1.5; $P < 0.001$) under the fixed-effects model.

CONCLUSIONS: Individuals in the general population with TS ≥50% vs <50% have an increased risk of premature death.

There is evidence for increased mortality in patients with clinically overt hereditary hemochromatosis *(1–5)*. Early diagnosis and instigation of appropriate treatment with repeated venesections can prevent the consequences of hereditary hemochromatosis and restore normal life expectancy *(1–4)*. Although total mortality according to increased transferrin saturation (TS),[7] as a biochemical proxy for iron overload, has been examined in a single previous population-based study *(6)*, it is largely unknown whether increased TS in the general population is associated with increased mortality.

We tested the hypothesis that increased TS at baseline, as a proxy for iron overload, is associated with increased risk of total mortality in 2 Danish population-based follow-up studies comprising 45 159 individuals, of whom 4568 died. Follow-up was up to 18 years and was 100% complete.

Materials and Methods

By using 2 similar but independent white Danish population-based follow-up studies, the Copenhagen General Population Study (CGPS) 2003–2007 examination *(7)* and the Copenhagen City Heart Study (CCHS) 1991–1994 and 2001–2003 examinations *(8, 9)*, we examined 36 419 and 8740 individuals, respectively. Individuals in the 2 studies were ascertained and examined similarly. The studies were approved by Herlev Hospital and the Danish ethics committees (KF-100.2039/91, KF-01-144/01, and H-KF-01-144/01). Written informed consent was obtained from all participants in both studies. The studies complied with the Declaration of Helsinki.

TRANSFERRIN SATURATION

TS (%) was determined as iron concentration (in μmol/L) divided by 2 × transferrin concentration (in μmol/L) × 100. Transferrin was measured by turbi-

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[7] Nonstandard abbreviations: TS, transferrin saturation; CGPS, Copenhagen General Population Study; CCHS, Copenhagen City Heart Study; BMI, body mass index.

Table 1. Baseline characteristics of participants in 2 population-based follow-up studies.				
	CGPS mortality[a,b]		CCHS mortality[a,b]	
	Alive	Dead	Alive	Dead
n	35 294	1125	5297	3443
Women, %	54	38[c]	60	52[c]
Age, years	58 (47–67)	74 (66–80)[c]	53 (40–63)	70 (64–76)[c]
BMI >25 kg/m^2, %	57	58	47	59[c]
Total tobacco consumption, pack-years >10,[d] %	24	32[c]	46	65[c]
Current smoker, %	23	32[c]	44	54[c]
Plasma cholesterol >5 mmol/L, %	75	66[c]	79	88[c]
Antihypertensive medication, %	19	37[c]	8	19[c]
Alcohol >84 g/week (i.e.>7 units/week), %	52	52	41	39
Physically inactive,[e] %	7	13[c]	8	19[c]

[a] CGPS had a median follow-up of 4 years (interquartile range 3–4 years). CCHS had a median follow-up of 15 years (interquartile range 10–16 years). Variables expressed as median (± interquartile range) or proportion were collected at the 2003–2007 examination of the CGPS and the 1991–1994 examination of the CCHS.

[b] Statistical comparisons were made using 2-sided Mann–Whitney *U*-test and Pearson χ^2 test as appropriate.

[c] $P < 0.001$.

[d] One pack-year is equivalent to smoking 20 cigarettes each day in 1 year.

[e] Physical activity was leisure-time physical activity (almost completely inactive, some activity, regular activity, or regular hard physical training).

dimetry and iron by colorimetry (Konelab autoanalyzer; ThermoFisher Scientific). A threshold level of TS ≥50% was chosen as suggestive of increased TS, in accordance with accepted clinical practice *(10–12)*.

To explore a graded relationship, TS was divided into 8 categories: TS <20%, TS ≥20% but TS <30%, TS ≥30% but TS <40%, TS ≥40% but TS <50%, TS ≥50% but TS <60%, TS ≥60% but TS <70%, TS ≥70% but TS <80%, and TS ≥80%.

OTHER CHARACTERISTICS

Individuals were questioned about alcohol consumption, smoking habits, antihypertensive medication, and physical activity. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Plasma total cholesterol was measured enzymatically *(13)*.

END POINT

Information on mortality was obtained from the Danish Civil Registration System *(14)* from time of blood sampling until May 9, 2009, in both studies. Follow-up in the CGPS was from 2003–2007 through May 2009, and follow-up in the CCHS was from 1991–1994 through May 2009. Follow-up information was acquired for all participants.

STATISTICS

The Stata/SE 10.0 statistical software package was used for statistical analysis. Mann–Whitney *U*-tests and Pearson χ^2 tests were used for continuous and categorical variables, respectively. Two-sided *P* values <0.05 were considered significant. A priori, we stratified main analyses by sex, because penetrance of clinically manifest hemochromatosis differs markedly in the 2 sexes. In an explorative analysis, we also stratified participants into 8 groups of TS as described above.

Cumulative survival was plotted by using Kaplan-Meier curves, and differences between TS levels were examined by log-rank tests. Cox proportional hazards regression was used to estimate hazard ratios with 95% CIs. We analyzed age at event by using left truncation (or delayed entry) and age as time scale, thereby allowing automatic adjustment for age. The assumption of proportional hazards was tested with the use of Schoenfeld residuals, and no violations were observed. Interaction of TS levels with other risk factors was evaluated by including 2-factor interaction terms, 1 at a time, in the multifactorial Cox regression model. With use of age as the time scale, we could not study the effects of age itself. Therefore, for the test of interaction of age with TS levels, we used years of follow-up as the time scale analyzing time to event. No significant or clinically relevant interactions were observed.

Multifactorial adjustments included age, alcohol consumption (intake of ≤7 drinks/week vs >7 drinks/week), smoking habits (current vs nonsmoker; pack-years of smoking: 0, >0 but ≤10 pack-years, and >10 pack-years; 1 pack-year is equivalent to smoking 20 cigarettes/day for 365 days/year), leisure-time physical

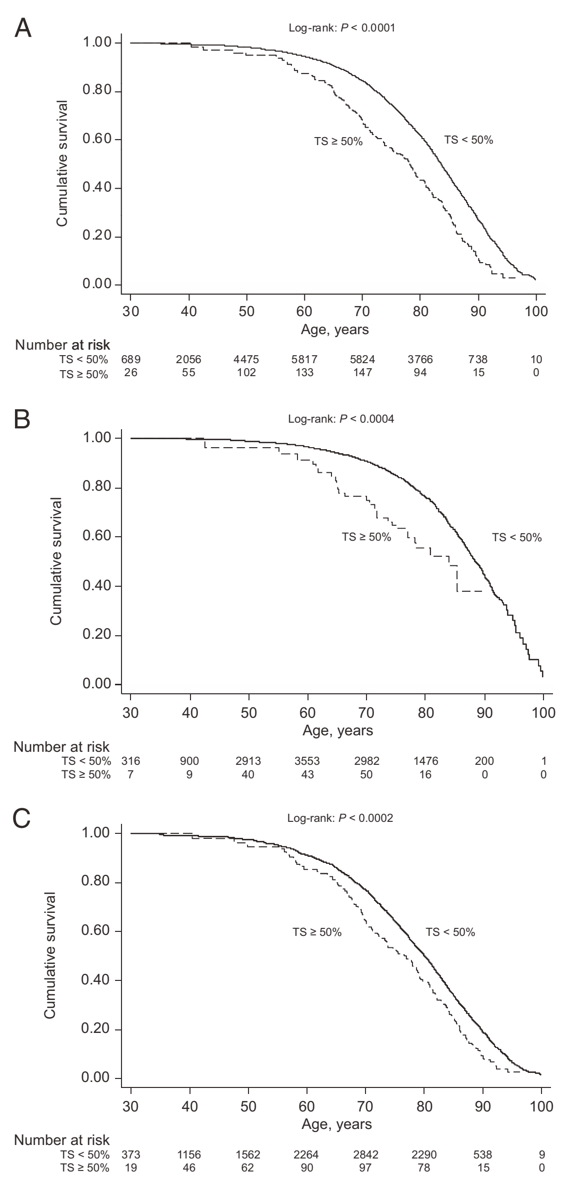

Fig. 1. Total mortality in individuals with TS ≥50% vs <50%.
(A), CGPS and CCHS combined. (B), CGPS. (C), CCHS.

activity (almost completely inactive, some activity, regular activity, regular hard physical training) *(15)*, BMI ($<$25 vs $\geq$25 kg/m^2), cholesterol ($<$5 vs $\geq$5 mmol/L), antihypertensive medication (yes vs no), and sex (except in sex-stratified analyses). Multifactorially adjusted models included time-dependent covariates from the 1991–1994 and 2001–2003 examinations for the CCHS.

Population-attributable risk was estimated as: $[f(\text{HR} - 1)]/[1 + f(\text{HR} - 1)]$, where f is the frequency of TS $\geq$50% in the population, and HR is the hazard ratio for total mortality *(16)*.

METAANALYSIS

A priori search strategy and selection criteria. We included prospective studies on the risk of total mortality by increased TS in a general population that were published before June 17, 2010. Relevant studies were identified through PubMed searches and by examination of reference lists of articles.

The keywords used were "transferrin saturation" and ("survival" or "mortality" or "longevity") and "follow-up" (giving 32 hits); 4 studies were retrieved *(17–20)*. Another 2 references *(6, 21)* were retrieved from manual searches of journals.

Studies were included if they were conducted prospectively and had follow-up data (i.e., incidence) of total mortality as an end point and provided risk estimates with confidence limits or tabular data *(6)*. Studies were excluded if they provided only disease-specific mortality *(17–21)*. We also included results from the CGPS and CCHS.

Data abstraction. The following information was abstracted from each study according to a fixed protocol: authors, year of publication, country, follow-up in years, ethnicity, sex, study name, number of participants, age, end point, TS interval, risk estimate, and confidence limits.

Statistical analysis. Only 1 study *(6)* met the inclusion criteria. Thus, we ended up with individual participant data in 2 studies (CCHS and CGPS) and published tabular data in a third study (Mainous et al.) *(6)*. We performed and reported the individual analyses and then combined the summary findings from the 3 studies in a metaanalysis. Statistical analyses were performed with use of the Stata Meta command to calculate both fixed and random effect measures from reports of effect measures and CIs *(22)*. Statistical heterogeneity was assessed by the Q statistic with a corresponding P value, although lack of power may be an issue owing to the limited number of studies *(23)* ($P < 0.05$ was considered significant). Because only 3 studies were included in the metaanalysis [CGPS, CCHS, and a study by Mainous et al. *(6)*], it was not possible to assess publication bias. Although we had decided a priori to assess methodological heterogeneity by stratification on sex, this was not possible, because 1 study was not sex stratified *(6)*.

Results

GENERAL POPULATION STUDIES

Table 1 lists characteristics of participants at study entry. The median follow-up time was 4 years (interquar-

Table 2. Sex- and study-specific total mortality according to transferrin saturation in 2 Danish population–based follow-up studies combined.[a]

	Transferrin saturation	Participants, n	Events, n	HR[c] (95% CI)	*P*
All sexes	<50%	44 306	4391	1.0	
	≥50%	853	177	1.4 (1.2–1.6)	<0.001
Sex-specific					
Men	<50%	20 146	2237	1.0	
	≥50%	569	126	1.3 (1.1–1.6)	0.003
Women	<50%	24 160	2154	1.0	
	≥50%	284	51	1.5 (1.1–2.0)	0.005
Study-specific					
CGPS[b]	<50%	35 900	1097	1.0	
	≥50%	519	28	1.8 (1.2–2.6)	0.003
CCHS[b]	<50%	8406	3294	1.0	
	≥50%	334	149	1.2 (1.0–1.4)	0.02

[a] Based on the CGPS and CCHS.
[b] The CGPS had a median follow-up of 4 years (interquartile range 3–4 years). The CCHS had a median follow-up of 15 years (interquartile range 10–16 years).
[c] HR, hazard ratio with 95% CI adjusted for age, sex (not for sex-stratified analyses), BMI, tobacco consumption, smoking habits, cholesterol, antihypertensive medication, alcohol consumption, and physical activity as listed in Table 1.

tile range 3–5 years) in the combined studies, 4 years (interquartile range 3–4 years) in the CGPS, and 15 years (interquartile range 10–16 years) in the CCHS.

In the 2 studies combined, the cumulative survival was reduced in individuals with TS ≥50% vs <50% (log-rank $P < 0.0001$) (Fig. 1A). In the 2 studies combined, multifactorially adjusted hazard ratios for total mortality for TS ≥50% vs <50% was 1.4 (95% CI 1.2–1.6; $P < 0.001$) overall, 1.3 (1.1–1.6; $P = 0.003$) in men, and 1.5 (1.1–2.0; $P = 0.005$) in women (Table 2). Study-specific results for the CGPS and CCHS separately with multifactorially adjusted hazard ratios were 1.8 (1.2–2.6; $P = 0.003$) and 1.2 (1.0–1.4; $P = 0.02$) overall (Table 2). In Fig. 1B and C, it is shown that the relative risks in a population with a lower rate of TS ≥50% during a median of 4 years of follow-up (CGPS) and one with a higher rate of TS ≥50% during a median of 15 years of follow-up (CCHS) are not entirely comparable. Stepwise increasing levels of TS were associated with a stepwise increased risk of total mortality (trend-test, log-rank: $P < 0.0001$) (Fig. 2). The highest risk was conferred by TS ≥80% vs TS <20% with a hazard ratio of 2.2 (1.4–3.3; $P < 0.001$).

On the basis of a frequency of TS ≥50% of 1.9% overall, 2.8% in men, and 1.1% in women in the 2 studies combined, and on multifactorially adjusted hazard ratios of 1.4, 1.3, and 1.5, respectively, for total mortality, the corresponding population-attributable risks were 0.8% overall, 0.8% in men, and 0.6% in women.

METAANALYSIS

The odds ratio for total mortality for TS of approximately ≥50% vs approximately <50% was 1.3 (95% CI 1.2–1.5; $P < 0.001$) under the fixed effects model and 1.4 (1.1–1.9; $P = 0.005$) under the random effects model (heterogeneity $Q = 5.1$, $P = 0.08$) (Fig. 3).

Discussion

In 2 Danish population–based follow-up studies comprising 45 159 individuals, and in a metaanalysis comprising 55 873 individuals, we showed that individuals with the threshold level of TS ≥50% vs <50% have an increased risk of premature death; we had individual participant data in the 2 Danish studies and published tabular data in one-third, performed and reported the individual analyses, and then combined the summary findings from the 3 studies in a metaanalysis. Moreover, a stepwise increased risk of total mortality was observed for stepwise increasing levels of TS, with the highest risk conferred for TS ≥80%. This study is the largest and most comprehensive study to date estimating risk of total mortality by increased TS.

Our study confirmed a previous finding *(6)* that increased TS is associated with increased total mortality overall. However, in the current study, we also demonstrated an increased mortality in sexes separately and a stepwise increased risk of early death with stepwise increased TS.

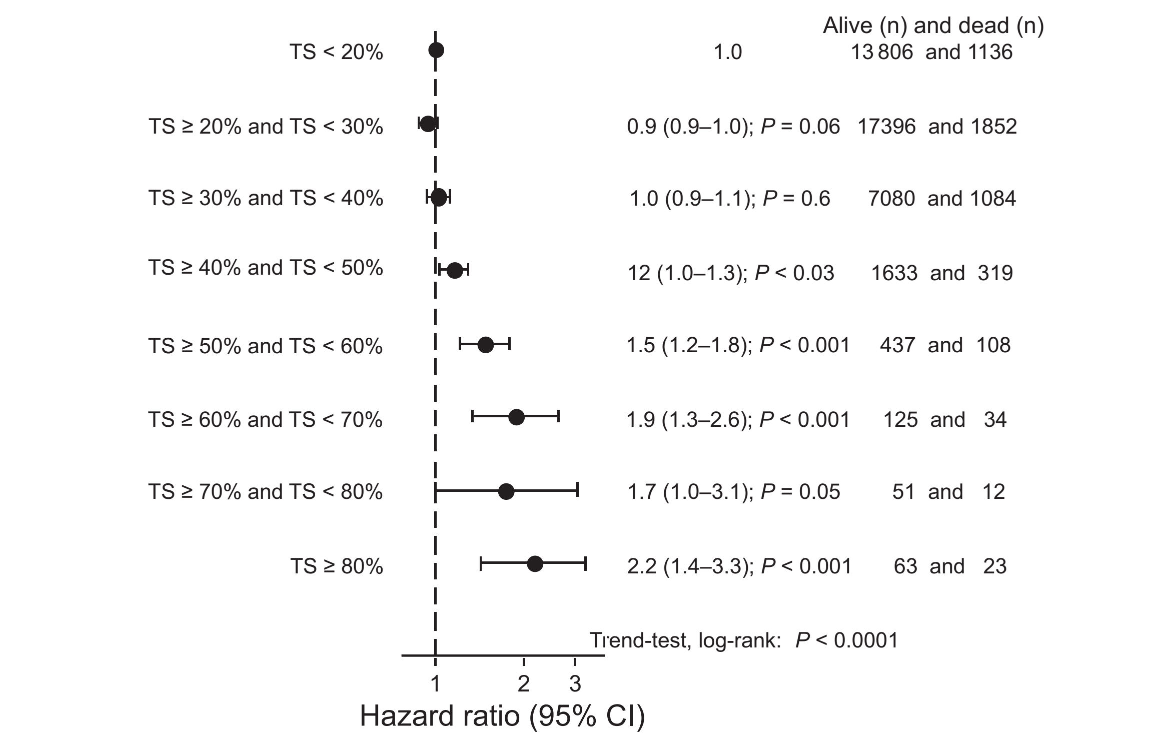

Fig. 2. Total mortality by stepwise increasing TS in individuals in the general population.
Results are shown for CGPS and CCHS combined.

The graded relationship between TS and total mortality suggests no evidence of a threshold effect, but rather a continuum, with the most extreme being TS ≥80%. This result is in accordance with a previously published study also showing a stepwise increase in risk of being on antihypertensive medication by stepwise increasing levels of TS *(24)*.

The biological mechanism for the relationship between increased TS and premature death may be a result of the Fenton reaction causing oxidative stress, which may affect survival *(25, 26)*. We estimated total mortality, because this is a more unbiased measure than disease-specific mortality *(27)*. We focused on total mortality and its relationship with the biochemical marker TS, an intermediate step between clinically silent hemochromatosis and clinical hemochromatosis *(28)*. Mortality among advanced cases of untreated hemochromatosis is high and usually due to liver cirrhosis and diabetes mellitus *(2, 4)*; however, whether clinically unnoticed increases in TS also lead to premature death has hitherto been unknown. In support of the present findings, a recent study of total mortality in hemochromatosis patients compared to controls showed a hazard ratio of 2.2 (1.6–3.0) *(5)*, close to the estimates in the present study for TS ≥80% vs TS <20%.

The population-attributable risk for total mortality in individuals with TS ≥50% vs <50% showed that the public health significance was small. Nonetheless, it is important, untreated hemochromatosis carries a poor prognosis, and early diagnosis and aggressive treatment of hemochromatosis improves survival and may lead to long-term survival similar to that in the general population *(2–4, 29–31)*.

There were certain limitations to our study. We cannot exclude that individuals with high TS did not have bone marrow suppression, hepatocellular injury, or a transient nonspecific rise in TS instead of increased iron stores. Ferritin concentrations were not measured, and there were no repeated measures of TS. Furthermore, information on recent heavy alcohol use or oral iron supplementation was not recorded. Errors in measurement and within-person variation of TS, however, should result in bias toward the null. None of the individuals in the CCHS with genotypes associated with hereditary hemochromatosis developed overt hemochromatosis *(32)*; however, we have no specific hemochromatosis follow-up on individuals in the CGPS. Furthermore, we do not have any information on blood donation.

The practical implication of our findings seems to be that undiagnosed and untreated hemochromatosis

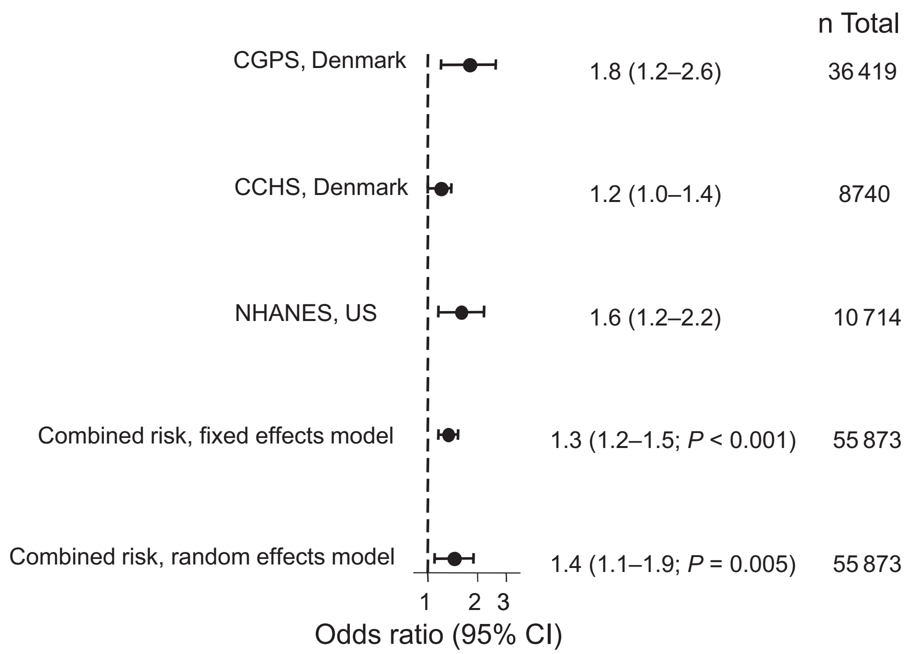

Fig. 3. Metaanalysis of risk of total mortality by TS in prospective studies of the general population.
TS is approximately ≥50% vs <50%. Heterogeneity $Q = 5.1$, $P = 0.08$. Horizontal lines indicate CIs, and the filled circles represent the risk estimates. NHANES, National Health and Nutrition Examination Survey.

(the presumptive interpretation of the TS of ≥50% or even ≥40%) is undesirable because it is associated with increased mortality. In 1956, Denham Harman wrote a "free-radical theory of aging" about endogenous oxidants resulting in cumulative damage *(33–35)* and hence premature death. Much of the early evidence was based on a correlation between oxidative stress and aging *(35)*; however, recent research suggests a more causal relationship between oxidative stress and aging *(35, 36)*. Thus, we suggest that hemochromatosis and even modest iron overload could provide a model for reactive oxygen species production through the Fenton reaction, thus leading to premature death. It is unclear whether it is the absolute level of oxidative stress or the response to oxidative stress that determines life expectancy *(35)*; however, we have shown that risk of premature death increases stepwise with stepwise increasing levels of TS, even at intervals lower than the clinically agreed upon threshold level of TS ≥50% *(10–12)*. This result raises the question as to whether individuals without hemochromatosis are at risk of premature death from modest iron excess.

If there are free-living individuals at risk of early death due to modest or severe iron excess (for example, because they have hemochromatosis) but having no awareness of their iron stores, as suggested in the present study, should one then recommend population screening? This is indeed an important but difficult question to answer. Clinical penetrance is essential in considering screening for a disease: penetrance of genotypes sometimes leading to hereditary hemochromatosis is low *(37, 38)* and such screening is not yet recommended by experts in the field *(39)*; however, our results address the broader question of whether individuals having no awareness of common increased iron stores and being at increased risk of premature death should be screened with a simple blood test of TS rather than a genotype test. Determining the answer to this question will naturally require further scrutiny of the diagnostic values of TS tests, the population of individuals who would benefit from screening, and the cost-effectiveness of TS-screening strategies. Our present results may be of value if and when the screening issue is reevaluated.

If screening is recommended, a likewise important but also difficult question is whether the screening test should be TS or ferritin. Indeed, debate exists as to whether TS *(10–12)* or ferritin *(40)* is the best first iron overload indicator for hemochromatosis *(41)*. The present results unfortunately do not enable us to solve this issue, except to demonstrate that TS is an important indicator of increased risk for premature death. We hope that future studies will examine in parallel the predictive value of TS and ferritin levels to better understand which test is the optimal one.

In conclusion, individuals in the general population with TS ≥50% vs <50% have an increased risk of premature death. These data may be useful in the continued discussion of whether or not to screen for hemochromatosis or even modest iron overload. They may also prove useful in the discussion of whether common use of iron supplement tablets is advisable.

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