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Smoking is associated with increased resting energy expenditure in the general population: The NEO study

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Abstract

Introduction

Animal studies and human studies in small selected populations have shown a positive association between nicotine smoking and resting energy expenditure (REE), but data in large cohorts are lacking. We aimed to investigate the association between smoking behaviour and REE in a large, population-based study.

Methods

In this cross-sectional analysis of baseline measurements from the Netherlands Epidemiology of Obesity (NEO) study (n=6,673), we included participants with REE measurement by indirect calorimetry who were not using lipid or glucose lowering drugs (n=1,189). We used linear regression analysis to examine the association of smoking status (never, former, occasional, current smoker) and smoking quantity (pack years) with REE per kilogram (kg) fat free mass (FFM) and with REE adjusted for FFM. Models were adjusted for age, sex, ethnicity, educational level, physical activity, energy intake and body mass index (BMI).

Results

Mean (standard deviation, SD) age was 55.2 (5.9) years and BMI was 26.3 (4.4) kg/m^2. 60% of the participants were women. Mean (SD) REE/FFM (kcal/day/kg FFM) was for male never smokers 25.1 (2.0), male current smokers 26.4 (2.8), female never smokers 28.9 (2.5) and female current smokers 30.1 (3.7). After adjustment, only current smokers had a higher REE/FFM (mean difference 1.28, 95% CI 0.64, 1.92), and a higher REE adjusted for FFM (mean difference 60.3 kcal/day, 95% CI 29.1, 91.5), compared with never smokers. There was no association between pack years and REE/FFM (mean difference -0.01, 95% CI -0.06, 0.04) or REE adjusted for FFM (mean difference 0.2, 95% CI -2.4, 2.8) in current smokers.

Conclusion

Current smoking is associated with a higher resting energy expenditure compared with never smoking in a large population-based cohort.

Introduction

The effects of smoking on body composition have been studied extensively.[1–4] Cigarette smoking decreases body weight[1] and smoking cessation is associated with weight gain.[1,2] These effects of smoking on the body's energy balance can be explained by either a decrease in energy intake or an increase in energy expenditure and a possible inverse effect after cessation.

Nicotine is thought to be the most important mediator of the effects of smoking on body composition.[1,3] Nicotine acts by various mechanisms on the body's energy balance and affects both the central nervous system and peripheral tissues by regulating the release of a wide range of neurotransmitters and hormones.[1] Studies in mice suggest that the effects of nicotine on energy metabolism mostly involve the central nervous system. More specifically, a recent mouse study showed that nicotine-induced weight loss was associated with inactivation of hypothalamic AMP-activated protein kinase (AMPK). Inactivation of AMPK results in decreased orexigenic signalling and thus decreased food intake, while energy expenditure is increased as a result of increased locomotor activity and increased thermogenesis in brown adipose tissue.[4]

In humans, smoking has been shown to suppress appetite.[3] Smoking also seems to have a direct effect on energy expenditure. A study with eight healthy cigarette smokers showed an increase in total 24-hour energy expenditure after the participants smoked 24 cigarettes in 24 hours, compared with a 24-hour period without cigarette smoking.[5] In another study with 18 male smokers there was an increase in resting energy expenditure (REE) demonstrated directly after a nicotine dose via nasal spray, compared with placebo.[6] Further studies have shown that cigarette smoking increases REE in small (i.e. $n \geq 147$) selected patient populations.[7–10]

Overall, the studies mentioned above found a positive association between smoking and REE, which may contribute to the lower body weight of smokers and weight gain after smoking cessation.[1,2,5,6] However, such a relationship has not been confirmed in a large cohort, neither have long-term effects of smoking been investigated at the population level. The aim of the present study, therefore, was to examine the association between smoking and REE in a large population-based cohort.

Materials and Methods

Study design and study population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based cohort study. Participants were recruited from September 2008 until September 2012, resulting in a co-

hort of 6,673 individuals with an oversampling of individuals with overweight or obesity. Men and women aged between 45 and 65 years with a self-reported body mass index (BMI) of 27 kg/m^2 or higher living in the greater area of Leiden (in the West of The Netherlands) were eligible to participate in the NEO study. In addition, all inhabitants aged between 45 and 65 years from one municipality (Leiderdorp) were invited, irrespective of their BMI, allowing for a reference distribution of BMI. Baseline data were collected at the NEO study center of the Leiden University Medical Center (LUMC). Prior to the NEO study visit, participants completed a questionnaire about demographic and clinical information and fasted for at least 10 hours. Participants came to the research site in the morning to undergo several baseline measurements including anthropometric measurements and blood sampling. Records were made of all medication used in the month preceding the visit to the study center. More extensive measurements were taken in random subsets of participants, including magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), indirect calorimetry, dual energy X-ray absorptiometry (DXA) and accelerometry.

More detailed information on the study design and data collection has been described elsewhere.[11] This study was approved by the medical ethics committee of the Leiden University Medical Center (LUMC) and all participants gave written informed consent.

For the present analyses we included the random subsample of participants of whom REE was measured by indirect calorimetry (n=1,434) and of those we excluded the participants who were using lipid or glucose lowering drugs (n=245), as there are indications in literature that these classes of drugs affect energy expenditure.[12–15] This brings the total study population to 1,189 NEO study participants, of which six participants had missing data on fat free mass (FFM) (one non-smoking participant and five former smokers).

Data collection

Questionnaires on health and lifestyle factors were sent to all participants. Smoking status was classified as never-smoker, former smoker, occasional smoker or current smoker on the basis of a questionnaire, in which the participants could answer the question “Do you smoke?” as either: “No, I never smoked”; “No, but I did smoke in the past”; “Yes, occasionally” or “Yes, currently”. Long-term tobacco exposure was expressed in pack years of smoking, calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person smoked. A pack year is defined as twenty cigarettes smoked every day for one year. Questionnaires were used to assess energy intake (semi-quantitative food frequency questionnaire, FFQ[16]) and physical activity (Short Questionnaire to Assess Health-enhancing physical activity, SQUASH[17]). Energy intake was calculated from the FFQ using the 2011 version of the Dutch food composition table (NEVO-2011). Physical

activity was expressed as metabolic equivalents of task (MET)*hours/week, with MET indicating the intensity of an activity. Weight was measured without shoes and one kilogram (kg) was subtracted to correct for the weight of clothing. BMI was calculated by dividing the weight in kilograms by the height in meters squared. FFM was calculated from body weight and body fat percentage estimated with the Tanita bio impedance balance (TBF-310, Tanita International Division, UK).

Resting energy expenditure

The basal metabolic rate (BMR) is responsible for approximately 60% of total energy expenditure in persons with sedentary occupations.[18] REE, which is more feasible to assess, can be used as a representation of BMR. Generally, REE is within 10% of the corresponding BMR.[18] REE was measured for 30 minutes by indirect calorimetry (ventilated hood system, Oxycon Pro), which was performed after an overnight fast and a resting period of 15 minutes. During the measurement, participants were awake while lying on a bed in a quiet room. The volume of oxygen inspired and carbon dioxide exhaled were measured every minute. REE and substrate oxidation rates were calculated using standard formulas.[11]

Statistical analysis

Because individuals with a BMI of 27 kg/m^2 or higher were oversampled in the NEO study adjustments were made to correctly represent associations for the general population.[19] This was done by weighting individuals according to the BMI distribution of participants from the Leiderdorp municipality[20], whose BMI distribution was similar to the BMI distribution of the general Dutch population[21]. All presented results are based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of participants with a BMI of 27 kg/m^2 or higher.

Baseline characteristics were expressed as mean (standard deviation, SD) or percentage. Results concerning or related to body composition and REE are presented for men and women separately, as these variables are influenced by sex. Differences in baseline characteristics between the four categories of smoking status were tested by a chi-squared test for categorical variables and by analysis of variances (ANOVA) for continuous variables with post hoc Bonferroni correction if statistical differences in baseline characteristics between the four smoking categories were found.

Since the amount of metabolically active tissue is the major determinant of REE, REE is often adjusted for FFM by using the ratio of REE and FFM (REE/FFM).[22–26] Therefore, weighted linear regression analysis was performed to evaluate the association between smoking status and REE/FFM, and the association between long-term tobacco exposure,

expressed as pack years, and REE/FFM. Alternatively, linear regression analyses have frequently been performed with REE as dependent variable and FFM included as independent variable in the models.[23,27,28] This method was therefore additionally used to assess the association of REE adjusted for FFM with both smoking status and tobacco exposure. For all linear regression analyses mean differences and corresponding 95% confidence intervals (CI) were reported. Crude models (model 1: not adjusted in models using REE/FFM; adjusted for FFM in models using REE) were adjusted for sex (model 2), age, ethnicity and educational level (model 3). In a fourth model (model 4) physical activity, energy intake and BMI were added as potential confounding variables. Analyses to assess statistical interaction between smoking behaviour and sex, between smoking behaviour and age and between smoking behaviour and physical activity were performed. This was done by including product terms into the models. All analyses were performed using STATA Statistical Software (Statacorp, College Station, Texas, USA), version 12.0.

Results

Table 8.1 shows the demographic and clinical characteristics of the study population (n= 1,189. Overall, women had a considerably lower REE than men (mean difference -368 kcal/day; 95% CI -396, -339). In men mean BMI and total body fat were significantly different between the four categories of smoking status. Post-hoc analyses showed that only the difference in BMI between former and never smokers was statistically significant ($P<0.001$), and that the difference in total body fat was significantly different between former smokers and never smokers ($P<0.001$) and between current smokers and never smokers ($P=0.033$). The difference in total body fat between current and never smokers disappeared after adjustment for age, ethnicity and educational level ($P=0.105$). Unadjusted REE was not clearly different between never, occasional, former and current smokers for both men and women. REE/FFM was higher in current smokers than never smokers for both men and women (Figure 8.1).

In Table 8.2 the mean and difference in REE/FFM of the association between smoking status and REE/FFM are presented. After adjustment for sex (model 2) the REE/FFM of current smokers was 1.25 kcal/day/kg FFM higher compared with never smokers. Further adjustment for age, ethnicity and educational level (model 3) showed a similar higher REE/FFM for current smokers. Likewise, model 4 (further adjusted for physical activity, energy intake and BMI) showed a higher REE/FFM of 1.28 kcal/day/kg FFM for current smokers. This corresponds to a 4.7% higher REE/FFM relative to the adjusted mean of REE/FFM of never smokers. There was no statistical interaction with sex, age or physical

activity for the association between smoking status and REE/FFM.

No difference in REE/FFM was demonstrated between never and occasional smokers in the adjusted models (Table 8.2). There was also no difference in REE/FFM between never and former smokers, neither unadjusted nor in the adjusted models (Table 8.2). Pack years ranged from 0 to 139. There was no association between long-term tobacco exposure (expressed as pack years) and REE/FFM, neither in former smokers, occasional smokers or current smokers (Table 8.3).

Table 8.1: Demographic and clinical characteristics of the NEO study population stratified by smoking status.

Characteristic Characteristic	**Never smokers (40.2%)**	**Occasional smokers (4.5%)**	**Former smokers (44.6%)**	**Current smokers (10.7%)**	**P-value**
Sex (% women)	63	43	61	52	0.1337
Ethnicity (% whites)	96	97	96	92	0.5500
Age (year)	54.6 (5.9)	55.1 (5.6)	55.8 (5.9)	54.7 (6.0)	0.1544
Quantity of smoking (pack years)	-	7.0 (9.4)	13.0 (14.4)	24.6 (13.2)	<0.0001
Educational level (% academic degree)	15	9	13	5	0.2650
Net monthly income (% high[a])	13	13	11	16	0.5832
Alcohol use (% non-users)	14	15	8	9	0.1802
BMI (kg/m^2)					
Men	25.7 (3.1)	26.8 (4.9)	27.5 (4.0)	26.9 (3.6)	0.0007
Women	25.9 (4.6)	26.3 (5.7)	26.1 (4.9)	25.2 (4.7)	0.7709
Total body fat (%)					
Men	22.6 (4.8)	24.0 (7.9)	25.8 (5.9)	25.2 (5.0)	0.0002
Women	36.8 (6.0)	36.9 (6.7)	36.3 (7.7)	35.8 (7.8)	0.9211
FFM (kg)					
Men	66.0 (6.0)	65.3 (6.8)	66.1 (7.4)	64.7 (6.6)	0.6800
Women	45.2 (4.3)	46.1 (6.0)	45.1 (4.5)	44.3 (4.9)	0.6356
Physical activity (MET hours/week)					
Men	39.7 (37.7)	36.8 (27.0)	36.3 (34.8)	28.9 (25.8)	0.3042
Women	35.0 (27.4)	23.8 (13.9)	35.1 (26.9)	26.7 (25.3)	0.0035
REE (kcal/day)					
Men	1,658 (199)	1,655 (202)	1,689 (233)	1,703 (235)	0.5699
Women	1,304 (167)	1,283 (197)	1,312 (171)	1,334 (217)	0.7437
REE/FFM (kcal/day/kg FFM)					
Men	25.1 (2.0)	25.4 (2.3)	25.6 (2.6)	26.4 (2.8)	0.0310
Women	28.9 (2.5)	27.8 (2.3)	29.1 (2.5)	30.1 (3.7)	0.0058

Results are based on weighted analyses with n=1,189 (n=1,183 when characteristic comprises measurements of fat (free) mass). Values are presented as mean (SD) or percentage.

[a] High net monthly income: >€3,000/month.

BMI, body mass index; FFM, fat free mass; MET, metabolic equivalents of task; NEO, Netherlands Epidemiology of Obesity; REE, resting energy expenditure.

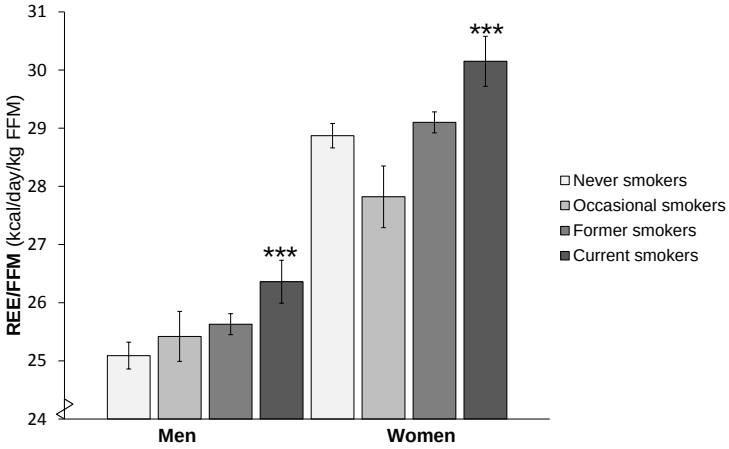

Figure 8.1: Association between mean REE/FFM and smoking status stratified by sex. Results are based on weighted analyses (n= 1,183). *** p ≤ 0.001 compared with non-smokers in linear regression analysis adjusted for sex (model 2). Error bars represent standard errors. FFM, fat free mass; REE, resting energy expenditure.

The associations between REE and smoking status and REE and long-term tobacco exposure, using linear regression to adjust REE for FFM, are presented in Table 8.4 and 8.5, respectively. Results were similar to those obtained with the REE/FFM ratio method (Table 8.2 and 8.3). Table 8.4 shows that, compared with never smokers, only current smokers had a higher REE of 60.3 kcal/day, even after adjustment for confounding variables. This corresponds to a 3.9% higher REE relative to the adjusted mean of never smokers. No association between pack years and REE adjusted for FFM was found (Table 8.5).

Table 8.2: Mean and difference in REE/FFM of never smokers compared with occasional smokers, former smokers and current smokers.

	Model 1	Model 2	Model 3	Model 4
REE/FFM, adjusted mean of never smokers (95% CI) kcal/day/kg FFM	27.49 (27.15, 27.83)	27.28 (27.02, 27.54)	27.28 (27.02, 27.54)	27.52 (27.27, 27.76)
Occasional smokers[a]				
Difference in REE/FFM[b](95% CI), kcal/day/kg FFM	-1.03 (-1.96,-0.11)	-0.30 (-1.05, 0.46)	-0.29 (-1.06, 049)	-0.28 (-1.02, 0.46)
P-value	0.029	0.441	0.471	0.455
Former smokers[a]				
Difference in REE/FFM[b](95% CI), kcal/day/kg FFM	0.28 (-0.24, 0.80)	0.34 (-0.06, 0.75)	0.35 (-0.06, 0.75)	0.32 (-0.09, 0.73)
P-value	0.293	0.094	0.093	0.124
Current smokers[a]				
Difference in REE/FFM[b](95% CI), kcal/day/kg FFM	0.85 (0.01, 1.69)	1.25 (0.61, 1.89)	1.24 (0.60, 1.89)	1.28 (0.64, 1.92)
P-value	0.046	<0.001	<0.001	<0.001

Results are based on weighted analyses (n=1,183).Model 1: unadjusted (n=1,183). Model 2: adjusted for sex (n=1,183). Model 3: adjusted for sex, age, ethnicity and educational level (n=1,173; ethnicity was missing in 1 participant, 9 missing data on educational level). Model 4: adjusted for sex, age, ethnicity, educational level, physical activity, energy intake and BMI (n=1,159; 14 missing data on physical activity).

[a] Compared with never smokers.

[b] Beta coefficient from weighted linear regression.

BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure.

Table 8.3: Association between pack years of smoking and REE/FFM for occasional smokers, former smokers and current smokers separately.

	Model 1	Model 2	Model 3	Model 4
Occasional smokers				
Difference in REE/FFM per pack year[a] (95% CI), kcal/day/kg FFM	-0.02 (-0.07, 0.04)	0.02 (-0.03, 0.06)	0.01 (-0.05, 0.06)	-0.01 (-0.07, 0.06)
P-value	0.605	0.543	0.791	0.813
Former smokers				
Difference in REE/FFM per pack year[a] (95% CI), kcal/day/kg FFM	-0.02 (-0.04, 0.01)	0.01 (-0.01; 0.03)	0.01 (-0.00, 0.03)	0.01 (-0.01, 0.03)
P-value	0.210	0.174	0.141	0.200
Current smokers				
Difference in REE/FFM per pack year[a] (95% CI), kcal/day/kg FFM	-0.01 (-0.06,0.05)	-0.01 (-0.05, 0.03)	-0.01 (-0.06, 0.03)	-0.01 (-0.06, 0.04)
P-value	0.767	0.738	0.535	0.628

Results are based on weighted analyses (current smokers: n=126; occasional smokers: n=53; former smokers n= 555). Model 1: unadjusted. Model 2: adjusted for sex. Model 3: adjusted for sex, age, ethnicity and educational level (former smokers: ethnicity was missing in 1 participant, 4 missing data on educational level; current smokers: 2 missing data on educational level). Model 4: adjusted for sex, age, ethnicity, educational level, physical activity, energy intake and BMI (former smokers: 7 missing data on physical activity).

[a] Beta coefficient from weighted linear regression.

BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure.

Table 8.4: Mean and difference in REE adjusted for FFM of never smokers compared with occasional smokers, former smokers and current smokers.

	Model 1	Model 2	Model 3	Model 4
REE, adjusted mean of never smokers (95% CI), kcal/day	1,504 (1,489, 1,519)	1,516 (1,502, 1,530)	1,513 (1,499, 1,527)	1,539 (1,526, 1,551)
Occasional smokers[a]				
Difference in REE[b] (95% CI), kcal/day	-19.1 (-61.1, 23.0)	-12.0 (-53.4, 29.4)	-11.0 (-53.3, 31.4)	-13.4 (-50.9, 24.1)
P-value	0.374	0.571	0.611	0.484
Former smokers[a]				
Difference in REE[b] (95% CI), kcal/day	17.7 (-3.7, 39.2)	18.5 (-2.0, 39.0)	18.9 (-1.24, 39.1)	13.7 (-6.3, 33.8)
P-value	0.105	0.078	0.066	0.180
Current smokers[a]				
Difference in REE[b] (95% CI), kcal/day	53.5 (18.6, 88.4)	63.2 (29.7, 96.7)	61.7 (28.2, 95.2)	60.3 (29.1, 91.5)
P-value	0.003	<0.001	<0.001	<0.001

Results are based on weighted analyses (n=1,183). Model 1: adjusted for FFM (n=1,183). Model 2: adjusted for FFM and sex (n=1,183). Model 3: adjusted for FFM, sex, age, ethnicity and educational level (n=1,173; ethnicity was missing in 1 participant, 9 missing data on educational level). Model 4: adjusted for FFM, sex, age, ethnicity, educational level, physical activity, energy intake and BMI (n=1,159; 14 missing data on physical activity).

[a] Compared with never smokers.

[b] Beta coefficient from weighted linear regression.

BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure.

Table 8.5: Association between pack years of smoking and REE adjusted for FFM, for occasional smokers, former smokers and current smokers separately.

	Model 1	Model 2	Model 3	Model 4
Occasional smokers				
Difference in REE per pack year[a] (95% CI), kcal/day	1.9 (-1.3, 5.1)	1.7 (-1.5, 4.9)	1.2 (-2.2, 4.6)	-0.1 (-3.7, 3.5)
P-value	0.237	0.297	0.481	0.967
Former smokers				
Difference in REE per pack year[a] (95% CI), kcal/day	1.0 (-0.2, 2.1)	1.0 (-0.1, 2.1)	1.1 (-0.1, 2.2)	0.8 (-0.3, 1.9)
P-value	0.115	0.081	0.061	0.152
Current smokers				
Difference in REE per pack year[a] (95% CI), kcal/day	-0.3 (-2.0, 1.4)	-0.5 (-2.2, 1.3)	-0.8 (-2.9, 1.3)	0.2 (-2.4, 2.8)
P-value	0.690	0.614	0.475	0.899

Results are based on weighted analyses (current smokers: n=126; occasional smokers: n=53; former smokers n= 555). Model 1: adjusted for FFM. Model 2: adjusted for FFM and sex. Model 3: adjusted for FFM, sex, age, ethnicity and educational level (former smokers: ethnicity was missing in 1 participant, 4 missing data on educational level; current smokers: 2 missing data on educational level). Model 4: adjusted for FFM, sex, age, ethnicity, educational level, physical activity, energy intake and BMI (former smokers: 7 missing data on physical activity).

[a] Beta coefficient from weighted linear regression.

BMI, body mass index; FFM, fat free mass; REE, resting energy expenditure.

Discussion

In this large population-based cohort study we observed a higher REE/FFM (1.28 kcal/-day/kg FFM) and a higher REE adjusted for FFM (60.3 kcal/day) in current smokers compared with never smokers, after adjustment for age, sex, ethnicity, educational level, physical activity, energy intake and BMI. These results corroborate findings in previous studies investigating short-term effects of smoking on energy expenditure in selected study populations. These studies also observed a positive association between smoking or nicotine administration and REE.[5–10] However, as the habit of smoking often lasts many years, the question is whether results from short-term intervention studies are generalizable to prevalent smokers in the general population. The results of the current study show that in the general population smoking is associated with a higher REE.

The observed association between smoking and REE could contribute to a lower body weight in smokers and weight gain after smoking cessation. Compared with never smokers the REE adjusted for FFM was 3.9% higher in current smokers. In the literature, a range of 3-10% increase in REE has been reported directly after nicotine administration,[6] which is in line with our findings.

The mechanisms underlying the effects of nicotine on the body's energy balance have not yet been fully elucidated. It has been recognized for a long time that nicotine has sympathoadrenal stimulatory effects, thereby increasing energy substrate utilization, especially by enhancing the mobilization of free fatty acids through lipolysis in white fat tissue.[2,29] Besides this adrenergic pathway, nicotine also directly enhances lipolysis in white fat cells via nicotinic cholinergic receptors.[30] More recently it has been proposed that nicotine also increases energy combustion in brown adipose tissue (BAT).[4] BAT generates heat by burning triglycerides and glucose and thereby contributes to total energy expenditure.[31] We recently showed that in smoke-exposed mice, compared with air-exposed mice, plasma triglyceride-derived fatty acids were more rapidly taken up by BAT (MR Boon & PCN Rensen, unpublished). In addition, there are indications that the metabolic effects of nicotine change during physical activity in comparison with resting state. A study with thirty male participants, investigating the effects of smoking on energy expenditure during physical activity, concluded that smoking during light physical activity doubles the enhancing effect of smoking on energy expenditure.[32] Furthermore, there is still dissension about the contribution of a possible decreased REE after smoking cessation to body weight and composition, as some studies reported a decrease in REE after smoking cessation,[2,33] while others found no decline in REE.[2,33]

In this study there was no association between long-term tobacco exposure (pack years)

and REE/FFM or REE adjusted for FFM, neither in occasional smokers, former smokers or current smokers. These findings suggest that the effects of smoking on REE are primarily acute. The finding that never and former smokers, and especially never and occasional smokers did not differ in REE strongly supports this hypothesis that smoking has no long-term effects on REE. However, the current data did not allow us to confirm this hypothesis. There were no data available concerning the timespan between the last moment a participant smoked and the moment of blood sampling. Literature about this subject is scant. A small study examining the acute effects of smoking on energy expenditure, found that an acute cigarette-induced increase of energy expenditure ceased the morning after smoking.[5]

The main strength of our study is the extensive phenotyping of a large population-based cohort. It should be considered though that the NEO cohort is predominantly white, and different results might be obtained in other ethnic groups. Literature about the effect of race on the association between smoking and REE is scarce and ambiguous. It has been suggested that smoking increases REE to a greater extent in African American women compared to white women,[34] but others found no effects of racial differences on the association between smoking and REE.[8]

NEO study participants underwent a wide range of examinations and measurements and completed a variety of questionnaires, giving detailed information about smoking habits, REE and body composition. This made it possible to include many confounding factors into the models. However, we could not completely rule out the occurrence of any residual confounding. The reasons for this are that the study was set up as an observational cross-sectional study and that some confounding variables were self-reported (educational level, physical activity and dietary intake) which could have resulted in some extent of misclassification by under or overreporting. It should also be taken into consideration that pack years were self-reported, possibly leading to underreporting. Underreporting of pack years would lead to an underestimation of the association with REE/FFM or REE adjusted for FFM. However, there was no association between pack years and REE/FFM or REE adjusted for FFM in occasional, former and current smokers and we do not expect that some extent of underreporting would alter these highly non-significant results.

We conclude that current smoking is associated with an increased resting energy expenditure per kg fat free mass and an increased resting energy expenditure adjusted for fat free mass, compared with never smoking. The magnitude of the difference in resting energy expenditure between never and current smokers shows the considerable effect of smoking on the body's energy balance. There was no difference in resting energy expenditure per kg fat free mass or resting energy expenditure adjusted for fat free mass between never

and former smokers or between never and occasional smokers and no association with pack years, which implies that smoking has primarily acute effects on resting energy expenditure. To support this hypothesis future research into the duration and persistence of a smoking-induced increase in resting energy expenditure is necessary.

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