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Influence of smoking and snuff use on electrolytes, adrenal and calcium regulating hormones

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Little is known about the effects of snuff use on health. We have investigated electrolyte levels, adrenocortical and calcium regulating hormones in three groups of healthy young men, including 18 non-tobacco users, 21 snuff users and 19 smokers with similar age and body mass index. Smoking and snuff use was positively associated with alcohol and coffee consumption and inversely related to physical activity. Compared to non-tobacco users, smokers had significantly increased levels of serum sodium and magnesium, plasma calcitonin, urinary cortisol and potassium levels and decreased serum sex hormone-binding globulin as well as serum and urinary creatinine values. However, only decreased sexual hormone-binding globulin and urinary creatinine and increased serum phosphate and urinary potassium levels were seen in snuff users. Among tobacco users we noted that smokers differed from snuff users in that they had higher serum sodium (1.4 mmol/l, $p<0.01$), plasma calcitonin (3.3 pmol/l, $p<0.05$) and urinary cortisol (41 nmol/24 h, $p<0.05$) but lower serum creatinine (5.8 μmol/l, $p<0.01$). We conclude that chronic snuff use appears to have less influence on hormone and electrolyte balance than does smoking, and that some of the abnormalities seen in smokers do not seem to be mediated by nicotine.

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Cigarette smoking has been suggested to be associated with osteoporosis and to have an anti-estrogen effect (1). There have been few studies on the influence of smoking upon calcium regulating hormones and electrolytes, although increased levels of adrenal steroids among smokers have been reported (2–4). The use of smokeless tobacco in the form of moist, oral snuff is increasing among men in Northern America and Scandinavia (5, 6). No data have yet been published on the effects of snuff use on hormone or serum electrolyte levels. We compared levels of adrenal and sex hormones, calcium regulating hormones and electrolytes in healthy male, young smokers and snuff users with matched non-tobacco users.

Subjects and methods

Study samples

Male volunteers who were snuff users, smokers or non-tobacco users were recruited mainly from populations of university students and teachers by advertisement in the university magazine. Inclusion criteria included having never used tobacco (non-users), at least two years' use of at least one can (50 g) of moist snuff a week (snuff users) or of smoking at least 10 cigarettes a day (smokers). Subjects with body mass index (BMI) > 28 kg/m^2 or who were more than 31 years of age were excluded. Participants: Non-tobacco users—18 subjects who had never used tobacco; snuff users—21 subjects who used snuff daily, 5 of whom were ex-smokers and had stopped smoking 2 to 8 years before the study; smokers—19 subjects smoked cigarettes daily, one of whom had used snuff until 6 years prior to the study. No subject was suffering from any acute or chronic illness or was taking any medication or vitamin supplementation. All gave informed consent to participate in the study, which had been approved by the Ethics Research Committee of Umeå University.

All subjects underwent a physical examination and completed a questionnaire regarding lifestyle habits. Present and lifetime cumulative tobacco consumption was calculated (one cigarette = one gram of tobacco, one can of moist snuff = 50 grams of tobacco). Alcohol consumption was calculated in g of absolute alcohol consumed per month. Height and weight were measured wearing indoor clothing, without shoes and BMI was calculated as weight (kg)/height (m)^2.

The study was performed from November to April. Examination took place at 08.00, after an overnight fast and abstention from tobacco and after 24 h abstention from alcohol. Blood samples were drawn with minimal venous occlusion after 30 min supine rest. Samples were centrifuged free of formed elements and sera and plasma were stored at −70°C until analysis. Twenty-four hour urine collections were collected under everyday condi-

tions with tobacco use ad libitum and were delivered to the laboratory immediately upon completion. Urinary electrolytes were analysed the same day. Samples were frozen for later analysis of aldosterone and cortisol. A total urinary volume of more than 500 ml was considered as an adequate collection. One participant delivered only 400 ml of urine and was therefore excluded. Urine samples were analysed from 16 subjects in each group; the remaining participants did not fulfil this due to the inconvenience of study and work conditions. Concentrations of electrolytes and hormones are reported as ratios to urinary creatinine concentration.

Assay methods

Serum potassium, sodium, calcium, alkaline phosphatase and creatinine were analysed in a Technicon SMA II. Potassium and sodium in urine were assayed on a IL flame-photometer. Urinary calcium was measured using a complexometric method on a Conning Calcium Analyzer. Phosphate in serum and urine as well as creatinine in urine were analysed on an IL Multistat and serum and urinary magnesium were analysed on an LKB 7400 photometer. Serum and urine cortisol were analysed with a RIA (Pharmos Diagnostica, Oulunsalo, Finland).

Urinary aldosterone was measured with a RIA (Abbot Diagnostic Products GmbH, Wiesbaden, Germany). Calcitonin (CT) was measured in duplicate by a RIA as described previously (7). Synthetic human CT (Peninsula Laboratory Inc., California) was used as standard and for radioiodination. The antiserum raised in rabbits against synthetic human CT (Cibacalcin) was directed against the carboxyterminal of CT. The detection limit (based on 2 SD below maximal binding) of the RIA was 0.23 pmol/tube or 0.0023 pmol/l. Intraassay variation was $<10\%$ and interassay variation $<16\%$ at all concentrations. Serum concentrations of osteocalcin (bone gla-protein) were determined by a RIA using antibovine osteocalcin antibody (OSTK-PR, Compagnie Oris, Industrie SA, France). All analyses were performed in duplicate and in a single assay. The sensitivity of this assay was 0.06 nmol/l. The intraassay variation was 6.5%. Insulin-like growth factor I (IGF-1 Somatomedin C) concentrations were determined by a RIA using a polyclonal rabbit anti-somatomedin antibody (Somatomedin C ^{125}I-RIA, Incstar Corp. Stillwater, Maryland). All analyses were performed in duplicate and in a single assay. The detection limit was 2.0 nmol/l and the intraassay variation 9.1%. Sex hormone-binding globulin (SHBG) concentrations were determined by RIA using a monoclonal mouse anti-SHBG antibody and a rabbit anti-SHBG serum (SHBG [^{125}I], Farmos Diagnostica, Oulunsalo, Finland). All analyses were performed in duplicate and in a single assay. The detection limit was 0.5 nmol/l, the intraassay variation 2.2%.

Testosterone concentrations were determined by a RIA using rabbit testosterone anti-serum (Direct testosterone [^{125}I] Farmos Diagnostica). All analyses were performed in duplicate and in a single assay. The detection limit was 0.3 nmol/l, the intraassay variation 4.5%. A ratio of testosterone/SHBG was calculated as an index of free testosterone.

Intact parathyroid hormone (PTH) (1–84) was quantified using an immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Interassay variation was 6.2% at the level of 3.7 pmol/l.

Total estrone was determined by a RIA after enzymatic hydrolysis with purified Helix pomatia enzyme and ether extraction (8). The method determines the sum of unconjugated estrone and conjugated estrone hydrolysed by Helix pomatia enzyme. Estrone sulphate accounts for more than 85% of the values obtained. Intra and interassay variations were 7 and 9% for total estrone.

Dehydroepiandrosterone sulphate (DHAS) was measured with a RIA (9). Intra- and interassay variations were 8 and 12%, respectively. Plasma nicotine and cotinine (the major metabolite of nicotine) were determined with a single step liquid–liquid extraction followed by capillary gas chromatography. Nicotine and cotinine were detected by a nitrogen sensitive detector. The detection limit for nicotine (MW 162.23) was 4 nmol/l and for cotinine (MW 176.21) 8.5 nmol/l.

Intraassay variation for nicotine was 4.8% at 59 nmol/l level and for cotinine 3.6% at 160 nmol/l level.

Statistics

Results are presented as means and standard deviations (SD). Comparisons between all three groups were made with ANOVA or Kruskal-Wallis one-way analysis of variance, depending on a Bartlett's test for homogeneity of variance. For p-values below 0.10, groups were compared two by two with the two-tailed Student's *t*-test or the Mann-Whitney test. P-values were considered significant if below 0.05. Ninety-five percent confidence intervals (95% CI) for differences between groups are given where appropriate. The computer program Epi Info 5.01a was used (Center for Disease Control, Atlanta, Georgia).

Results

Background variables

The three groups were similar in age and BMI (Table 1). Lifestyle habits were closely linked to tobacco habits (Fig. 1). Smokers drank more coffee and alcohol and exercised less than non-tobacco users. Snuff users' alcohol and exercise habits were intermediate between the other two groups.

Smokers and snuff users had similar tobacco exposure measured as total amount consumed, present consumption or duration of use (Table 1). Plasma nicotine levels were low or undetectable in non-users, the highest value (14.8 nmol/l) was well below the means for tobacco

users. One smoker had a plasma nicotine of 80.2 nmol/l, otherwise all values for tobacco users were below 62 nmol/l (i.e. 10 μg/l). Snuff users had significantly higher levels of plasma cotinine than smokers. Levels in non-users were all below 45 nmol/l (i.e. 8 μg/l).

Electrolytes and creatinine

Smokers had higher serum sodium levels than non-smokers (difference 1.8 mmol/l, 95% CI 1.0 to 3.0) and snuffers (Table 2). No differences were seen in serum potassium or calcium. Snuff users had higher serum phosphate than non-users (difference 0.12 mmol/l, 95% CI 0.01 to 0.23). Smokers had higher serum magnesium than non-users (difference 0.04 mmol/l, 95% CI 0.01 to 0.07). This difference was not significant (p=0.07) in snuff users compared to non-users. No differences in alkaline phosphatase activity or serum albumin levels were noted (data not shown).

Serum creatinine was lower among smokers (difference against non-users 7.1 μmol/l; 95% CI 0 to 12.0, against snuffers 5.8, 95% CI 2.0 to 12.0). Urinary potassium was higher in snuff users than among non-users (difference 1.7 mmol/mmol creatinine, 95% CI 0.3 to 2.3) and in smokers; although significant, the 95% confidence interval included zero.

There were no differences in calcium, phosphate or magnesium levels in urine between the groups. Twenty-four hour urinary creatinine was significantly higher among non-users than smokers (difference 3.3 mmol/24 h, 95% CI 0.9 to 5.7) and snuff users (2.2 mmol/24 h, 95% CI 0.2 to 4.2). This was not due to inadequate sampling among tobacco users as they had higher 24 h urine volume than non-users (data not shown).

Table 1. Background variables given as mean (SD). Non-tobacco users (N=18), snuff users (N=21), smokers (N=19).

Variable	Non-users	Snuff users	Smokers
Age (years)	24.4 (2.6)	24.1 (3.4)	25.3 (3.8)
BMI (kg/m^2)	22.2 (1.6)	22.0 (1.7)	23.0 (3.3)
Tobacco use: duration (years)	0	7.0 (3.8)	9.1 (3.8)
Tobacco consumption: present (g/week)	0	146 (60)	134 (29)
Tobacco consumption: cumulative (kg)	0	52.2 (35.1)	65.9 (35.4)
P-nicotine (nmol/l)	5.6 (3.1)	19.7[a] (8.0)	20.3[b] (16.7)
P-cotinine (nmol/l)	11.6 (11.6)	1852[a] (642)	1347[b,c] (579)

[a]Snuff users vs non-users p<0.001. [b]Smokers vs non-users p<0.001. [c]Snuff users vs smokers p<0.05.

Steroid hormones

Serum cortisol was insignificantly higher among snuff users (Table 3). Urinary excretion of cortisol was higher among smokers than non-users (difference 2.3 nmol/mmol creatinine, 95% CI 0.8 to 4.7). Total cortisol excretion (i.e. not adjusted for urinary creatinine—data not shown) did not differ between smokers and non-users but smokers had significantly higher excretion

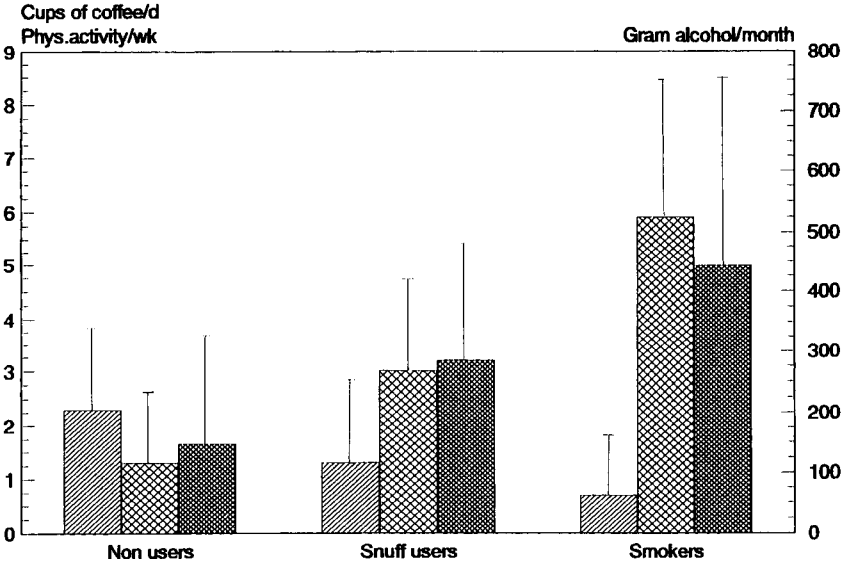

Fig. 1. Means and SD (bars) of lifestyle variables. Physical activity (▨) measured as number of strenuous exercise occasions of more than 30 min duration per week. Coffee consumption (▩) measured as the number of cups of coffee consumed per day. Alcohol consumption (▩) measured as grams of pure alcohol consumed per month. For coffee, all group differences were significant (p<0.01); for alcohol, tobacco users differed from non-users (p<0.01). Physical activity differed between non-users and snuffers (p<0.05) and between non-users and smokers (p<0.001).

Table 2. Electrolytes and creatinine in serum and urine, means (SD). Non-tobacco users (N = 18, urine 16), snuff users (N = 21, urine 16), smokers (N = 19, urine 16).

Variable	Non-users	Snuff users	Smokers
S-Na (mmol/l)	139.5 (1.0)	139.9[a] (1.4)	141.3[b] (1.9)
S-K (mmol/l)	3.95 (0.28)	3.94 (0.22)	4.05 (0.25)
S-Ca (mmol/l)	2.32 (0.10)	2.34 (0.08)	2.35 (0.07)
S-Phosphate (mmol/l)	1.18 (0.20)	1.30[c] (0.15)	1.22 (0.19)
S-Mg (mmol/l)	0.84 (0.05)	0.88 (0.05)	0.88[d] (0.04)
S-creatinine (μmol/l)	93.0 (11.5)	91.7[a] (7.0)	85.9[d] (6.4)
U-Na (mmol/mmol u-crea)	11.2 (4.8)	13.8 (5.0)	12.9 (4.5)
U-K (mmol/mmol u-crea)	5.2 (1.6)	6.9[c] (3.4)	6.3[d] (1.5)
U-Ca (mmol/mmol u-crea)	0.37 (0.23)	0.34 (0.14)	0.38 (0.21)
U-Phosphate (mmol/mmol u-crea)	2.2 (1.2)	2.3 (0.7)	2.3 (0.7)
U-Mg (mmol/mmol u-crea)	0.34 (0.11)	0.40 (0.14)	0.36 (0.07)
U-creatinine (mmol/24 h)	16.1 (3.3)	12.8[c] (3.4)	13.9[d] (2.2)

[a]Snuff users vs smokers $p<0.01$. [b]Smokers vs non-users $p<0.01$. [c]Snuff users vs non-users $p<0.01$. [d]Smokers vs non-users $p<0.05$.
crea: creatinine.

Table 3. Steroid hormones and SHBG in serum, plasma and urine, means (SD). Non-tobacco users (N = 18, urine 16), snuff users (N = 21, urine 16), smokers (N = 19, urine from 16).

Variable	Non-users	Snuff users	Smokers
S-cortisol (nmol/l)	439 (131)	496 (117)	466 (127)
U-cortisol (nmol/mmol u-crea)	11.4 (4.0)	12.7[a] (7.8)	13.7[b] (3.1)
U-aldosterone (nmol/mmol u-crea)	0.28 (0.21)	0.34 (0.21)	0.62* (0.62)
S-estrone (nmol/l)	2.2 (1.1)	2.1 (1.1)	2.1 (0.8)
S-DHAS (mmol/l)	7.0 (1.4)	6.4 (1.7)	7.4 (3.3)
S-testosterone (nmol/l)	28.4 (5.4)	26.4 (7.2)	29.5 (8.6)
S-SHBG (nmol/l)	28.6 (8.3)	21.2[c] (6.1)	22.9[d] (5.2)
Testosterone/ SBHG ratio	1.03 (0·24)	1.30[e] (0·39)	1.33[f] (0·45)

[a]Snuff users vs smokers $p<0.05$. [b]Smokers vs non-users $p<0.01$. [c]Snuff users vs non-users $p<0.01$. [d]Smokers vs non-users $p<0.05$. [e]Snuff users vs non-users $p<0.05$. [f]Smokers vs non-users $p<0.05$.
Note: * Sample lacking from one smoker.
Crea: creatinine.

Table 4. Calcium regulating hormones and IGF-I in serum and plasma, means (SD). Non-tobacco users (N = 18), snuff users (N = 21), smokers (N = 19).

Variable	Non-users	Snuff users	Smokers
P-calcitonin (pmol/l)	6.4 (5.1)	5.7[a] (2.7)	9.0[b] (4.9)
S-osteocalcin (nmol/l)	1.82 (0.37)	1.85 (0.34)	1.73 (0.46)
P-PTH (pmol/l)	3.0 (1.7)	2.4 (1.0)	2.2 (0.5)
S-IGF-1 (nmol/l)	30.1 (8.6)	34.2 (7.5)	37.5 (17.4)

[a]Snuff users vs smokers $p<0.05$. [b]Smokers vs non-users $p<0.05$.

than snuff users (means 190 vs 149 nmol/24 h, $p<0.05$). Smokers tended to have higher urinary aldosterone excretion, even when adjusted for urinary sodium (data not shown), although the difference was not significant.

No differences between the groups were found for estrone, DHAS or testosterone in serum. Serum levels of SHBG were lower among both smokers (difference 5.7 nmol/l, 95% CI 1.1 to 10.3) and snuff users (7.4 nmol/l, 95% CI 2.7 to 12.1). Tobacco users had higher ratios of testosterone/SHBG than non-users.

Calcium regulating hormones

Plasma CT was significantly higher among smokers than non-users (difference 2.6 pmol/l, 95% CI 0.6 to 5.3) (Table 4). One non-tobacco user had a very high plasma level of calcitonin (22.5 pmol/l). If he is excluded the mean for non-users falls to 5.5 (±3.3) pmol/l and the difference is even more marked against smokers. We found no group differences in osteocalcin. PTH values tended to be lower among snuff users and smokers ($p=0.10$ between smokers and non-users). Slightly higher levels of IGF-I were found among tobacco users than among non-users, but the difference was not significant.

Discussion

While men in Western societies are smoking fewer cigarettes, the use of smokeless tobacco, mainly in the form of moist oral snuff, is increasing (5). The health hazards of snuff use have been poorly studied and mainly confined to dental health. A consensus conference of the American Medical Association considered studies in

other fields urgent (6). No data concerning snuff and hormones have yet been published. We studied three groups of healthy young men with similar age and BMI. Their tobacco habits were closely related to lifestyle patterns with possible confounding effects.

Plasma levels of cotinine and nicotine confirmed self-reported tobacco habits and verified abstention from acute tobacco exposure at the examination.

Does tobacco use influence calcium homeostasis?

Smoking and osteoporosis are associated (1). The lower body weight of smokers might partly be responsible but direct effects of smoking on bone are also likely. Smoking has been shown to influence calcium-phosphate balance and PTH secretion in young women (10).

Snuff users had higher serum phosphate levels than non-users in our study. One other study has examined indices of bone turnover and parathyroid function in smoking and non-smoking fertile women and found higher phosphate levels among smokers (10). The slightly higher serum magnesium among smokers has not previously been described.

As CT suppresses bone resorption it has been suggested that CT deficiency would contribute to osteoporosis. However, a recent case-control study lent no support to this hypothesis (11). Smokers with mucopurulent bronchitis had higher serum levels of CT than non-smoking matched controls (12). Smoking of two cigarettes increased serum CT by 20% both in healthy and thyroidectomized subjects (13). The source of the smoking-released CT has been suggested to be the neuroendocrine cells in the lung. We found 40% higher plasma CT among smokers but no significant elevation among snuff users. Our findings do not support that circulating nicotine leads to CT-release.

Men with idiopathic osteoporosis have been reported to have lower plasma levels of IGF-I than healthy controls (14). We have found no reported data on the effect of smoking on IGF-I levels. In our study, higher IGF-I among tobacco users was found, the highest level among smokers, but statistical significance was not achieved.

Serum levels of osteocalcin, a marker of osteoblast activity, have been shown to correlate with bone loss in a study of perimenopausal women (15). No differences in 24 h serum osteocalcin levels were noted between smoking and non-smoking middle-aged men and women in a recent study (16). Levels of osteocalcin did not differ between the groups in our study. Gudmundsson et al. found significantly reduced PTH levels among smoking women, although the reduction was only 5% (10). No studies in men have been published. We noted lower values among smokers and snuff users compared to non-users, but the differences were not significant.

Thus we found no major abnormalities in calcium regulation among tobacco users which would help to explain a possible association with osteoporosis.

Tobacco, adrenocortical hormones and electrolytes

Intensive smoking has repeatedly been shown to cause cortisol release, probably by way of ACTH stimulation (2–4). We found that smokers excreted significantly more cortisol than both snuffers and non-users, thus signifying a slight hypercortisolism which may contribute to osteoporosis.

In hypertensive patients plasma aldosterone increased after smoking but no changes were noted in plasma renin activity (4). We noted that smokers had slightly higher urinary aldosterone excretion. High aldosterone levels would be expected to promote increased potassium losses in urine, which we could verify among tobacco users. Benowitz noted higher urinary potassium levels among tobacco chewers and also among snuff users (17). No such effect was seen among smokers.

In our study, smokers had higher serum sodium levels. This could possibly be due to lower plasma volume, which may in part explain smokers' polycythemia (18). A lower serum creatinine level in smokers has been described previously (19), and this was confirmed in our study, but no significant decrease was seen among snuff users.

Tobacco and sex hormones

There have been conflicting reports concerning smoking and sex hormones in men (20). Higher serum androstenedione and DHAS levels among smoking men are the most consistent findings. These were confirmed in the MRFIT trial and the Rancho Bernardo study even after adjustment for obesity and other confounders (21, 22). Serum estrone levels have been found to be both elevated (22) and normal (21) in smokers. We found no differences in serum levels of either DHAS or in estrone between tobacco users and non-users.

In a cohort of 1640 middle-aged men, serum SHBG levels were influenced by age and BMI but not by smoking or alcohol (23). In one study, SHBG was negatively correlated with alcohol and body fat and positively with the waist–hip ratio, but not with smoking (24). Our findings of lower serum SHBG levels in both smokers and snuff users could not be explained by differences in age or BMI. However, higher alcohol consumption could contribute. The men we studied were younger than those reported in other studies and it is possible that tobacco use may have a greater impact in younger subjects. Concentrations of both total and free serum testosterone have been reported to be higher in smokers than in non-smokers (21, 22, 25). The difference was most pronounced in the youngest age group. The free testosterone index was clearly elevated among tobacco users in our study.

In conclusion, we could confirm some of the previously reported abnormal hormonal and electrolyte findings in smokers. In addition, this group had higher serum sodium and magnesium levels and urinary excretion of cortisol than non-tobacco users. In contrast to the smoking group, snuff users showed only a few abnormal findings, i.e. decreased serum SHBG and increased serum phosphate and urinary potassium levels. Smokers had higher serum sodium, plasma calcitonin and urinary cortisol and lower serum creatinine than snuff users. Thus it seems that regular use of moist oral snuff clearly has less impact on hormonal and electrolyte balance than smoking. The laboratory abnormalities seen in smokers but not in snuff users do not seem to be caused by circulating nicotine, since the groups had comparable plasma levels of nicotine and cotinine. The only plausible connection between smoking and osteoporosis in our study was higher cortisol excretion.

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