Journal of the American Heart Association

ORIGINAL RESEARCH

High-Sensitivity Cardiac Troponin I and T Response Following Strenuous Activity is Attenuated by Smokeless Tobacco: NEEDED (North Sea Race Endurance Exercise Study) 2014

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BACKGROUND: Use of snus, a smokeless tobacco product, is increasing in Scandinavia. Strenuous physical activity is associated with an acute increase in high-sensitivity cardiac troponin (hs-cTn) concentrations. Current smoking is associated with lower hs-cTn, but whether this also holds true for smokeless tobacco and whether tobacco affects the hs-cTn response to exercise remain unknown.

METHODS AND RESULTS: We measured hs-cTnI and hs-cTnT concentrations in 914 recreational athletes before and 3 and 24 hours after a 91-km bicycle race. Self-reported snus tobacco habits were reported as noncurrent (n=796) and current (n=118). The association between snus use and change in log-transformed hs-cTnI and hs-cTnT concentrations (ie, the differences between concentrations at baseline and 3 hours and 24 hours) were assessed by multivariable linear regression analysis. Concentrations of hs-cTn at baseline were lower in current than in noncurrent snus users (hs-cTnI median, 1.7 ng/L; Q1 to Q3: 1.6–2.3 versus 2.0 ng/L; Q1 to Q3: 1.6–3.2 [*P*=0.020]; and hs-cTnT: median, 2.9 ng/L, Q1 to Q3: 2.9–3.5 versus 2.9 ng/L, Q1 to Q3: 2.9–4.3 [*P*=0.021]). In fully adjusted multivariable models, use of snus was associated with lower change in hs-cTn concentrations from baseline to 3 hours (hs-cTnI: –29% [*P*=0.002], hs-cTnT: –18% [*P*=0.010]) and 24 hours (hscTnI: –30% [*P*=0.010], hs-cTnT –19%, [*P*=0.013]).

CONCLUSIONS: Resting hs-cTn concentrations are lower and the exercise-induced cardiac troponin response is attenuated in current users of smokeless tobacco compared with nonusers. Further insight into the pathophysiological processes underlying the attenuated cardiac troponin response to exercise in tobacco users is needed.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT02166216.

Key Words: exercise ■ troponin ■ smokeless tobacco ■ nicotine ■ snus

Strenuous exercise is associated with an acute and transient increase in circulating cardiac troponin (cTn) concentrations.[1] Although elevated concentrations of cTn in healthy individuals following strenuous activity in most cases is considered to be a benign phenomenon, the underlying pathophysiological mechanisms and clinical relevance remain unclear.[1–4] The dominating theory for the exercise-related

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Supplementary Materials for this article are available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.017363

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For Sources of Funding and Disclosures, see page 8.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In this cohort of healthy, trained individuals, we demonstrate lower concentrations of circulating cardiac troponin I and cardiac troponin T in users of snus, a moist oral tobacco product in the resting state.
- Current snus users had a smaller increase in cardiac troponin concentrations 3 and 24 hours postexercise compared with noncurrent users of snus.

What Are the Clinical Implications?

- The increasing use of alternative tobacco products is a public health concern, and the current study demonstrates that moist oral tobacco may impact the release or degradation of cardiac troponins.
- Use of smokeless tobacco is associated with lower circulating cardiac troponin concentrations both in the resting state and after exercise, and tobacco use should be taken into account when interpreting cardiac troponin test results.

Nonstandard Abbreviations and Acronyms

cTn	cardiac troponin
cTnI	cardiac troponin I
cTnT	cardiac troponin T
NEEDED	North Sea Race Endurance Exercise Study

cTn response, however, is release of loosely bound troponin from a rapidly releasable pool combined with a reversible increase in membrane permeability.[5,6]

A broad range of smokeless tobacco products are used by >300 million adults around the world, and Swedish snus is the dominating smokeless tobacco in Scandinavia. In contrast to the marked decline in tobacco smoking, the consumption of snus has increased both in Scandinavia and in the United States during the past decades.[7,8] Despite no demonstrable effect of snus use on exercise performance, the use of smokeless tobacco is common and also increasing among elite and recreational athletes.[9] It is well documented that tobacco smoking is associated with an increased incidence of cardiovascular disease.[10] The association between snus tobacco and cardiovascular risk, however, remains unclear. Raised blood pressure and heart rate are described as acute hemodynamic effects of snus,[11] and some data suggest that quitting snus after a myocardial infarction is associated with reduced mortality.[12] However, no increased risk of nonfatal ischemic heart disease has been found among Swedish snus users.[13]

Stable, low-grade elevation in high-sensitivity cTn (hs-cTn), even within the normal concentration range, is associated with increased risk of nonfatal and fatal cardiovascular events.[14,15] Surprisingly, lower concentrations of hs-cTn in smokers have been demonstrated in 2 large population-based cohorts.[16,17] However, the influence of smokeless tobacco on baseline and exercise-induced concentrations is unknown. We hypothesized that snus use is inversely associated with hs-cTn concentrations in the resting state and an attenuated hs-cTn response after strenuous exercise. To test these novel hypotheses, we assessed the association between snus use and circulating levels of hs-cTnI and hs-cTnT at rest and following strenuous exercise in a large cohort of healthy, trained individuals.

METHODS

The data that support the findings of this study are available from Dr Stein Ørn on reasonable request.

Study Overview and Participants

This is a substudy of NEEDED (North Sea Race Endurance Exercise Study) 2014. Details of the NEEDED design and the principal results have been previously reported.[1] In brief, this is a prospective observational study of the biomarker response in healthy recreational cyclist participating in a 91-km bicycle race (North Sea Race) in Norway. Race participants without previous known cardiac disease, hypertension, and diabetes mellitus were invited to participate in the study. Study recruitment was performed with an electronic form distributed through the official website of the North Sea Race (www.nordsjorittet.no). The Regional Committee for Medical Research Ethics approved the study, and all participants provided informed written consent.

Baseline Data

Clinical examinations were performed in a standardized manner and included height, weight, blood pressure, and resting ECG. Information on health, lifestyle, fitness, and training-related items were gathered from self-reported electronic questionnaires. Data on snus use and cigarette smoking were also collected via the electronic questionnaire. The questionnaire was submitted before the race.

Blood Sampling Procedures and Biochemical Assays

As previously reported, samples of nonfasting venous blood were collected 24 hours before the race

(baseline) and at 3 and at 24 hours following the race. The serum samples were stored at 4°C and transported to Stavanger University Hospital, Norway. hs-cTnI concentrations were analyzed within 24 hours with the Abbott Diagnostics STAT High Sensitive Troponin assay on Architect i2000SR (Abbott Diagnostics), with a lower detection limit of 1.6 ng/L. Additional serum was frozen at −80°C and shipped to Haukeland University Hospital, Bergen, Norway, for measurement of hs-cTnT on a Cobas e602 device (Roche Diagnostics), with a level of blank of 3 ng/L and lower detection limit of 5 ng/L. During the period in which the NEEDED samples were analyzed, the cTnT assay had a total analytical coefficient of variation (CV_A) of 13% at 4.5 ng/L, 3.6% at 18 ng/L, and 2.1% at 93 ng/L. The cTnI assay had a total CV_A of 10% at 6 ng/L, 7% at 27 ng/L, and 5% at 140 ng/L. Information concerning the fraction of deltas that were higher than the reference change value are presented in Data S1.

Statistical Analysis

Means and SDs were reported for continuous variables with a symmetric distribution, while median and 25th to 75th percentile were reported for variables with a markedly skewed distribution. We used the Shapiro–Wilk test to test for normality. Numbers and percentages were used to report frequencies. The Student *t* test, Mann–Whitney *U* test, chi-square test, or the Fisher exact test was used for comparison of groups, as appropriate. A *P* value of <0.05 was considered significant. To assess the relationship between use of smokeless tobacco and the troponin increase, the delta values were used in multiple linear regression analysis. Potential confounding variables were selected a priori, based on factors known to influence cardiovascular risk and/or exercise-induced cTn release.

Model 1 was unadjusted, model 2 was adjusted for sex and age, model 3 was adjusted for model 2 and systolic blood pressure, body mass index, low-density lipoprotein cholesterol, and estimated glomerular filtration rate, and model 4 was adjusted for the same variables as in model 3 but also race duration and resting heart rate. Residual plots were deemed satisfactory after ln transformation of the dependent variables. Missing data attributable to a negative delta value were <5% in the smokeless tobacco group at any time, while the nonsmokeless tobacco group had 0.3%/2.5% missing for delta cTnI at 0 to 3 hours/0 to 24 hours, and 2.3%/5.4% for delta cTnT at 0 to 3 hours/0 to 24 hours. To evaluate the robustness of our findings and to assess consistency, we also performed all analyses using the absolute cTn concentrations at 3 and 24 hours rather than the delta value as the dependent variable in the full sample. For the statistical analysis, the software programs SPSS version 24 (SPSS Inc) and GraphPad Prism version 8 (GraphPad Software) were used.

RESULTS

Characteristics at Baseline

Overall, 914 race participants with cTn results and data concerning snus use were included in the study; 711 (78%) were men and the median age was 46.7 years (quartile 1 to quartile 3 [Q1 to Q3] 40.2–52.4 years). Among the participants, 118 (13%) were current snus users and 794 (87%) were noncurrent users of snus. Current users of snus were younger and had higher estimated glomerular filtration rate than nonusers. The prevalence of smoking habits differed according to snus use. Current users of snus were more likely to be former and current smokers than never users. However, training history and race performance did not differ between current and noncurrent users of snus (Table 1).

Associations Between Snus Use and hs-cTnI and hs-cTnT at Baseline

Concentrations of cTn in the resting state differed according to snus use (Figure). In unadjusted analyses, current snus use was associated with significantly lower concentrations of hs-cTnI (current users of snus versus noncurrent: median, 1.7 ng/L; Q1 to Q3: 1.6–2.3 versus 2.0 ng/L; Q1 to Q3: 1.6–3.2; *P*=0.020) and hs-cTnT (current users of snus versus noncurrent: median, 2.9 ng/L; Q1 to Q3: 2.9–3.5 versus 2.9 ng/L; Q1 to Q3: 2.9–4.3; *P*=0.021) before the race.

Association Between Snus Use and the Magnitude of the cTn Response to Exercise

Concentrations of cTn 3 and 24 hours after exercise also differed according to snus use (Figure). The change in concentrations of hs-cTnI from baseline to 3 hours postexercise (median, 35.4 ng/L; Q1 to Q3: 23.2–76.1 ng/L) was significantly lower in current than noncurrent users of snus (median, 50.2 ng/L; Q1 to Q3: 31.1–87.9 ng/L [*P*=0.001]). A similar pattern was observed for the change in concentrations of hs-cTnT from baseline to 3 hours postexercise: concentrations were lower in current users of snus (median, 25.9 ng/L; Q1 to Q3: 16.5–43.0 ng/L) than in noncurrent users (median 32.3 ng/L; Q1 to Q3: 21.6–50.1 ng/L [*P*=0.010]). The inverse association between current snus use and the change in concentrations from baseline to 24 hours following the race was significant for hs-cTnI (*P*=0.009), but not for hs-cTnT (*P*=0.058).

Table 1. Baseline Characteristics According to Snus Status

Variable	Noncurrent Snus Users n=796	Current Snus Users n=118	*P* Value
Baseline characteristics			
Men, No. (%)	609 (76.5)	102 (86.4)	0.015
Age, y	47.0 (41.1–53.0)	43.1 (36.5–49.6)	<0.001
Weight, kg	82.1 (74.5–89.3)	81.1 (73.4–90.2)	0.798
Body mass index, kg/m^2	25.3 (23.8–27.4)	25.1 (23.5–27.5)	0.564
Waist circumference	86.0 (80.0–92.9)	84.8 (80.0–91.0)	0.508
Systolic blood pressure, mm Hg	136.5 (127.0–148.0)	135.0 (120.0–145.0)	0.153
Diastolic blood pressure, mm Hg	80.0 (74.0–86.0)	79.0 (73.0–85.0)	0.691
Resting heart rate	59.0 (53.0–66.0)	61 (52–67)	0.545
Medical history			
Family history of cardiovascular disease, No. (%)*	151 (19.5)	31 (26.3)	0.045
Never smoker, No. (%)	519 (66.8)	40 (33.9)	
Former smoker, No. (%)	248 (31.9)	64 (54.2)	
Current smoker, No. (%)	10 (1.3)	3 (2.5)	<0.001
Blood tests			
eGFR, mL/min per 1.73 m^2	91.0 (82.2–99.8)	94.1 (84.4–103.5)	0.003
LDL cholesterol, mmol/L	3.2 (2.6–3.7)	3.3 (2.7–3.8)	0.291
HDL cholesterol, mmol/L	1.5 (1.3–1.7)	1.5 (1.3–1.8)	0.970
CRP, mg/L	0.8 (0.4–1.4)	0.6 (0.3–1.0)	0.004
Troponin measurements			
cTnI, baseline, ng/L	2.0 (1.6–3.2)	1.7 (1.6–2.3)	0.020
Delta cTnI, 3 h postexercise-baseline, ng/L	50.2 (31.1–87.9)	35.4 (23.2–76.1)	0.001
Delta cTnI, 24 h postexercise-baseline, ng/L	7.6 (4.0–16.7)	5.7 (2.7–12.7)	0.009
cTnT, baseline, ng/L	2.9 (2.9–4.3)	2.9 (2.9–3.5)	0.021
Delta cTnT, 3 h postexercise-baseline, ng/L	32.3 (21.6–50.1)	25.9 (16.5–43.0)	0.010
Delta cTnT, 24 h postexercise-baseline, ng/L	6.4 (3.4–11.1)	5.4 (2.3–10.0)	0.058
Training history			
No. of endurance competitions in the past 5 y	7.0 (3.0–15.0)	5.0 (3.0–10.0)	0.075
MET, h/wk†	51.2 (30.8–80.4)	49.8 (28.9–80.0)	0.454
Race performance			
Race duration, h	3.8 (3.4–4.3)	3.7 (3.4–4.1)	0.427

CRP indicates C-reactive protein; cTnI, cardiac troponin I; cTnT, cardiac troponin T; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and MET, metabolic equivalent.

*Women: first-degree relative with cardiovascular disease before the age of 65, men: first-degree relative with cardiovascular disease before the age of 60.

†Obtained by the International Physical Activity Questionnaire Short Form.

Tables 2 and 3 show the relationship between snus use and circulating concentrations of delta hs-cTnI and hs-cTnT at 3 and 24 hours in a series of multivariable models.

After adjustment for potentially cofounding factors, the inverse association between current snus tobacco use and change in hs-cTnI concentrations from baseline to 3 hours postexercise remained significant (β coefficient, −0.31; 95% CI, −0.49 to −0.13 [*P*=0.001]). After additional adjustment for race duration and heart rate, the relationship between snus tobacco use and hs-cTnI was not markedly changed. Furthermore, the change in hs-cTnI concentrations between baseline and 24 hours were 30% lower in current users of snus compared with noncurrent snus users in fully adjusted models (*P*=0.010) (Table 2; multivariable model 3 and 4).

Compared with noncurrent users of snus, the change in hs-cTnT levels from baseline to 3 hours was 18% lower in current snus users in fully adjusted models (*P*=0.010). The change in hs-cTnT values from baseline to 24 hours was also significantly lower in users of snus compared with noncurrent users after adjusting for conventional risk factors (β coefficient, −0.21; 95% CI, −0.36 to −0.05 [*P*=0.009]) and after adding race duration and resting heart rate to the model (β coefficient, −0.19; 95% CI, −0.34 to −0.04; *P*=0.013) (Table 3).

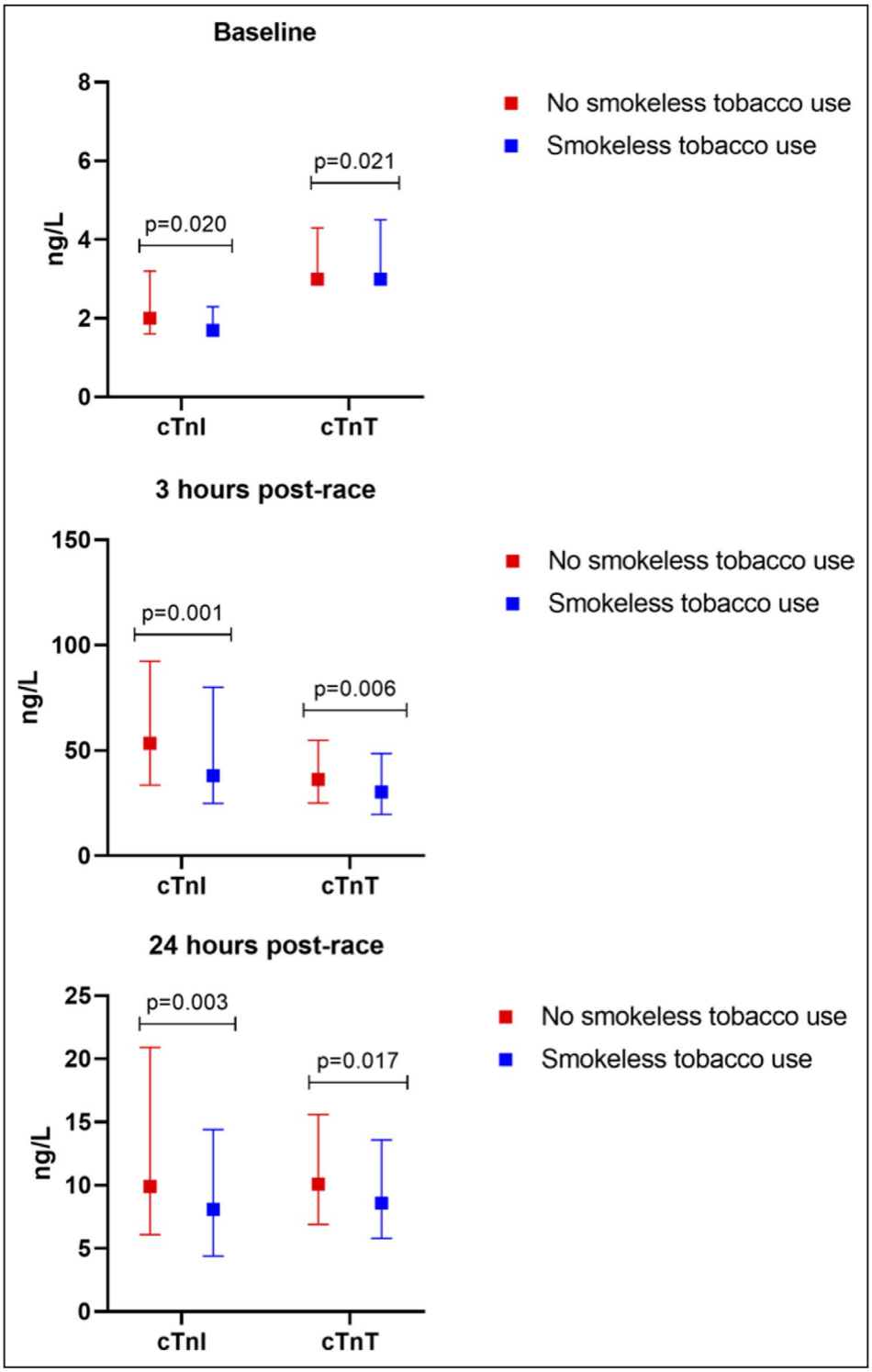

Figure. Concentrations of high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT) at baseline and at 3 and 24 hours following the race.
Median with 25th to 75th percentile. Red boxes represent current users of snus and blue boxes represent noncurrent users of snus.

Sensitivity Analyses

The inverse association between current snus use and cTn remained significant in analyses with absolute troponin concentrations at 3 and 24 hours in the total sample postexercise as the dependent variable. In fully adjusted models, users of snus had lower concentrations of cTn both at 3 hours

Table 2. Association Between Snus Use and Concentrations of Delta cTnI

	β Coefficient (95% CI)			
	Model 1	Model 2	Model 3	Model 4
Delta hs-cTnI 3 h—baseline				
Current snus	−0.28 (−0.46 to −0.10), *P*=0.003	−0.33 (−0.51 to −0.14), *P*=0.001	−0.31 (−0.49 to −0.13), *P*=0.001	−0.29 (−0.47 to −0.11), *P*=0.002
Delta hs-cTnI 24 h—baseline				
Current snus	−0.38 (−0.62 to −0.15), *P*=0.002	−0.36 (−0.59 to −0.12), *P*=0.003	−0.32 (−0.56 to −0.09), *P*=0.007	−0.30 (−0.54 to −0.07), *P*=0.010

Model 1 unadjusted; model 2 adjusted for sex and age; model 3 adjusted for the same variables in model 2 but also systolic blood pressure, body mass index, low-density lipoprotein cholesterol, and estimated glomerular filtration rate; and model 4 adjusted for the same variables as in model 3 but also race duration and resting heart rate. cTnI indicates cardiac troponin I; and hs-cTnI, high-sensitivity cardiac troponin I.

(hs-cTnI: β coefficient, −0.28; 95% CI, −0.44 to −0.11 [*P*=0.001] and hs-cTnT: β coefficient, −0.17; 95% CI, −0.29 to −0.05 [*P*=0.005]) and 24 hours (hs-cTnI: β coefficient, −0.23; 95% CI, −0.43 to −0.04 [*P*=0.020] and hs-cTnT: β coefficient, −0.14; 95% CI, −0.26 to −0.02 [*P*=0.019]) postexercise, compared with noncurrent snus users.

Despite the low number of current smokers in our sample (n=13), we also performed a sensitivity analysis excluding all current smokers to eliminate the potential confounding effects of current smoking. This did not change the results (Tables S1 and S2).

DISCUSSION

The new and important information derived from the current study is that tobacco consumption is not only associated with lower cTn concentrations in the resting state but also reduces the exercise-induced cTn response. Specifically, the current study demonstrates that users of snus tobacco have both lower circulating concentrations of hs-cTnI and hs-cTnT in the resting state and a smaller increase in concentrations of cTn following termination of strenuous exercise than nonusers of snus. The longitudinal experimental design, which allows the participants to serve as their own controls, the consistent results for both hs-cTnI and hs-cTnT in a large cohort of healthy individuals, and measurements of troponins at baseline and 3 and 24 hours after termination of exercise support the validity of our findings. Although the clinical applicability of the results is unknown, it signals that smokeless tobacco use may confound the association between cTn and cardiovascular health.

Association Between Smokeless Tobacco Use and cTn Concentrations

In the current study, we demonstrate for the first time an association between current snus tobacco use and lower cTn concentrations in the resting state. This lends further support to the validity of the finding of an inverse association between tobacco smoking and cTn observed in the population-based setting.[16,17]

An increase in concentrations of cTn following intense exercise is commonly observed.[2] Activity-related changes in serum enzymes have been known for several decades,[18,19] and, in line with these observations, exercise-induced troponin increase has traditionally been considered a benign and physiological phenomenon. However, recent observations demonstrating associations between the magnitude of postexercise troponin response and cardiovascular disease and outcome[20,21] challenge this concept. Moreover, it highlights that more detailed information is needed

Table 3. Association Between Snus Use and Concentrations of Delta cTnT

	β Coefficient (95% CI)			
	Model 1	Model 2	Model 3	Model 4
Delta hs-cTnT 3 h—baseline				
Current snus	−0.17 (−0.31 to −0.03), *P*=0.019	−0.21 (−0.35 to −0.06), *P*=0.005	−0.20 (−0.34 to −0.05), *P*=0.008	−0.18 (−0.32 to −0.04), *P*=0.010
Delta hs-cTnT 24 h—baseline				
Current snus	−0.20 (−0.36 to −0.04), *P*=0.015	−0.23 (−0.39 to −0.08), *P*=0.004	−0.21 (−0.36 to −0.05), *P*=0.009	−0.19 (−0.34 to −0.04), *P*=0.013

Model 1 unadjusted; model 2 adjusted for sex and age; model 3 adjusted for the same variables as in model 2 but also systolic blood pressure, body mass index, low-density lipoprotein cholesterol, and estimated glomerular filtration rate; and model 4 adjusted for the same variables as in model 3 but also race duration and resting heart rate. cTnT indicates cardiac troponin T; and hs-cTnT, high-sensitivity cardiac troponin T.

on determinants of the magnitude of transient elevations of cTn following exercise. In the current study, we add to the existing knowledge by providing data on the associations between snus tobacco and the exercise-induced troponin response. Our results indicate that substances in tobacco may modulate the acute release and/or degradation of cTn in the exercise-induced release setting.

Potential Mechanisms

The use of nicotine is high and increasing within the field of sports,[9] and from 2012 nicotine has been on the World Anti-Doping Agency´s Monitoring Program. Nicotine is one of the main active substances in tobacco, and the total nicotine exposure is similar for cigarette smokers and users of snus tobacco.[22,23] Psychostimulatory, sympathetic nervous system, and cardiovascular effects are observed following nicotine delivery.[24] The evidence for performance-enhancing effects of nicotine in sports, however, is low,[9] and race duration did not differ between users and nonusers of snus in our analyses. In a prospective study of patients with coronary artery disease, nicotine patch therapy and subsequent higher nicotine concentrations used to promote smoking cessation improved myocardial perfusion.[25]

Although the performance-enhancing effects of nicotine are unlikely to explain the differences observed, higher myocardial perfusion as a consequence of nicotine use could be a possible mechanism for lower circulating concentrations of cTn in snus users.

Nicotine effects on degradation and clearance of cTn could be another explanation for lower concentrations of cTn in users of snus. Katrukha et al[26] recently demonstrated the degradation of cTnT by the coagulation enzyme thrombin in an experimental study. Tobacco smoking is associated with an increase in prothombotic factors.[27] Tissue factor, which initiates formation of platelet-dependent thrombin, has been found to be higher in cigarette smokers.[28] Less is known about the effects of snus on circulating markers of thrombogenesis; however, significantly increased thrombin has been seen after adding nicotine or cotinine to platelet-rich plasma of nonsmokers.[29] Given this, increased thrombin-mediated proteolysis of cTn might be one possible mechanism for lower cTn concentrations in current users of snus.

Associations between smoking and improved short-term outcomes have been described for several cardiovascular disorders.[30–32] The mechanisms underlying the "smokers' paradox" are unknown, but a possible explanation could be that tobacco protects myocytes by preconditioning.[31,32] Whether this apparently cardioprotective effect of smoking also holds true for snus is unknown. Furthermore, if the smokers' paradox is the result of biological effects of tobacco or could be explained by selection and/or unmeasured bias is an ongoing discussion.[30,33]

Strengths and Limitations

The large sample size, quantification of troponin with 2 high-sensitive troponin assays, and multiple troponin sampling times are strengths of this study. This study also has several limitations. First, because data on tobacco habits are self-reported by the participants and not validated by biochemical tests, underestimating may have happened. However, the correlation between self-reported tobacco/nontobacco use and nicotine exposure, assessed by blood cotinine and nicotine, has been shown to be high.[34] Second, unknown factors associated with snus use could theoretically explain our findings. However, the experimental design in which participants served as their own controls and measurement of troponin at 3 different time points in each study patient reduce the potential for residual confounding. Third, our study includes a White cohort and the sample mainly included snus-using men. The findings may not be generalizable to individuals of other ethnic groups or to women. Fourth, cardiac imaging data may have provided better understanding of the mechanisms underlying the difference in cTn concentrations. Finally, although the current findings suggest that smokeless tobacco use should be taken into account when interpreting resting and postexercise cTn values, it remains unclear whether smokeless tobacco use impacts the prognostic value of cTn measurements. Prespecified follow-up studies to assess the prognostic value of the exercise-induced cTn response are planned 5, 10, and 20 years following inclusion.

CONCLUSIONS

The findings from the present prospective observational study of recreational cyclist participating in a 91-km bicycle race demonstrate lower resting concentrations of hs-TnI and hs-cTnT in healthy, trained snus users than in nonusers. Significant differences between users and nonusers of snus were also observed in hs-cTn concentrations 3 and 24 hours postrace. Moreover, use of snus was associated with a lower hs-cTnI and hs-cTnT response following strenuous exercise. The current results, combined with data from prior reports observing an inverse association between cigarette smoking and concentrations of cTn, suggest that the effect is real and underscore the need for further experimental research exploring the potential underlying mechanisms for this apparently paradoxical phenomenon.

ARTICLE INFORMATION

Received May 2, 2020; accepted July 28, 2020.

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Sources of Funding

NEEDED has been supported by an operating grant from North Sea Race ("Nordsjørittet), Abbot Diagnostics, and the Lærdal Foundation (Stavanger, Norway). Dr Skranes was supported by a PhD fellowship from South-Eastern Norway Regional Health Authority.

Disclosures

Dr Skadberg has received consulting fees from Abbott Diagnostics. Dr Omland reports consulting fees and research grant support via Akershus University Hospital from Abbott Laboratories, CardiNor, Novartis, Roche Diagnostics, Singulex, and SomaLogic. The remaining authors have no disclosures to report.

Supplementary Materials

Data S1
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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Information concerning the fraction of deltas that were higher than the reference change value

The fraction of delta values that are higher than the reference change value, may be important for the interpretation of the results. We have earlier described the 6-hour RCVs for healthy individuals in a steady-state [35]. In this study participants were sampled during morning hours (similar time of the day as the North Sea Race), and the 6-hour RCV includes analytical, within subject biological and diurnal (cTnT) variation. Analytical variation in the study is similar to the one obtained when the NEEDED samples were analyzed (see above). Accordingly, we evaluated the deltas seen during the NEEDED study towards the 6-hour positive RCVs (95% CI) for cTnT (22%) and cTnI (Abbott) (64%) as was demonstrated in the biological variation study.

Using this RCV data we found the following:

Three hours after the North Sea Race 906/913 (99.2%) and 894/897 = 99.7% of participants increased their concentrations above the RCV limit for cTnI (64%) and cTnT (22%), respectively. There was no difference between the snus and the non-snus groups. For cTnI: 1 participant in the snus (0.8%) vs. 6 participants (0.8%) in the non-snus group showed cTnI increase lower than 64% (p-value for difference 1.00). For cTnT: 1 subject in the snus (0.9%)

and 2 subjects in the non-snus (0.3%) showed cTnT increase below 22% (p-value for difference 0.34).

Twenty-four hours after the North Sea Race 833/914 (91.1%) and 849/906 (93.7%) of participants increased their concentrations above the RCV limit for cTnI (64%) and cTnT (22%), respectively. There was no difference between the snus and non-snus groups. For cTnI: 15 participants in the snus (12.7%) vs 66 participants (8.3%) in the non-snus group showed cTnI increase less than 64% (p-value for difference 0.12). For cTnT: 11 subjects in the snus (9.4%) and 46 subjects in the non-snus (5.8%) showed cTnT increase less than 22% (p-value for difference 0.14).

Table S1. Association between snus use and concentrations of delta cTnI, smokers (n=13) excluded.

B (95% CI)				
	Model 1	**Model 2**	**Model 3**	**Model 4**
Delta Hs-cTnI 3 hours - baseline				
Current snus	-0.32 (-0.50 to -0.14), p=0.001	-0.36 (-0.54 to -0.17), p<0.001	-0.34 (-0.52 to -0.15), p<0.001	-0.32 (-0.50 to -0.14), p=0.001
Delta Hs-cTnI 24 hours – baseline				
Current snus	-0.42 (-0.66 to -0.18), p=0.001	-0.39 (-0.63 to -0.15), p=0.001	-0.35 (-0.59 to -0.12), p=0.004	-0.34 (-0.57 to -0.10), p=0.005

Model 1 unadjusted; model 2 adjusted for sex, age; model 3 adjusted for model 2 and systolic blood pressure, body mass index, low-density lipoprotein cholesterol, and estimated glomerular filtration rate; model 4 adjusted for the same variables as in model 3 but also Race duration and resting heart rate

Table S2. Association between snus use and concentrations of delta cTnT, smokers (n=13) excluded.

B (95% CI)				
	Model 1	**Model 2**	**Model 3**	**Model 4**
Delta Hs-cTnT 3 hours - baseline				
Current snus	-0.20 (-0.34 to -0.05), p=0.008	-0.23 (-0.37 to -0.08), p=0.002	-0.21 (-0.36 to -0.07), p=0.004	-0.21 (-0.35 to -0.06), p=0.004
Delta Hs-cTnT 24 hours - baseline				
Current snus	-0.23 (-0.39 to -0.06), p=0.007	-0.26 (-0.41 to -0.10), p=0.001	-0.23 (-0.39 to -0.07), p=0.004	-0.22 (-0.37 to -0.06), p=0.006

Model 1 unadjusted; model 2 adjusted for sex, age; model 3 adjusted for model 2 and systolic blood pressure, body mass index, low-density lipoprotein cholesterol, and estimated glomerular filtration rate; model 4 adjusted for the same variables as in model 3 but also Race duration and resting heart rate