Effect of Low-Energy Diets Differing in Fiber, Red Meat, and Coffee Intake on Cardiac Autonomic Function in Obese Individuals With Type 2 Diabetes

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OBJECTIVE

The autonomic nervous system (ANS) regulates both the cardiovascular system and energy balance and is disturbed in diabetes and obesity. The effect of different approaches of caloric restriction on ANS function has not been assessed in individuals with diabetes. Thus, we sought to determine whether low-energy diets differing in fiber, red meat, and coffee intake exert differential effects on cardiac autonomic function.

RESEARCH DESIGN AND METHODS

In this randomized parallel-group pilot trial, obese patients with type 2 diabetes were randomly allocated to consume either a diet high in cereal fiber, free of red meat, and high in coffee (n = 13) or a diet low in fiber, high in red meat, and coffee free (n = 15) over 8 weeks. Eight measures of heart rate variability (HRV) indicating vagal and/or sympathetic modulation over 3 h and inflammatory markers were determined during a hyperinsulinemic-euglycemic clamp.

RESULTS

After 8 weeks, both dietary interventions resulted in a mean weight loss of 5–6 kg, a mean decline in heart rate of 4–6 bpm, and improvement in vagally mediated HRV. However, the changes in HRV parameters from baseline to 8 weeks did not differ between the groups. In the entire study cohort, incremental HRV from baseline to 8 weeks was associated with enhanced oxidative glucose utilization (P < 0.05), but not with insulin sensitivity and inflammatory markers.

CONCLUSIONS

In obese patients with type 2 diabetes, energy restriction per se over 8 weeks contributed to improved cardiac vagal function in relation to improved oxidative glucose utilization. This preliminary finding should be verified in a confirmatory trial.

Cardiovascular autonomic neuropathy detected by reduced heart rate variability (HRV) affects ~20% of individuals with diabetes (1). HRV measures the fluctuations in autonomic inputs to the heart (2) and is considered a prognostic marker in various conditions including diabetes (1), metabolic syndrome (3), and cardiovascular disease (2). Spectral analysis of HRV indicates that efferent vagal activity is a major contributor to the high-frequency component, while the low-frequency component

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has been considered to be a marker of sympathetic modulation or to be under both sympathetic and vagal influences (2). Vagus nerve signaling plays a paramount role in the regulation of feeding behavior and metabolic homeostasis that is aimed at preserving energy balance and preventing fluctuations in body weight and metabolism (4). The metabolic homeostasis is regulated by the vagus nerve via controlling heart rate, gastrointestinal motility and secretion, pancreatic endocrine and exocrine secretion, and endogenous glucose production. In addition, efferent vagus nerve–mediated cholinergic signaling controls innate immune responses and inflammation during pathogen invasion and tissue injury (4).

Obesity alters autonomic nervous system (ANS) activity, and HRV generally diminishes with increasing waist circumference (3). The sympathetic nervous system is a key player linking the development and progression of cardiovascular disease with obesity (5). Cardiac sympathovagal imbalance, estimated by the low-frequency/high-frequency ratio, which is characterized by a shift toward sympathetic predominance and diminished vagal activity, is a consequence of many primary cardiovascular disease states and can trigger arrhythmias (6).

Weight loss induced by caloric restriction appears to exert favorable effects on ANS activity via enhancing vagal activity and/or reducing sympathetic modulation in overweight or obese otherwise healthy individuals (7–12). However, the results across studies are inconsistent, and the mechanisms implicated in these effects are unclear. In glucose-tolerant obese subjects, chronic hyperinsulinemia was associated with persistent baroreflex downregulation and episodic (postprandial) sympathetic dominance, and reversal of these changes by weight loss suggested a causal role for insulin (13). However, in normoglycemic obese women, weight loss after Roux-en-Y gastric bypass independently improved HRV and glucose uptake (10). Caloric restriction in overweight individuals over 6 months was accompanied by improved vagal activity and reduced sympathetic activity only when combined with exercise despite weight loss comparable to caloric restriction only (11).

Improvement in HRV due to weight loss in patients with diabetes could hypothetically indicate reduced cardiovascular risk, but the susceptibility of the ANS toward this intervention could be compromised due to cardiovascular autonomic neuropathy. Nonetheless, in an uncontrolled study including overweight or obese subjects with type 2 diabetes on a 16-week energy-restricted diet, weight loss was associated with improvement in HRV (14). Notably, caffeine consumption over 2 weeks could also improve HRV in subjects with type 1 diabetes (15), and coffee ingestion over 5 days was associated with increased parasympathetic activity in patients with myocardial infarction (16). Moreover, epidemiological studies suggest that a diet high in fiber and coffee, but low in red meat, is associated with a reduced risk of type 2 diabetes (17–19).

The effects of distinct dietary modulations of caloric restriction on cardiac ANS function have not been assessed in diabetic subjects. We sought to determine whether low-energy diets differing in fiber, red meat, and coffee intake exert differential effects on cardiac autonomic function in obese subjects with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Participants

Prior to study inclusion, all participants gave their written informed consent. The study (clinical trial reg. no. NCT01409330, clinicaltrials.gov) was approved by the local ethics committee of Heinrich Heine University, Düsseldorf, Germany, and was performed according to the Declaration of Helsinki (2008 version). Inclusion criteria were age 18–69 years; BMI $\geq$30 kg/m^2; type 2 diabetes treated with diet, metformin, or acarbose; and known disease duration of $\leq$5 years. Exclusion criteria were HbA_{1c} >75 mmol/mol (>9.0%); type 1 or secondary diabetes types; presence of acute or chronic diseases, including cancer; and use of a medication affecting the immune system or insulin sensitivity, other than metformin. The screening included medical history, laboratory tests, anthropometry, and 12-lead ECG.

Study Design

The details of the study protocol have been reported elsewhere (20). In brief, volunteers invited to the German Diabetes Center were 1:1 randomly allocated to one of two dietary interventions using randomization blocks (four or six patients) with consecutive numbers. After randomization, participants maintained their dietary habits during the run-in period (18.8 $\pm$ 1.3 days) and were monitored by daily dietary weighing protocols to calculate average energy intake (Prodi expert 6.1; NutriScience, Hausach, Germany). Metformin treatment was stopped 3 days before the assessments. During the intervention, participants received individually calculated daily diet sheets providing a median 1,198 kJ (interquartile range [IQR] 694) reduction in total daily energy intake and a constant macronutrient distribution (50% of energy from carbohydrates, 30% from fat, and 20% from protein). The average daily reduction in energy intake has been calculated using the returned and amended dietary protocol of the participant, thus reflecting the true intake documented by the subject, which we considered as the best available source. The diet high in cereal fiber, free of red meat, and high in coffee (HF−RM+C) contained 30–50 g/day cereal fiber from wheat and rye (100 g of whole-grain crispbread and 250–300 g of wheat/rye whole-grain bread), five or more cups of coffee per day containing 7–8 g coffee powder each, and no red meat (although poultry was allowed). The diet low in fiber, high in red meat, and coffee free (LF+RM−C) contained $\leq$10 g/day whole-grain fiber, $\geq$150 g/day red meat (beef), and no coffee or tea. Patients were instructed to maintain their medication and physical activity, which were assessed by weekly protocols as previously reported, and were asked to document any changes and to return the completed sheet to monitor compliance. Dietary components specific for each diet were purchased by the participants themselves but were clearly defined (including manufacturer and detailed product information) in the respective dietary sheet. This additional expenditure by the participants has been reimbursed. Moreover, participants received a financial compensation for their time spent in the study center during baseline/follow-up visits. The weekly visits also included monitoring of body weight, hip and waist circumference, blood pressure, and blood sampling, as previously reported (20). Since this trial was designed as a pilot study, a statistical power calculation was not performed (20). Post hoc statistical power calculation revealed that 132 (80% power), 176 (90% power), and 217 (95% power)

subjects would be needed in each group to detect a significant difference for the change in the low-frequency/high-frequency ratio from baseline to 8 weeks between the groups. The study population comprised 30 individuals (15 in each group), 2 of whom allocated to the HF−RM+C group dropped out during or immediately after the baseline assessments because of time constraints, while 2 subjects randomized to the LF+RM−C group had incomplete HRV data, leaving 26 volunteers (HF−RM+C group n = 13, LF+RM−C group n = 13) for statistical analysis.

Modified Botnia Clamp

Endogenous glucose production, glucose-induced insulin secretion, and insulin sensitivity were assessed from the modified intravenous glucose/hyperinsulinemic-euglycemic clamp test with (6,6-^{2}H)glucose as previously described and validated (21). M value was calculated to quantify whole-body insulin sensitivity.

Indirect Calorimetry

Indirect calorimetry was performed in the canopy mode (Vmax Encore 29n; CareFusion, Höchberg, Germany) during fasting and steady-state clamp conditions for 20 min followed by a postexposure recalibration procedure (22). VO_2 and VCO_2 were measured, and substrate oxidation rates were calculated as previously described (23). Nonoxidative glucose disposal was calculated as the difference between R_d and oxidative glucose utilization.

Laboratory Analyses

Serum triacylglycerols and cholesterol were analyzed using a Cobas c311 analyzer (Roche Diagnostics, Mannheim, Germany). Serum cytokines were assayed using the Quantikine HS (for interleukin [IL]-6) and Quantikine (for total adiponectin and IL-1 receptor antagonist [RA]) ELISA kits (R&D Systems, Wiesbaden, Germany), and an IL-18 ELISA kit (MBL, Nagoya, Japan) (24). Serum amino acids were measured by gas chromatography–mass spectrometry (GC-MS) using an EZ:faast kit (Phenomenex, Torrance, CA) with norvaline as an internal standard. Plasma coffee-derived compounds (caffeine and its metabolites, theophylline, theobromine, and paraxanthine, and the polyphenols caffeic acid and its metabolites, dihydrocaffeic acid, ferulic acid, dihydroferulic acid, isoferulic acid, dihydroisoferulic acid, dihydro-3-coumaric acid, 3-(3,4-dimethoxyphenyl)propionic acid, 3,4-dimethylcaffeic acid, and 3-coumaric acid) were determined using high-performance liquid chromatography and GC-MS (25). The two main urinary alkylresorcinol metabolites, 3,5-dihydroxybenzoic acid and 3-(3,5-dihydroxyphenyl)-propanoic acid, were analyzed using GC-MS, as previously reported (20).

HRV

R-R intervals were recorded during the clamp at baseline and after 8 weeks using a digital SpiderView Holter recorder with seven electrodes to record three-channel ECGs (Sorin Group, Munich, Germany). HRV was analyzed from the Holter monitor recordings using commercially available software (SyneScope version 3.00 analysis system; Sorin Group). The sampling rate of the ECG signal was 200 Hz (5-ms resolution). The system automatically edits all artifacts and ectopic beats, and obtains a regular signal by linear interpolation of the heart rate tachogram. Time-domain and frequency-domain parameters of HRV were computed according to guidelines of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (2). Time-domain parameters included normal-to-normal (NN) mean R-R interval, the root mean square of successive differences (RMSSD), the SD of NN averages over 5 min (SDANN), SD of 1-h histograms (SDNN), and the percentage difference between two consecutive NN intervals >50 ms (pNN50). Frequency-domain indices included the low-frequency band (0.04–0.15 Hz), the high-frequency band (0.15–0.4 Hz), and the low-frequency/high-frequency ratio.

Statistical Analysis

Continuous data were expressed as the mean ± SEM for normally distributed parameters or the median (IQR) in the case of skewed data distribution. Data showing skewed distribution were $\log_e$ transformed, and were analyzed using a two-sided Student t test for paired and independent samples. P values were adjusted for multiple comparisons using the Bonferroni correction. Pearson correlation coefficients were computed to analyze bivariate correlations. Multiple linear regression analyses with adjustments for sex, age, and BMI were performed to determine associations between two variables. The primary outcome for this pilot study was the change in whole-body insulin sensitivity to obtain effect size and variance estimates for both diets (20). The primary outcome measures were pNN50 (representing the time-domain HRV measures) and low-frequency/high-frequency ratio (representing the frequency-domain HRV measures). All analyses were performed using SPSS version 22 (IBM, Armonk, NY) and Prism version 6.04 (GraphPad, La Jolla, CA) statistical software. All tests were two sided, and the level of significance was set at α = 0.05.

RESULTS

At the screening visit, individuals in the HF−RM+C group were 53.5 ± 1.8 years old, 6 individuals were male, the mean waist circumference was 116 ± 3.3 cm, the mean diabetes duration was 2.4 ± 0.3 years, and 10 received metformin treatment. Participants in the LF+RM−C group were 53.7 ± 2.6 years old, 8 were male, the mean waist circumference was 114 ± 3.1 cm, the mean diabetes duration was 2.0 ± 0.5 years, and 11 received metformin treatment. No significant differences between the groups were noted for any of these variables. After 8 weeks compared with the screening visit, the mean weight loss was 6.17 ± 1.37 kg in the HF−RM+C group and 5.15 ± 0.86 kg in the LF+RM−C group (P = NS between groups). The mean values of anthropometric and clinical data at baseline and 8 weeks after the intervention are given in Table 1. After 8 weeks, weight, BMI, HbA_{1c}, total energy, and fat intake decreased, while protein and carbohydrate intake increased in both groups compared with baseline (all $P < 0.05$). As expected, cereal fiber and white meat intake, plasma caffeine, and urinary alkylresorcinol metabolites increased with the HF−RM+C diet and decreased with the LF+RM−C diet, while red meat intake dropped to zero with the HF−RM+C diet and increased with the LF+RM−C diet compared with baseline. Total cholesterol, branched chain amino acid, and IL-18 concentrations declined significantly with the HF−RM+C diet ($P < 0.05$) and remained unchanged with the LF+RM−C diet compared with baseline. Fasting glucose and insulin levels declined, while histidine

Table 1—Anthropometric and clinical data at baseline and 8 weeks after intervention

	HF−RM+C (n = 13)			LF+RM−C (n = 13)		
	Baseline	8 Weeks	Δ	Baseline	8 Weeks	Δ
Weight (kg)	107 ± 5.0	103 ± 4.5[c]	−4.17 ± 0.86	100 ± 3.7	96.0 ± 3.3[c]	−3.95 ± 0.75
BMI (kg/m^2)	35.4 ± 1.1	34.1 ± 0.9[c]	−1.36 ± 0.26	33.5 ± 1.0	32.2 ± 1.0[c]	−1.31 ± 0.24
Systolic BP (mmHg)	135 ± 3.6	134 ± 4.3	−1.54 ± 4.50	141 ± 4.6	135 ± 5.1	−5.33 ± 3.98
Diastolic BP (mmHg)	87.0 ± 2.6	87.2 ± 3.4	0.23 ± 3.96	87.7 ± 2.4	82.6 ± 2.6	−5.08 ± 2.46
Oxidative glucose utilization (mg/kg/min)	2.26 (1.02)	2.25 (1.17)	−0.25 (1.38)	2.54 (1.32)	2.51 (0.98)	−0.45 (1.64)
Lipid oxidation (mg/kg/min)	0.17 (0.31)	0.11 (0.32)	0.07 (0.59)	0.21 (0.46)	0.24 (0.41)	0.14 (0.76)
Total energy intake (kJ/day)	9,378 (2,786)#	8,239 (2,311)*[c]	−1,191 (600)	9,272 (2,977)#	7,844 (1,733)*[c]	−1,206 (778)
Protein intake (% energy)	18.5 (4.5)#	21.3 (1.7)*[a]	2.8 (4.4)	17.0 (3.5)#	20.8 (1.3)*[b]	2.8 (3.7)
Carbohydrate intake (% energy)	45.7 ± 1.8#	51.9 ± 0.3*[b]	6.23 ± 1.70	44.2 ± 1.8#	51.1 ± 0.7*[b]	6.88 ± 1.75
Fat intake (% energy)	36.5 (8.2)#	28.7 (1.2)*[b]	−7.9 (13.7)	38.0 (10.2)#	31.3 (1.2)*[c]	−7.00 (9.5)
Cereal fiber intake (g/day)	9.8 (4.5)#	34.9 (4.1)*[c]	26.1 (4.9)	8.4 (7.0)#	4.6 (1.1)*[c]	−2.8 (8.0)[c]
Red meat intake (g/day)	124 (64)#	0*		118 (98)#	208 (35)*[c]	77 (84)
White meat intake (g/day)	52.0 (39.5)#	126 (41.0)*[c]	73.2 (31.4)	21.0 (40.5)#	0*	
Plasma caffeine (μmol/L)	3.37 (2.21)	6.47 (4.30)[a]	1.87 (5.20)	1.99 (2.60)	0.46 (0.21)[c]	−1.44 (3.72)[c]
Urinary AR metabolites (μmol/24 h)	29.9 (35.8)	113 (106)[c]	63 (97)	41.6 (30.8)	15.0 (20.4)[c]	−14.9 (21.3)[c]
Triacylglycerols (mmol/L)	1.52 (0.32)	1.10 (0.79)	−0.19 (0.91)	1.58 (2.16)	1.61 (1.06)	−0.22 (0.83)
Total cholesterol (mmol/L)	5.13 ± 0.21	4.79 ± 0.21[a]	−0.35 ± 0.15	5.40 ± 0.23	5.19 ± 0.20	−0.20 ± 0.27
LDL cholesterol (mmol/L)	3.23 ± 0.20	3.03 ± 0.18	−0.20 ± 0.13	3.39 ± 0.24	3.40 ± 0.17	−0.05 ± 0.20
HDL cholesterol (mmol/L)	1.34 ± 0.10	1.25 ± 0.07	−0.09 ± 0.05	1.21 ± 0.08	1.18 ± 0.08	−0.04 ± 0.06
Fasting glucose (mmol/L)	5.88 (1.14)	5.25 (1.27)	−0.25 (0.89)	6.24 (1.79)	5.25 (1.41)[b]	−0.64 (1.66)
Fasting insulin (pmol/L)	94 (80)	83 (39)	−23.8 (59.1)	105 (59)	93 (44)[a]	−13.7 (29.8)
HbA_{1c} (%)	6.40 (0.55)	5.80 (1.05)[b]	−0.60 (0.85)	6.10 (1.15)	5.80 (0.95)[c]	−0.50 (0.67)
HbA_{1c} (mmol/mol)	46.4 (6.0)	39.9 (11.5)[b]	−6.56 (9.3)	43.2 (12.1)	39.9 (6.4)[c]	−5.46 (7.37)
BCAA (μmol/L)	508 ± 29	442 ± 16[a]	−66.2 ± 24.2	502 ± 46	464 ± 29	−38.3 ± 37.6
Histidine (μmol/L)	48.7 ± 4.8	46.9 ± 3.8	−1.83 ± 2.62	46.5 ± 2.8	60.3 ± 4.7[c]	13.8 ± 3.0[c]
IL-6 (pg/mL)	2.70 (1.66)	2.12 (1.64)	−0.20 (1.89)	2.39 (1.51)	2.81 (2,12)	0.26 (2.33)
IL-18 (pg/mL)	248 (104)	190 (100)[b]	−39 (105)	300 (201)	267 (180)	−33 (86)
Adiponectin (μg/mL)	4,854 (3,136)	5,252 (3,740)	598 (1,472)	3,526 (3,199)	3,376 (3,355)	56 (811)
IL-1RA (pg/mL)	717 (818)	742 (450)	−37 (397)	602 (419)	463 (516)	3.0 (565)

Data are expressed as the mean ± SEM or median (IQR). Data were compared using a two-tailed Student t test for paired (before and after intervention) and unpaired (comparisons between groups) samples. AR, alkylresorcinol; BCAA, branched chain amino acids; BP, blood pressure. [a]$P < 0.05$, [b]$P \leq 0.01$, and [c]$P \leq 0.001$ (note: P value footnotes for the "Baseline" and "8 Weeks" columns are for within-group differences, whereas P values footnotes for the "Δ" columns are for between-group differences). *Total energy intake during the intervention. #Total energy intake during the run-in period.

concentrations increased with the LF+RM−C diet ($P < 0.05$) and remained unchanged with the HF−RM+C diet after 8 weeks compared with baseline. No significant changes over baseline in either group during the study were observed for systolic and diastolic blood pressure, oxidative glucose utilization, lipid oxidation, and levels of triacylglycerols, LDL and HDL cholesterol, IL-6, adiponectin, and IL-1RA. When comparing the differences from baseline to 8 weeks between the groups, cereal fiber intake, plasma caffeine, and urinary alkylresorcinol metabolites increased ($P < 0.05$) with the HF−RM+C versus LF+RM−C diet, while histidine increased with the LF+RM−C versus the HF−RM+C diet ($P \leq 0.001$). No differences between the groups for the changes over baseline were noted for the remaining variables listed in Table 1.

Heart rate and HRV indices at baseline and 8 weeks after the intervention are shown in Table 2. The changes from baseline to 8 weeks did not differ significantly between the groups. After 8 weeks, heart rate decreased and SDANN increased intraindividually in both groups compared with baseline ($P < 0.05$), while pNN50 and high-frequency power increased and the low-frequency/high-frequency ratio declined with the HF−RM+C diet only ($P < 0.05$). After Bonferroni correction, only the low-frequency/high-frequency ratio remained significant ($P = 0.012$), and pNN50 was at the significance limit ($P = 0.05$) after the HF−RM+C diet. No other changes in HRV measures from baseline to 8 weeks were observed.

In the entire study population, the changes in BMI from baseline to 8 weeks correlated positively with the changes in HbA_{1c} ($\beta = 0.535$, $P = 0.004$) and inversely with the changes in M value ($\beta = -0.457$, $P = 0.014$). Table 3 shows the associations between the changes in HRV indices, lipid oxidation, and oxidative glucose utilization from baseline to 8 weeks in the entire population studied. After adjustment for sex, age, and ΔBMI, the changes in SDNN, pNN50, RMSSD, low-frequency power, and high-frequency power were inversely associated with the changes in lipid oxidation, and were positively associated with oxidative glucose utilization after 8 weeks

Table 2—HRV indices at baseline and 8 weeks after intervention

	HF−RM+C (n = 13)			LF+RM−C (n = 13)		
HRV indices	Baseline	8 Weeks	Δ	Baseline	8 Weeks	Δ
Heart rate (bpm)	65.7 ± 2.7	61.9 ± 2.6[b]	−3.81 ± 1.11	71.9 ± 2.1	66.1 ± 2.3[b]	−5.76 ± 1.57
pNN50 (%)	6.2 (14.0)	15.1 (12.6)[c]	3.2 (9.8)	2.34 (6.8)	9.5 (28.4)	8.6 (14.9)
RMSSD (ms)	29.9 (16.3)	40.5 (11.1)	8.1 (17.7)	23.4 (25.4)	34.4 (39.4)	16.9 (19.6)
SDANN (ms)	51.7 ± 3.3	71.4 ± 9.4[(a)]	19.6 ± 8.7	41.2 ± 4.0	55.2 ± 4.3[a(a)]	14.0 ± 5.3
SDNN (ms)	57.0 (21.6)	68.0 (29.5)	2.4 (17.5)	45.1 (29.5)	55.6 (38.3)	12.0 (13.1)
Total power (ms^2)	3,265 (2,413)	4,253 (3,805)	592 (2,370)	1,863 (2,302)	2,587 (3,577)	1,125 (1,235)
Low-frequency power (ms^2)	739 (493)	924 (554)	154 (730)	464 (549)	681 (843)	175 (395)
High-frequency power (ms^2)	221 (151)	311 (400)[a(a)]	79 (437)	129 (243)	269 (399)	136 (298)
Low-frequency/high-frequency ratio	3.46 (1.60)	2.35 (2.08)[b]	−1.14 (1.80)	3.23 (3.30)	2.77 (2.09)	−0.40 (2.85)

Data are expressed as the mean ± SEM or median (IQR). Data were compared using a two-tailed Student t test for paired (before and after intervention) and unpaired (comparisons between groups) samples. [a]$P < 0.05$, [b]$P \leq 0.01$, [(a)]statistically not significant after Bonferroni correction, and [c]$P = 0.05$ after Bonferroni correction.

compared with baseline (all $P < 0.05$). Figure 1 illustrates the correlations of the changes in SDNN, RMSSD, low-frequency power, and high-frequency power with changes in lipid oxidation and oxidative glucose utilization from baseline to 8 weeks in the entire group studied. No correlations were observed between the changes in HRV indices from baseline to 8 weeks and any other anthropometric and metabolic parameters and biomarkers of subclinical inflammation (data not shown).

CONCLUSIONS

This randomized, controlled pilot study conducted over 8 weeks in obese individuals with type 2 diabetes demonstrates that two low-energy diets differing in fiber, red meat, and coffee intake resulted in comparable weight loss and decline in heart rate. However, the changes in HRV during the trial were not different between both low-energy diets. After 8 weeks, increased vagal activity was not associated with the changes in insulin sensitivity and subclinical inflammation but was associated with enhanced oxidative glucose utilization and diminished fat oxidation.

Whether distinct energy-restricted diets may differentially influence cardiac ANS function has not been previously assessed in obese patients with diabetes. Various dietary modifications have been shown to augment vagal activity without inducing weight loss. In normotensive premenopausal women with normal body weight, HRV increased after a low-fat diet compared with a high-fat diet after 2 weeks, while weight remained unchanged (26). After an Atlantic salmon diet three times per week provided to men over 5 months, vagal activity was increased compared with a control diet with an alternative meal (e.g., chicken, pork, beef) without fish (27). In contrast, in individuals with coronary heart disease, diets rich in fatty or lean (white) fish did not result in changes in HRV after 8 weeks (28). In individuals with type 2 diabetes, a moderate-fat diet (33% fat) containing pistachios (20% of total energy) for 4 weeks improved some HRV measures compared with a control diet that was low in fat (27% fat) and high in carbohydrates (29).

The effect of a single energy-restricted diet (6–7 MJ/day) has been studied by Sjoberg et al. (14) in an uncontrolled 16-week study including overweight and obese patients with type 2 diabetes. These authors reported a decline in heart rate and increases in SDNN, low-frequency power, and total power, which correlated with the changes in BMI, whereas high-frequency power and low-frequency/high-frequency ratio did not change significantly during the dietary intervention. In contrast, we did not observe any relationship between the improvement in HRV and weight loss. The reasons for this discrepancy may be that, in the previous study, the reduction in body weight was 11 kg after 16 weeks versus only 5.7 kg after 8 weeks in our study. It is conceivable that a longer study duration with more pronounced weight loss is required to demonstrate such an association. Moreover, in contrast to Sjoberg et al. (14), we show an intraindividual decline in low-frequency/high-frequency ratio, indicating an improvement in sympathovagal balance toward diminished sympathetic activity in participants on the HF−RM+C diet, but since those on the LF+RM−C diet also showed a nonsignificant numerical decline after 8 weeks, the changes in low-frequency/high-frequency ratio during the study did not differ between the groups. Although the concept of sympathovagal balance remains a matter of ongoing discussion (30), a shift of cardiac ANS activity toward sympathetic predominance estimated by an increased

Table 3—Associations between the changes from baseline to 8 weeks in indices of HRV and lipid oxidation and oxidative glucose utilization in the entire group studied

	ΔLipid oxidation		ΔOxidative glucose utilization	
HRV indices	β	P value	β	P value
ΔSDNN	−0.538	0.013	0.477	0.030
ΔpNN50	−0.518	0.012	0.445	0.036
ΔRMSSD	−0.499	0.018	0.437	0.042
Δlow-frequency power	−0.595	0.006	0.582	0.008
Δhigh-frequency power	−0.507	0.022	0.455	0.044

All parameters are adjusted for sex, age, and ΔBMI.

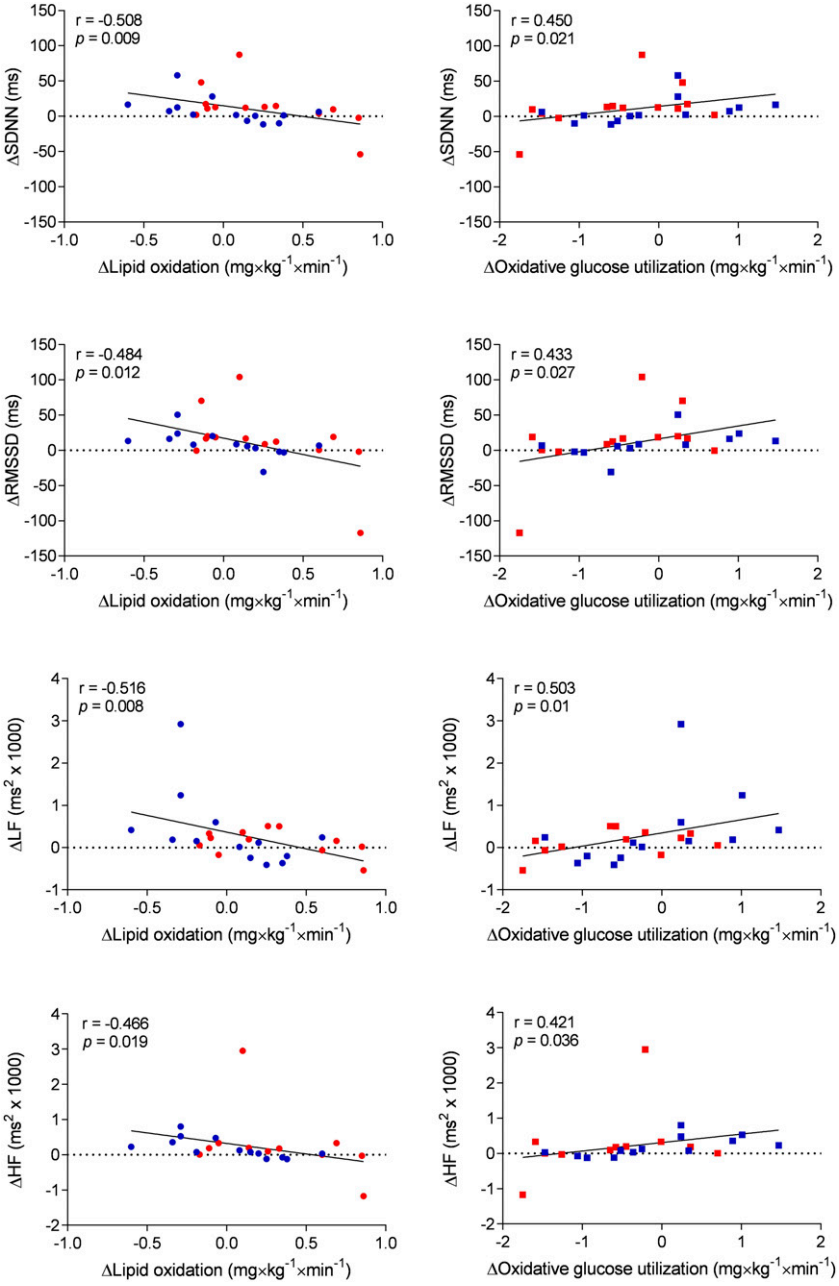

Figure 1—Pearson correlation coefficients of the changes in HRV indices with changes in lipid oxidation and oxidative glucose utilization after 8 weeks vs. baseline in the entire group studied. Red LF+RM−C, blue HF−RM+C. HF, high frequency; LF, low frequency.

low-frequency/high-frequency ratio has been linked to the amount of visceral fat (31), increased free fatty acid (FFA) concentrations (32), insulin resistance (33), and several components of the metabolic syndrome (3). However, whether an HF−RM+C diet could exert favorable effects on cardiovascular risk via restoring cardiac sympathovagal balance remains open. Moreover, our results may only apply to the well-controlled individuals with short-term type 2 diabetes that were studied herein, whereas in poorly controlled patients who have chronic diabetes, diminished HRV may be less susceptible to any intervention.

Another novel finding is the correlation of increasing vagal activity with rising oxidative glucose utilization and diminishing fat oxidation after weight loss over 8 weeks. The ANS plays an important role in controlling the selection of fuel by the heart, since the rate of myocardial glucose oxidation is markedly impaired in the chronically denervated heart (34), which likely is due to a decline in myocardial pyruvate dehydrogenase (35). It has been suggested that the decline in myocardial glucose oxidation is compensated for by an enhanced contribution of fatty acid oxidation to overall oxidative energy conversion (36). Our findings are compatible with this pattern and with recent data indicating that experimental vagal stimulation reduces FFA oxidation during mild β-adrenergic stress (37). A similar pattern was described in diabetic cardiomyopathy, which is characterized by structural and functional alterations in the diabetic heart muscle that are not directly attributable to coronary artery disease or hypertension and ultimately lead to heart failure (38). Metabolic disturbances in diabetic cardiomyopathy are characterized by increased myocardial lipid oxidation, intramyocardial triglyceride accumulation, and reduced glucose utilization (38). The heart has the capacity to adapt to various pathophysiological conditions by adjusting its relative metabolism of carbohydrates and FFA. The diabetic heart relies almost exclusively on mitochondrial fatty acid β-oxidation as the sole fuel source. Reduced myocardial glucose uptake and utilization due to altered insulin signaling may account for the loss of this metabolic flexibility, which is associated with pathological cardiac hypertrophy and heart failure. Despite the higher FFA oxidation rate that occurs in the diabetic heart, myocardial lipid accumulation is one of the hallmarks of diabetic cardiomyopathy (38).

The parasympathetic nervous system controls innate immune responses and inflammation during pathogen invasion and tissue injury. The physiological mechanism through which the parasympathetic nervous system regulates immune function and inhibits excessive proinflammatory cytokine production has been termed the “inflammatory reflex” (4). It is well established that weight reduction lowers the levels of several systemic inflammatory factors, and, apart from weight reduction, high dietary fiber intake was associated with decreases in inflammatory markers such as IL-6 (39). However, we observed only a decline in the proinflammatory cytokine IL-18 after the HF−RM+C diet, but no change in IL-6, IL-1RA, and adiponectin. It is possible that the moderate extent of weight loss or the relatively small sample size

precluded the detection of subtle changes in systemic inflammation.

The strengths of the current study are its controlled design and the detailed phenotyping using metabolic, immunologic, and functional measures, but there are also some limitations. First, due to the relatively small sample size, this pilot study was not adequately powered to detect differences in HRV changes between the groups. Post hoc statistical power calculation revealed that 132 subjects would be needed in each group to achieve 80% power to detect a significant difference for the change in the low-frequency/high-frequency ratio from baseline to 8 weeks between the groups. Second, the study duration was relatively short, and the degree of weight loss was moderate. Third, the possible effects of three dietary components modified in each of the two diets cannot be distinguished.

In conclusion, this 8-week randomized, controlled pilot study in obese individuals with type 2 diabetes showed that two low-energy diets differing in fiber, red meat, and coffee intake resulted in similar weight loss, declines in heart rate, and changes in HRV from baseline to 8 weeks. Improvements in vagal activity after 8 weeks were associated with enhanced oxidative glucose utilization and diminished fat oxidation, but not with changes in insulin sensitivity and subclinical inflammation. Large-scale controlled studies are now required to confirm these findings and to determine whether favorable modulation of autonomic tone toward reducing sympathetic drive can be translated into a reduction of cardiovascular end points in people with diabetes.

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