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Sympathetic nervous system activation and lipid metabolism: the NEO study

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ABSTRACT

Introduction

Mouse studies have revealed an important role for the sympathetic nervous system (SNS) in lipid metabolism, which may offer novel therapeutic modalities to treat dyslipidaemia. However, the role of the SNS in lipid metabolism in humans is insufficiently studied. Therefore, we aimed to study the association between SNS activation, measured as heart rate or heart rate variability, and serum triglyceride, non-high-density lipoprotein concentrations, high-density lipoprotein concentrations, and intrahepatic triglyceride content.

Methods

This is a cross-sectional analysis of baseline data of the Netherlands Epidemiology of Obesity study. Heart rate was estimated from a 10 second 12-lead electrocardiogram and parameters of heart rate variability were calculated over 24 hours. Fasting serum cholesterol concentrations were measured. Serum triglycerides were measured after fasting and 30 and 150 min after a liquid mixed meal. Intrahepatic triglyceride content was measured by ^{1}H-magnetic resonance spectroscopy. We performed linear regression analyses and adjusted for age, sex, smoking, education, physical activity, meal time, heart rate lowering medication, lipid lowering medication, body mass index, total body fat and HOMA-IR.

Results

After exclusion of individuals with missing data (n=496), 6174 participants were included: 44% men, mean (SD) age: 56 (6) years and mean BMI 26 (4) kg/m^2. Per 10 beats/min, total cholesterol concentration was 0.06 mmol/L (95% CI: 0.02, 1.10) higher and fasting serum triglyceride concentration was 5.8% (3.9, 7.8) higher. Resting heart rate was not associated with serum HDL-cholesterol or LDL-cholesterol concentrations. Postprandial triglyceride concentrations were 5.2% (3.5, 6.9) higher 30 minutes, and 4.4% (2.6, 6.3) higher 150 minutes after the liquid mixed meal. Intrahepatic triglyceride content was 13.1% (7.7, 18.7) higher after adjustment for confounding factors. Associations with heart rate variability were similar.

Conclusion

SNS activation, measured as higher resting heart rate or lower heart rate variability, was associated with higher fasting total cholesterol and higher serum triglyceride concentrations, and with higher intrahepatic triglyceride content. These results imply that the SNS is involved in lipid metabolism in humans, and that dyslipidaemia may be targeted by lowering SNS activation

INTRODUCTION

The autonomic nervous system is an involuntary nervous system with a sympathetic and a parasympathetic branch. Heart rate is a reflection of autonomic nervous system function (sympathovagal balance) that can be estimated as the intrinsic heart rate multiplied by a sympathetic factor and a vagal factor.[1, 2] Heart rate variability is a manifestation of activity of the baroreflex, which purpose is to buffer blood pressure by adaptions in sympathetic and parasympathetic outflow.[3, 4] A shift in sympathovagal balance towards increased activity of the sympathetic nervous system (SNS) results in a higher heart rate and lower heart rate variability, which are associated with both cardiovascular risk factors and cardiovascular events.[5-8] The mechanism underlying the increased cardiovascular risk due to SNS activation has not been elucidated. Since dyslipidaemia is a prominent risk factor for cardiovascular disease, alterations in lipid metabolism possibly play a role.

Lipid metabolism is influenced by the SNS in several ways. First, the SNS innervates the adipose tissue.[9] Sympathetic stimulation of adipose tissue induces intracellular lipolysis, thereby increasing the amount of glycerol and free fatty acids in the circulation.[10] Second, sympathetic stimulation of the liver enhances hepatic de novo lipogenesis.[11] Experimental studies showed that selective sympathetic denervation of the liver in rats decreased both hepatic lipogenesis and decreased hepatic very low-density lipoprotein (VLDL)-triglyceride secretion.[12, 13] This indicates that activation of the SNS stimulates both lipogenesis and the secretion of VLDL-triglycerides in the liver.

In healthy individuals, activation of the SNS is an adaptive response, aimed at temporarily providing lipids as a substrate for energy metabolism in muscles during fasting or 'flight-fight-or-fright actions'. However, chronic SNS activation may result in an prolonged increased lipid production, by stimulation of lipolysis in adipose tissue and of de novo lipogenesis in the liver, even if lipids are not necessary as a substrate for energy metabolism. This may result in higher fasting serum triglycerides and non-high-density lipoprotein (non-HDL) cholesterol concentrations and possibly a lower HDL-cholesterol concentration in the circulation (Figure). Some previous studies have reported these associations.[14-16] However, no previous study has investigated the association between heart rate and postprandial serum triglyceride concentrations after a mixed meal. Furthermore, in addition to an increased concentration of lipids in the circulation, the chronic activation of the SNS may also result in accumulation of lipids in the liver.[17] Evidence on this association is also lacking. Therefore, we hypothesized that SNS activation is associated with a disadvantageous lipid metabolism, specifically with triglyceride levels in the circulation and the liver. To this extent, we studied the associa-

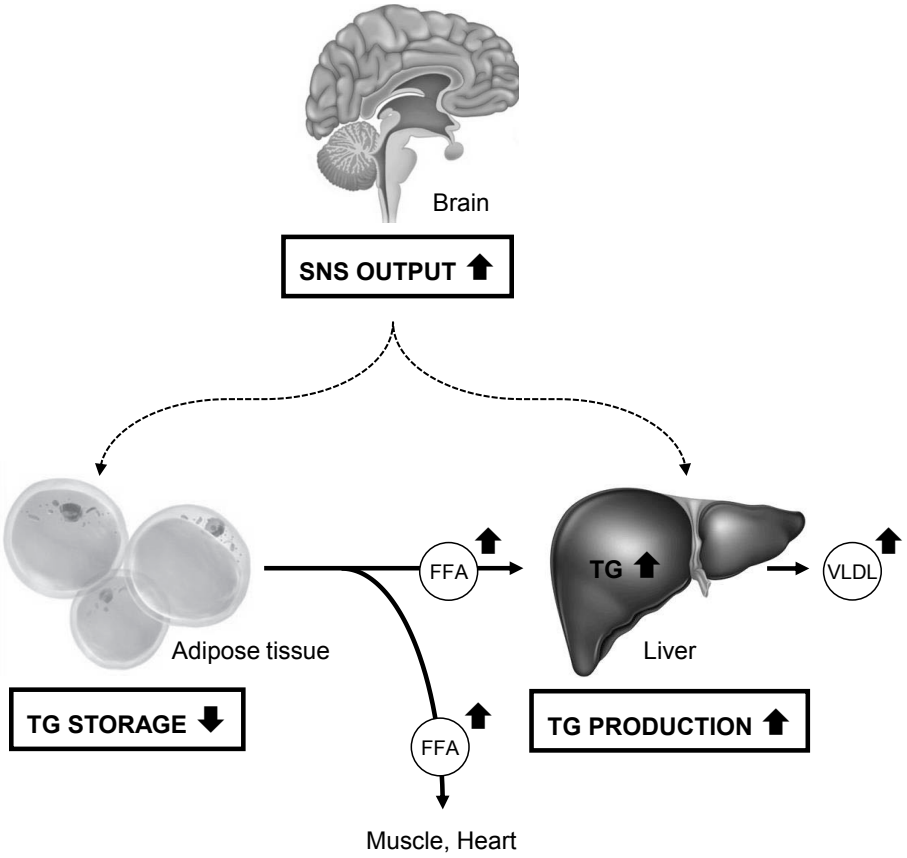

Figure. Sympathetic control of lipid metabolism
SNS=sympathetic nervous system; TG=triglyceride; FFA=free fatty acid; VLDL=very low-density lipoprotein; LDL=low-density lipoprotein

tions of resting heart rate and heart rate variability with fasting and non-fasting serum lipid concentrations and intrahepatic triglyceride content in a population-based study.

METHODS

Study design and population

The Netherlands Epidemiology of Obesity (NEO) study is a population-based prospective cohort study of 6673 individuals aged 45 to 65 years with an oversampling of individuals with a BMI ≥ 27 kg/m^2. The study design and data collection of the NEO study have been described previously.[18] In short, persons with a self-reported BMI of 27 kg/m^2 or higher were included between September 2008 and October 2012 from the greater area of

Leiden, the Netherlands. In addition, all inhabitants from one municipality (Leiderdorp) were invited irrespective of their BMI, allowing for a reference distribution of BMI. The present study is a cross-sectional analysis using the baseline measurements of the NEO study.

Participants were invited for a baseline visit at the NEO study centre of the Leiden University Medical Centre. Prior to this study visit, participants fasted overnight for at least 10 hours and completed a questionnaire on demographic, lifestyle, and clinical data in addition to questions on diet and physical activity. The participants were asked to bring all their current medication to the study centre. At the baseline visit, the participants underwent an extensive physical examination, including fasting and postprandial blood sampling, and a resting standard 10-second electrocardiogram (ECG). Heart rate variability and intrahepatic triglyceride content were measured in random subsamples of the baseline population. The NEO study was approved by the Medical Ethical Committee of the Leiden University Medical Centre. Before inclusion, all participants gave their informed consent.

Data collection

Participants reported their highest level of education in 10 categories according to the Dutch educational system. We defined low education as no education, primary education or lower vocational training) and used high education as the reference category in the analyses. Self-identified ethnicity of participants was reported in eight categories that we grouped into white (reference) and other. Tobacco smoking was categorized into current, former or never (reference) smokers. We estimated alcohol intake in grams per week by questionnaire. We measured physical activity using the Short Questionnaire to Assess Health-enhancing physical activity questionnaire and calculated the metabolic equivalent of task (MET) hours per week.[19]

We measured height and weight without shoes and one kilogram was subtracted to correct for the weight of clothing. Body Mass Index (BMI) was calculated by dividing the weight in kilograms by the height in meters squared. Total body fat was measured with a bio-impedance device (TBF-310, Tanita International Division, United Kingdom). Blood was sampled from the antecubal vein after an overnight fast of at least 10 hours, and 30 minutes and 150 minutes after consumption of a liquid mixed meal (400 mL containing 600 kcal, 16 % of energy (En%) derived from protein, 50 En% carbohydrates, and 34 En% fat). In the fasting blood samples, serum concentrations of triglycerides and total cholesterol were measured with the colorimetric method and HDL-cholesterol concentrations were measured with the homogenous HDLc method (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany). LDL-cholesterol concentra-

tions were calculated with the Friedewald formula.[20] Serum triglyceride concentrations were also measured 30 minutes and 150 minutes after the liquid mixed meal. Plasma glucose concentrations were measured with the enzymatic and colorimetric method (Roche Modular Analytics P800, Roche Diagnostics, Mannheim, Germany) and serum insulin concentrations were determined by an immunometric method (Siemens Immulite 2500, Siemens Healthcare Diagnostics, Breda, the Netherlands). Serum insulin concentrations below the detection limit of the assay (2.0 mU/L) were imputed using multiple imputation methods for left censored data, with 10 imputation datasets.[21] We calculated the updated Homeostasis Model Assessment Insulin Resistance (HOMA-IR), by entering fasting glucose and fasting insulin in a Microsoft Excel spreadsheet available on the internet.[22]

Heart rate and heart rate variability

To estimate the heart rate, a resting 12-lead ECG was obtained using a Mortara Eli-350 electrocardiograph (Mortara Instrument Inc., Best, Netherlands). Mean resting heart rate was calculated using the Matlab-based program BEATS.[23]

The Actiheart device, an accelerometer combined with a heart rate monitor (CamNtech Ltd, Cambridge, United Kingdom), was worn by a random subsample (14%) of the baseline population. We used the data from the heart rate monitor to calculate heart rate variability parameters. We selected only the Actiheart recordings with the least noise (<1%) for manual reparation and a valid estimation of heart rate variability. The heart rate data were processed to remove artefacts and non-sinus beats, and consequently heart rate variability was calculated over a 24 hour period according to a procedure that was described and applied previously.[2, 24, 25] In short, heart rate variability was calculated using a 5-minute moving window and all valid 5-minute values were averaged. We calculated the mean interbeat interval, the standard deviation of all normal intervals (SDNN) and the root mean square of the successive differences (RMSSD). Prior to spectral analysis of the recordings, the tachogram was processed with adjustment for linear trends, tachogram tapering and zero padding. A test of stationarity was performed, and we excluded non-stationary episodes from the analysis. For calculation of low frequency power (LF power) and high frequency power (HF power), a fast Fourier transformation was employed.

Intrahepatic triglyceride content

A random subsample (35%) of participants without contra-indications underwent proton (1H)-MR spectroscopy of the liver to assess hepatic triglyceride content. All imaging was performed on an MR system operating at a field strength of 1.5 Tesla (Philips Medical Systems, Best, Netherlands). Hepatic ^{1}H-MR spectra were obtained as

described previously.[26] In short, ^{1}H-MRS (magnetic resonance spectroscopy) of the liver was performed with an 8 mL voxel positioned in the right lobe of the liver, avoiding gross vascular structures and adipose tissue depots. Sixty-four averages were collected with water suppression. Spectra were obtained with an echo time of 26 milliseconds and a repetition time of 3000 milliseconds. Data points (1024) were collected using a 1000 Hz spectral line. Without changing any parameters, spectra without water suppression, with a repetition time of 10 seconds, and with four averages were obtained as an internal reference. ^{1}H-MRS data were fitted using Java-based magnetic resonance user interface software (jMRUI version 2.2, Leuven, Belgium), as described previously.[27] Intrahepatic triglyceride content relative to water was calculated as (signal amplitude of triglyceride) / (signal amplitude of water) * 100.

Statistical analyses

In the NEO study, individuals with a BMI of 27 kg/m^2 or higher were oversampled. To correctly represent associations in the general population, adjustments for the oversampling of individuals with a BMI ≥ 27 kg/m^2 were made.[28] This was done by weighting individuals towards the BMI distribution of participants from the Leiderdorp municipality, whose BMI distribution was similar to the BMI distribution of the general Dutch population.[29, 30] All results were based on weighted analyses. Consequently, the results apply to a population-based study without oversampling of participants with a BMI ≥ 27 kg/m^2.

Baseline characteristics were expressed as mean (standard deviation), median (25th- 75th percentiles) or as percentage by tertiles of heart rate. We performed weighted linear regression analysis with heart rate per 10 beats/min or heart rate variability per standard deviation as a determinant. As outcome variables we used total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting and postprandial (30 min and 150 min) serum triglyceride concentrations (mmol/L) and intrahepatic triglyceride content (%). The serum triglyceride concentrations and intrahepatic triglyceride content were ln-transformed. Regression coefficients were expressed as mmol/L or as percentage difference with 95% confidence intervals for ln-transformed variables. We adjusted our results for age, sex, tobacco smoking, education, physical activity, heart rate lowering medication and lipid lowering medication. The analyses including intrahepatic triglyceride content were also adjusted for time since ingestion of the mixed meal and alcohol intake. Additionally we adjusted for BMI and total body fat, and in a last step for HOMA-IR.

RESULTS

Baseline characteristics

The NEO study included 6673 participants. We consecutively excluded participants with missing data on heart rate (n=89), lipid concentrations (n=44), education (n=64), physical activity (n=116), body composition (n=31), HOMA-IR (n=148) and other confounders (n=7). The main analyses therefore included 6174 participants. Compared with excluded participants, included participants were more often men (44% versus 40%). Other variables were comparable: age was 56 (6) years and BMI was 26 (4) kg/m^2 in both excluded and included participants. Total cholesterol was 5.6 (1.1) mmol/L in excluded and 5.7 (1.1) mmol/L in included participants and fasting serum triglyceride concentration was 1.1 (0.8) mmol/L versus 1.2 (0.8) mmol/L.

Table 1 shows the weighted baseline characteristics of the 6174 participants included in our analyses, stratified by tertiles of heart rate. Participants with a higher heart rate had more body fat and were more often women.

Table 1 Characteristics of the study population by tertiles of heart rate

Beats/min	**30-59**	**60-67**	**68-157**
Age (year)	55 (6)	55 (6)	56 (6)
Sex (% men)	51	41	39
BMI (kg/m^2)	26 (4)	26 (4)	27 (5)
Total body fat (%) - Men - Women	 24 (5) 36 (6)	 26 (6) 37 (7)	 28 (7) 40 (7)
Tobacco smoking - Current (%) - Former (%)	 17 45	 16 48	 15 43
Education level (% low)	17	19	23
MET hour/week	124 (84-161)	123 (79-157)	109 (68-143)
Heart rate lowering medication (%)	14	11	11
Lipid lowering medication (%)	10	10	14
Fasting triglycerides (mmol/L)	1.0 (0.7-1.3)	1.0 (0.7-1.5)	1.2 (0.8-1.7)
30 min triglycerides (mmol/L)	1.1 (0.9-1.5)	1.2 (0.9-1.7)	1.4 (1.0-1.9)
150 min triglycerides (mmol/L)	1.6 (1.1-2.1)	1.6 (1.1-2.3)	1.8 (1.3-2.6)
Total cholesterol (mmol/L) LDL cholesterol (mmol/L)	5.6 (1.0) 3.5 (0.9)	5.7 (1.0) 3.6 (1.0)	5.8 (1.1) 3.6 (1.0)
HDL cholesterol (mmol/L)	1.6 (0.5)	1.6 (0.5)	1.5 (0.4)
HOMA-IR	0.5 (0.3-0.9)	0.6 (0.3-1.1)	0.8 (0.5-1.4)

Data are expressed as mean (standard deviation), mean (25th-75th percentile) or percentage
Results are based on weighted analyses (n=6174)

Heart rate and cholesterol

Per 10 beats/min, the total cholesterol concentration was 0.09 mmol/L higher (95% CI 0.05, 0.13) (Table 2). After adjustment for confounding factors, including BMI, total body fat and HOMA-IR, the difference was 0.06 mmol/L (0.02, 1.10). There was a positive association between heart rate and LDL-cholesterol concentration, and an inverse association between heart rate and HDL-cholesterol concentration. These associations attenuated after adjustment for confounding variables.

Table 2 Difference in cholesterol concentrations per 10 bpm in heart rate

		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Model 1	Crude	0.09 (0.05, 0.13)	−0.02 (−0.03, 0.00)	0.04 (0.01, 0.08)
Model 2	+ age, sex	0.06 (0.03, 0.10)	−0.04 (−0.06, −0.03)	0.03 (0.00, 0.07)
Model 3	+ confounders*	0.08 (0.04, 0.11)	−0.04 (−0.05, −0.02)	0.04 (0.01, 0.08)
Model 4	+ BMI, TBF	0.07 (0.03, 0.11)	−0.01 (−0.02, 0.00)	0.02 (−0.01, 0.06)
Model 5	+ HOMA-IR	0.06 (0.02, 0.10)	0.00 (−0.01, 0.02)	0.02 (−0.02, 0.05)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance

Results are based on weighted analyses (n=6174)

Heart rate and triglycerides

Heart rate was associated with fasting serum triglyceride concentration (Table 3). Per 10 beats/min, fasting serum triglyceride concentration changed with 7.8% (5.4, 10.3). After adjustments for confounders this was 5.2% (3.5, 6.9). Heart rate was also associated with postprandial serum triglyceride concentrations (30 minutes and 150 minutes after a liquid mixed meal) in multivariate analyses. However, when we additionally adjusted for fasting serum triglyceride concentration, these results attenuated (Table 3).

Heart rate and intrahepatic triglyceride content

In the NEO study, a random 2354 participants underwent magnetic resonance spectroscopy of the liver. After exclusion of missing data on heart rate (40), intrahepatic triglyceride content (n=266), education (n=17), physical activity (n=44), HOMA-IR (n=53) and other confounders (n=5), the analyses on intrahepatic triglyceride content included 1929 participants. Included participants were more often male (47%) than excluded participants (42%) and had a slightly higher intrahepatic triglyceride content (2.8% (1.4-6.4) versus 1.9% (1.1-5.0)). Age and body mass index were not different.

Heart rate was associated with intrahepatic triglyceride content. Per 10 beats/min, intrahepatic triglyceride content changed with 20.3% (13.0, 28.1). Part of this association was explained by confounders including BMI, total body fat and HOMA-IR. After all adjustments, this difference was 13.1% (7.7, 18.7) per 10 beats/min (Table 4).

Table 3 Percentage difference in triglyceride concentrations per 10 bpm in heart rate

		Fasting	30 minutes	150 minutes
Model 1	Crude	7.8 (5.4, 10.3)	6.6 (4.6, 8.7)	5.8 (3.6, 8.0)
Model 2	+ age, sex	9.4 (7.0, 11.8)	8.0 (5.9, 10.0)	7.3 (5.2, 9.5)
Model 3	+ confounders*	9.5 (7.2, 11.9)	8.1 (6.1, 10.1)	7.4 (5.3, 9.5)
Model 4	+ BMI, TBF	7.0 (4.7, 9.3)	6.1 (4.1, 8.0)	5.4 (3.4, 7.5)
Model 5	+ HOMA-IR	5.9 (3.9, 8.0)	5.2 (3.5, 6.9)	4.4 (2.6, 6.3)
Model 6	+ fasting TG		0.6 (−0.1, 1.2)	−0.1 (−1.0, 0.7)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance; TG, triglycerides

Results are based on weighted analyses (n=6174)

Table 4 Percentage difference in intrahepatic triglyceride content per 10 bpm in heart rate

		Intrahepatic triglyceride content
Model 1	Crude	20.3 (13.0, 28.1)
Model 2	+ age, sex	22.7 (15.5, 30.4)
Model 3	+ confounders*	21.5 (14.5, 29.0)
Model 4	+ BMI, TBF	13.5 (7.7, 19.5)
Model 5	+ HOMA-IR	13.1 (7.7, 18.7)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication, meal time, alcohol consumption

BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance

Results are based on weighted analyses (n=1929)

Heart rate variability and lipids

Of the 6673 participants included in the NEO study, an Actiheart device was carried by 955 participants. In 50 of this 955, the recording failed due to technical problems (e.g. broken battery, detached electrodes) or incorrect use by the participant (e.g. early removal). From the 905 remaining recordings, we selected 485 recordings with maximum quality of IBI data (<1% artefacts) to use for heart rate variability analysis. After exclusion of participants with missing data on education (n=5), physical activity (n=4), HOMA-IR (n=9) and other confounders (n=2), the present analysis included 465

participants. Of this subgroup, 147 participants were also included in the subgroup with data on intrahepatic triglyceride content. Included participants were comparable with excluded participants, only the percentage men was higher (56% in included versus 43% in excluded participants).

The associations between heart rate variability parameters and lipid concentrations in the circulation were comparable to the associations using heart rate as a determinant. Increased heart rate variability (reflecting reduced SNS activity) was associated with lower total cholesterol and serum triglyceride concentrations. Unfortunately, due to the smaller sample size, these results were all non-significant (Appendix).

DISCUSSION

This is the first study that shows a positive association between activation of the SNS, measured by heart rate and heart rate variability, and intrahepatic triglyceride content. Furthermore, we identified an association of SNS activity not only with fasting serum triglyceride concentrations, but also with serum triglycerides concentrations 30 and 150 minutes after a standardized mixed meal. Finally, we observed a small positive association between heart rate and serum fasting total cholesterol concentrations. The positive association of heart rate with LDL-cholesterol, and the inverse association with HDL-cholesterol attenuated after adjustment for confounders. The results from this study suggest that sympathetic nervous system activity is associated with alterations in lipid metabolism.

Studies in rodents clearly indicate as role for the autonomic nervous system in lipid metabolism, as vagatomy increases cholesterol concentrations in the circulation, whereas sympathectomy decreases cholesterol concentrations.[31] However, previous studies on activation of the SNS and cholesterol concentrations in humans have produced conflicting results. We found a positive association of heart rate, reflecting SNS activity, with total cholesterol. This has also been shown in some earlier studies.[14-16] We did not find an association of heart rate with LDL-cholesterol and HDL-cholesterol after adjustment for confounding variables. This is contradicting some studies,[14-16] but is in line with another study.[32] In line with our results, some earlier studies observed an association between activity of the SNS and serum triglyceride concentrations.[14, 16, 33] No study has yet evaluated the association between heart rate and postprandial serum triglycerides concentrations 30 and 150 minutes after a liquid mixed meal. We showed that heart rate was also associated with postprandial triglyceride concentrations, but that this association could be explained by higher fasting triglyceride concentrations

with higher heart rate. In addition to cross-sectional studies, one study prospectively investigated the relation of heart rate with changes in serum triglyceride and HDL-cholesterol concentrations over two years.[34] An association between heart rate and a decrease in HDL-cholesterol concentrations was observed in this study, possibly indicating that increased SNS activation precedes alterations in lipid metabolism. However, no prospective association between heart rate and serum triglycerides concentrations was found. Only one previous study investigated the association of SNS activation, measured as low heart rate variability, with intrahepatic triglyceride content and reported lower heart rate variability in individuals with non-alcoholic fatty liver disease compared with controls.[35] We extend this finding by showing a positive association between heart rate and intrahepatic triglyceride content in the general population. Per 10 beats/min in heart rate, the difference in intrahepatic triglyceride content was 13.1% (7.7, 18.7), independent of confounders including body mass index, total body fat and HOMA-IR.

The most important strength of the present study is the study size of 6174 participants with data on lipid concentrations in the circulation. Especially the association of heart rate with postprandial serum triglyceride concentrations after a standardized liquid mixed meal has not been published yet. Since most individuals are in a postprandial state for the larger part of the day, and elevated postprandial serum triglyceride concentrations are associated with increased cardiovascular risk, this seems to be an important finding.[36, 37] Furthermore, intrahepatic triglyceride content measured by MRS, was available in 1929 participants. This technique is expensive and time-consuming, and therefore usually not available in large cohorts such as the NEO study. A limitation of our study is the cross-sectional design which may have resulted in residual confounding and reverse causation. Because parameters of SNS activation and lipid concentrations were assessed at the same time, we cannot make any statements on the longitudinal association between both variables. Furthermore, the absence of the measurement of free fatty acids is a limitation. Another limitation of the present study may be that we did not measure brown adipose tissue volume present in the body or any markers for its activity. Besides an effect on white adipose tissue and the liver, stimulating lipolysis and de novo lipogenesis, the SNS also innervates brown adipose tissue. SNS activation stimulates the uptake of VLDL-triglycerides in brown adipose tissue for production of heat through the mechanism of uncoupling, i.e. non-shivering thermogenesis.[38] As a result, SNS activation of brown adipose tissue may (partly) counteract the triglyeride-raising and cholesterol-raising effect of SNS activation of white adipose tissue and liver. On the other hand, a divergence of autonomic nervous system activity towards different organs has also been proposed, implying that increased SNS activity to white adipose tissue or the liver does not necessarily have to be accompanied by increased activity towards brown adipose tissue, especially not at room temperature.[39]

Stimulation of the SNS seems to be regulated by the hypothalamus, for example by release of neuropeptide Y.[12] In addition, peripherally produced hormones such as leptin and glucagon-like peptide-1 may stimulate the hypothalamus and consequently the SNS.[40, 41] Activation of the SNS influences lipid metabolism in several ways. Experimental studies showed that selective sympathetic denervation of the liver in rats decreased both hepatic lipogenesis and decreased hepatic VLDL-triglyceride secretion.[12, 13] This indicates that SNS activation to the liver stimulates both lipogenesis and the secretion of VLDL-triglycerides. Furthermore, in white adipose tissue, stimulation of the SNS induces lipolysis, thereby increasing the amount of glycerol and free fatty acids in the circulation.[10] Since in a fasting state most triglycerides in the circulation are produced by the liver, increased lipogenesis could explain our results on heart rate and the serum fasting triglyceride concentration.

On the mechanism underlying the positive association we found between heart rate and intrahepatic triglyceride content we can only speculate. In rats, sympathetic hepatic denervation or adrenalectomy decreased VLDL-triglyceride production, but increased intrahepatic triglyceride content.[13] This finding is not in line with our results, that show a higher intrahepatic triglyceride content with a higher heart rate. Increased SNS activation could possibly induce the accumulation of intrahepatic triglyceride by the secretion of leptin and the inhibition of adiponectin. High leptin and low adiponectin are associated with both a higher SNS activation and a higher intrahepatic triglyceride content.[43]

Postprandial serum concentrations of triglycerides in the circulation are strongly influenced by insulin resistance. Insulin stimulates the expression of lipoprotein lipase in adipose tissue, which results in increased uptake and storage of free fatty acids in adipocytes.[42] In an insulin resistant state, clearance of lipids from the circulation is therefore inhibited. However, in our study we still observed approximately 5% higher postprandial serum triglyceride concentrations 30 and 150 minutes after a meal, independent of insulin resistance measured as HOMA-IR. This may reflect an association between SNS activation and serum postprandial triglyceride concentrations independent of insulin resistance. Triglycerides, especially postprandial serum triglycerides concentrations, are strongly associated with cardiovascular risk.[36, 37] Therefore, elevated postprandial serum triglycerides concentrations may be a mechanism underlying the association between increased SNS activation and cardiovascular disease.

The strength of the associations of heart rate with cholesterol concentrations is modest. The difference in total cholesterol per 10 beats/min was 0.06 mmol/L after adjustments for confounders. This difference is clinically irrelevant. The observed associations of

heart rate with serum triglyceride concentrations and intrahepatic triglyceride content, however, may be of clinical relevance. Per 10 beats/min, fasting triglyceride concentrations were 5.8% higher. For a participant with a fasting triglyceride concentration of 1.0 mmol/L, this would imply an additional 0.06 mmol/L, independent of all confounding variables. The difference in intrahepatic triglyceride content was 13.1% per 10 beats/min in heart rate. In a participant with 5% intrahepatic triglycerides, this would correspond to 0.7% more liver fat.

In conclusion, SNS activation, measured as higher resting heart rate or lower heart rate variability, was associated with higher fasting total cholesterol, higher fasting and postprandial serum triglyceride concentrations, and with higher intrahepatic triglyceride content. These results imply that the SNS is involved in lipid metabolism in humans, and that dyslipidaemia may be targeted by lowering SNS activation. Future research should elucidate whether abnormalities in lipid profile are a mechanism underlying the association between SNS activation and cardiovascular disease.

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APPENDIX

E-Table 1 Difference in cholesterol concentrations per standard deviation in SDNN

		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Model 1	Crude	−0.02 (−0.14, 0.09)	0.00 (−0.06, 0.06))	0.01 (−0.11, 0.13)
Model 2	+ age, sex	0.05 (−0.09, 0.18)	0.04 (−0.02, 0.10)	0.06 (−0.06, 0.19)
Model 3	+ confounders*	0.07 (−0.06, 0.20)	0.03 (−0.02, 0.09)	0.08 (−0.05, 0.20)
Model 4	+ BMI, TBF	0.08 (−0.05, 0.20)	0.02 (−0.03, 0.07)	0.09 (−0.03, 0.21)
Model 5	+ HOMA-IR	0.09 (−0.04, 0.21)	0.00 (−0.05, 0.06)	0.10 (−0.03, 0.22)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication
SDNN, standard deviation of all normal intervals; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance
Results are based on weighted analyses (n=465)

E-Table 2 Difference in triglyceride concentrations per standard deviation in SDNN

		Fasting triglycerides (%)	Triglycerides 30 minutes (%)	Triglycerides 150 minutes (%)
Model 1	Crude	−2.9 (−11.3, 4.9)	−2.7 (−9.9, 4.0)	−2.4 (−9.6, 4.3)
Model 2	+ age, sex	−5.6 (−14.9, 2.9)	−4.8 (−12.6, 2.5)	−4.3 (−11.7, 2.7)
Model 3	+ confounders*	−4.4 (−12.9, 3.5)	−4.2 (−11.5, 2.6)	−4.5 (−11.4, 2.0)
Model 4	+ BMI, TBF	−3.7 (−12.1, 4.0)	−3.6 (−10.7, 3.1)	−3.9 (−10.6, −2.5)
Model 5	+ HOMA-IR	5.9 (3.9, 8.0)	−0.8 (−6.5, 4.5)	−1.7 (−7.3, 3.5)
Model 6	+ fasting TG		−0.8 (−2.6, 1.0)	−1.1 (−4.2, −1.9)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication
SDNN, standard deviation of all normal intervals; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance; TG, triglycerides
Results are based on weighted analyses (n=465)

E-Table 3 Difference in cholesterol concentrations per standard deviation in RMSSD

		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Model 1	Crude	−0.11 (−0.22, 0.00)	0.01 (−0.04, 0.06)	−0.07 (−0.18, 0.04)
Model 2	+ age, sex	−0.10 (−0.22, 0.02)	0.01 (−0.05, 0.06)	−0.06 (−0.17, 0.06)
Model 3	+ confounders*	−0.07 (−0.17, 0.04)	0.01 (−0.03, 0.06)	−0.02 (−0.13, 0.09)
Model 4	+ BMI, TBF	−0.06 (−0.17, 0.05)	0.01 (−0.04, 0.05)	−0.02 (−0.12, 0.09)
Model 5	+ HOMA-IR	−0.05 (−0.16, 0.06)	−0.01 (−0.06, 0.03)	−0.01 (−0.11, 0.10)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication
RMSSD, root mean square of the successive differences; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance
Results are based on weighted analyses (n=465)

E-Table 4 Difference in triglyceride concentrations per standard deviation in RMSSD

		Fasting triglycerides (%)	Triglycerides 30 minutes (%)	Triglycerides 150 minutes (%)
Model 1	Crude	−5.8 (−13.6, 1.4)	−5.4 (−12.2, 0.9)	−4.8 (−11.0, 1.0)
Model 2	+ age, sex	−5.6 (−13.9, 2.1)	−5.2 (−12.4, 1.6)	−4.4 (−11.0, 1.9)
Model 3	+ confounders*	−5.6 (−13.8, 2.0)	−5.5 (−12.6, 1.3)	−4.8 (−11.7, 1.6)
Model 4	+ BMI, TBF	−5.8 (−13.8, 1.6)	−5.6 (−12.5, 0.8)	−4.9 (−11.5, 1.4)
Model 5	+ HOMA-IR	−2.2 (−7.3, 2.6)	−2.7 (−7.4, 1.9)	−1.7 (−6.4, 2.8)
Model 6	+ fasting TG		−0.7 (−2.3, 0.8)	0.3 (−2.3, 3.0)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

RMSSD, root mean square of the successive differences; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance; TG, triglycerides

Results are based on weighted analyses (n=465)

E-Table 5 Difference in cholesterol concentrations per standard deviation in LF power

		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Model 1	Crude	−0.02 (−0.14, 0.09)	0.01 (−0.04, 0.06)	−0.01 (−0.12, 0.10)
Model 2	+ age, sex	0.06 (−0.08, 0.20)	0.06 (0.00, 0.12)	0.05 (−0.07, 0.18)
Model 3	+ confounders*	0.07 (−0.07, 0.21)	0.05 (−0.01, 0.10)	0.06 (−0.07, 0.18)
Model 4	+ BMI, TBF	0.08 (−0.06, 0.21)	0.03 (−0.03, 0.08)	0.07 (−0.06, 0.19)
Model 5	+ HOMA-IR	0.09 (−0.05, 0.22)	0.01 (−0.04, 0.06)	0.08 (−0.05, 0.20)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

LF, low frequency; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance

Results are based on weighted analyses (n=465)

E-Table 6 Difference in triglyceride concentrations per standard deviation in LF power

		Fasting triglycerides (%)	Triglycerides 30 minutes (%)	Triglycerides 150 minutes (%)
Model 1	Crude	−4.2 (−11.1, 2.2)	−4.1 (−10.8, 2.3)	−4.3 (−11.1, 2.0)
Model 2	+ age, sex	−7.6 (−15.7, −0.1)	−6.6 (−14.1, 0.5)	−6.5 (−14.0, 0.5)
Model 3	+ confounders*	−5.5 (−13.3, 1.7)	−5.0 (−12.1, 1.7)	−6.1 (−13.4, 0.7)
Model 4	+ BMI, TBF	−5.1 (−13.0, 2.2)	−4.6 (−11.7, 2.0)	−5.8 (−13.1, 1.1)
Model 5	+ HOMA-IR	0.6 (−5.2, 6.7)	−0.3 (−5.9, 5.1)	−2.5 (−3.4, 7.9)
Model 6	+ fasting TG		−0.7 (−2.6, 1.1)	−2.4 (−5.9, 1.0)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

LF, low frequency; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance; TG, triglycerides

Results are based on weighted analyses (n=465)

E-Table 7 Difference in cholesterol concentrations per standard deviation in HF power

		Total cholesterol (mmol/L)	HDL cholesterol (mmol/L)	LDL cholesterol (mmol/L)
Model 1	Crude	−0.06 (−0.17, 0.05)	0.03 (−0.02, 0.09)	−0.06 (−0.17, 0.05)
Model 2	+ age, sex	−0.08 (−0.21, 0.04)	0.01 (−0.05, 0.06)	−0.06 (−0.17, 0.06)
Model 3	+ confounders*	−0.04 (−0.16, 0.08)	0.01 (−0.04, 0.07)	−0.02 (−0.13, 0.09)
Model 4	+ BMI, TBF	−0.04 (−0.16, 0.08)	0.01 (−0.04, 0.06)	−0.02 (−0.13, 0.09)
Model 5	+ HOMA-IR	−0.03 (−0.15, 0.09)	−0.01 (−0.05, 0.04)	−0.01 (−0.12, 0.10)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

HF, high frequency; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance

Results are based on weighted analyses (n=465)

E-Table 8 Difference in triglyceride concentrations per standard deviation in HF power

		Fasting triglycerides (%)	Triglycerides 30 minutes (%)	Triglycerides 150 minutes (%)
Model 1	Crude	−6.7 (−14.2, 0.3)	−6.3 (−13.1, 0.0)	−6.3 (−13.1, 0.0)
Model 2	+ age, sex	−4.9 (−12.8, 2.5)	−4.7 (−11.9, 2.0)	−4.4 (−11.5, 2.3)
Model 3	+ confounders*	−4.6 (−12.7, 2.8)	−4.8 (−12.1, 1.9)	−4.6 (−12.1, 2.4)
Model 4	+ BMI, TBF	−5.4 (−13.4, −2.0)	−5.6 (−12.7, 1.0)	−5.2 (−12.6, 1.7)
Model 5	+ HOMA-IR	−0.7 (−6.4, 4.6)	−1.5 (−6.7, 3.4)	−1.6 (−7.4, 3.9)
Model 6	+ fasting TG		−0.9 (−2.3, 0.6)	−0.9 (−4.3, 2.4)

*Confounders: smoking, education, physical activity, heart rate lowering medication and lipid lowering medication

HF, high frequency; BMI, body mass index; TBF, total body fat; HOMA-IR, updated Homeostasis Model Assessment Insulin Resistance; TG, triglycerides

Results are based on weighted analyses (n=465)