Hypocretin deficiency : neuronal loss and functional consequences

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Citation

Fronczek, R. (2008, January 30). *Hypocretin deficiency : neuronal loss and functional consequences*. Retrieved from https://hdl.handle.net/1887/12580

Increased Heart Rate Variability but Normal Resting Metabolic Rate in Hypocretin/ Orexin-deficient Human Narcolepsy

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Objective | We investigated the possible role of abnormalities in autonomic balance and resting metabolic rate to explain obesity in hypocretin/orexin-deficient narcoleptic subjects.

Methods | Resting metabolic rate (RMR) and variability in heart rate and blood pressure were determined in the fasted, resting state. Subjects were 15 untreated, hypocretin-deficient male narcoleptics and 15 male controls matched for age and body mass index.

Results | Spectral power analysis revealed greater heart rate and blood pressure variability in hypocretin-deficient male narcoleptic patients (heart rate: p=0.01; blood pressure systolic: p=0.02; diastolic: p<0.01). The LF/HF ratio was normal (p=0.48). Resting metabolic rate did not differ between hypocretin-deficient male patients and controls (controls=1767 ± 226 kcal/24h, patients: 1766 ± 227 kcal/24h, p=0.99).

Conclusion | Using indirect calorimetry we did not find a reduced resting metabolic rate in hypocretin-deficient narcoleptic men. However, heart rate and blood pressure variability was increased, which may point to a reduced sympathetic tone. The role of this latter finding in the pathophysiology of obesity in narcolepsy remains to be elucidated.

Introduction

Narcolepsy is a sleep disorder that affects 20-60 per 100,000 in western countries. The syndrome is classically characterized by the tetrad of excessive daytime sleepiness, cataplexy, sleep paralysis and hypnagogic hallucinations.[1] The first report of obesity as a metabolic feature of narcoleptic patients dates back as early as the 1930s[2, 3] and the observation was confirmed repeatedly since.[4-11] The identification of hypocretin/orexin deficiency as the cause of human narcolepsy with cataplexy and the potential role of hypocretin peptides in metabolic control has sparked interest in the pathophysiology of the obesity accompanying narcolepsy. Indeed, it not only is a consistent feature of human narcolepsy, but also in hypocretin-deficient animal models.[12, 13] Furthermore, patients suffering from idiopathic hypersomnia who are also suffering from excessive daytime sleepiness, but are not hypocretin-deficient, are not obese.[11]

Hypocretin peptides are involved in the control of autonomic nervous system activity, food intake and energy balance.[14-16] In particular, injection of hypocretins into the lateral cerebral ventricle stimulates food intake.[15] Accordingly, ablation of hypocretin neurons leads to hypophagia in mice[13] and narcoleptic humans eat less than age and sex matched controls.[17] Paradoxically, both in mice and men this is accompanied by increased body weight. To reconcile these apparently contradictory corollaries of hypocretin deficiency, it may be necessary to consider the effects of hypocretin peptides on wakefulness and sympathetic activity. In rats, injection of hypocretins into the lateral ventricle also stimulates arousal and activates the sympathetic nervous system to increase arterial blood pressure, heart rate, oxygen consumption, body temperature and plasma catecholamine levels.[18-21] Thus, hypocretin deficiency and daytime sleepiness may reduce physical activity, which could diminish energy expenditure. Also, hypocretin deficiency might directly reduce sympathetic tone and resting metabolic rate, and thereby induce obesity. Moreover, adipose tissue is under neuronal control and is innervated by both sympathetic (catabolic) and parasympathetic (anabolic) pathways[22] and autonomic imbalance could thus lead to fat accumulation.[23]

We studied resting metabolic rate and variation in blood pressure and heart rate in hypocretin-deficient narcoleptic subjects and healthy controls. We hypothesized that sympathetic tone might be diminished and that resting metabolic rate would be reduced in narcoleptic subjects.

Materials and Methods

Subjects

The study was approved by the local medical ethical committee. All narcoleptic patients were male and fulfilled the ICSD-2 criteria of narcolepsy with cataplexy.[24] They did not take any medication and hypocretin/orexin was undetectable in their cerebrospinal fluid, as measured by a standard radioimmuno-assay (Phoenix Pharmaceuticals, Inc., Belmont, CA). Healthy male controls were recruited using an advertisement in a local newspaper. Groups were matched for age and body mass index (BMI). As BMI is a very strong confounder of metabolic rate in itself, BMI-matching is mandatory. The pathogenesis of obesity is a multifactorial (increased caloric intake, sedentary lifestyle and predisposing genetic make-up). However, narcoleptics do not eat more or move less than healthy individuals, which led us to hypothesize that a lowered metabolic rate is the sole causative factor in narcolepsy. As said above, BMI is a strong determinant of metabolic rate by itself. In other words, our hypothesis was that narcoleptic subjects have a metabolic rate that is too low for a given BMI. Therefore, we matched the control group for BMI.

Metabolic Measurements

In fifteen patients and fifteen controls metabolic values were measured. Subjects were instructed to fast and drink only water from 22.00 hrs the night before until the metabolic measurement was performed. Subjects arrived at the hospital at 09.00 hrs and

lied down in a supine position for a 30-minute period. Special care was taken to keep subjects awake during this period by talking to them. RMR was measured by indirect calorimetry[25] using a computerized open-circuit ventilated hood system (Oxycon B; Jaeger, Breda, The Netherlands).[26] Because subjects have to get used to the recording circumstances, the first 10 minutes of the test period were discarded.

Oxygen consumption (VO_2 L/min) and carbon dioxide production (VCO_2 L/min) were used to calculate the respiratory quotient (RQ=VCO2/VO2). Resting metabolic rate and carbohydrate (C) and fat (F) combustion were calculated using the Weir formula and were expressed as kilocalories per 24h (per kilogram body weight) and grams per minute (per kilogram body weight) respectively.[27, 28] The following formulas were used:

$$RMR\ (kcal/24h) = (3.9\ x\ VO2) + (1.1\ x\ VCO2)\ x\ 1.44$$

$$C\ (g/min) = (4.55\ x\ VCO2) - (3.21\ x\ VO2)$$

$$F\ (g/min) = (1.67\ x\ VO2) - (1.67\ x\ VCO2)$$

Autonomic Measurements

Since measurement of autonomic function was added in a later stage, autonomic activity was measured in nine patients and nine controls, a subpopulation of the subjects included for the metabolic measurements. This was done simultaneously with the metabolic measurements. Heart rate was determined by ECG, measured continuously using standard Ag-AgCl electrodes. Beat-to-beat arterial blood pressure was noninvasively monitored (Finometer, TNO-Biomedical Instruments, The Netherlands). The hand used for these finger blood pressure measurements was held in a constant position at heart level. The complete last 20 minutes of the actual testing period were used to calculate heart rate and blood pressure.

Blood pressure and heart rate calculations were performed using software written in MatLab (MatLab v7.0, Mathworks, Massachusetts). Heart Rate Variability (HRV) was estimated by calculating the mean and SD of consecutive R-R intervals and with spectral analysis performed by interpolating the series of RR intervals by cubic splines, resampling the signal at 3 Hz and performing a Fast Fourier Transformation (FFT) using a Hamming-window.[29] Power was calculated for the following bands: Very Low Frequency (VLF, 0-0.04 Hz), Low Frequency (LF, 0.04-0.15 Hz), and High Frequency (HF, 0.15-0.4 Hz). Total power was calculated by adding the powers of the VLF, LF and HF bands. Increases in total power can be caused by a reduction in sympathetic tone.[30, 31] Furthermore, the LF/HF ratio was calculated. The LF band is usually considered to represent the sympathetic part of the baroreceptor reflex, while the HF band, largely derived from respiratory influences, mostly concerns parasympathetic activity. The

LF/HF ratio is generally used as another measure for the autonomic parasympathetic-sympathetic balance.[32] However, some authors regard it as an indication of sympathetic activity.[33] Mean systolic (SBP) and diastolic (DBP) blood pressures were calculated and the total power in their frequency spectrum (calculated similarly to the HRV spectrum) was taken as an estimate of variability. Note that finger blood pressure measurement using the aforementioned finometer has a tendency to underestimate blood pressure. This can be prevented by calibrating the device using using the Riva-Rocci method.[34] However, blood pressure values were not corrected in this study, since groups were compared.

Statistics

Differences between groups were calculated using Student's t-test for unpaired samples. Pearson's correlation coefficient was used to evaluate potential correlations. P-values below 0.05 were considered significant.

Results

Resting Metabolic Rate

Data are shown in Table 8.1. Patients and controls did not differ for age or BMI (Figure 8.1a). There were no significant differences in resting metabolic rate (RMR), oxygen consumption (VO2), carbodioxide consumption (VCO2), respiratory quotient (RQ) and carbohydrate or fat substrate combustion between narcoleptic patients and controls. Since the groups were matched for BMI, correcting RMR and carbohydrate or fat combustion for bodyweight in kilograms did not influence the results (Table 8.1).

Autonomic data

Data are shown in Table 8.2. Patients and controls had similar age and BMI. There were no significant differences in mean heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP). However, heart rate variability (HRV) and blood pressure variability differed between the two groups: the total power in the spectrum of both the diastolic and systolic blood pressure was significantly higher in patients compared to controls (systolic: $p < 0.02$, diastolic: $p < 0.001$; Figure 8.1c and 8.1d). A respiratory high frequency (HF) peak was seen in the HRV spectra of all subjects (Figure 8.2). Total power ($p < 0.01$), very low frequency (VLF) power ($p < 0.03$) and low frequency (LF) power ($p < 0.02$) were higher in hypocretin-deficient patients compared to controls, while high frequency (HF) power tended to be higher as well ($p = 0.05$, Figure 8.2). In contrast, the ratio between low frequency and high frequency power (LF/HF ratio) did not differ between patients and controls ($p = 0.48$).

Table 8.1 Metabolic Measures

	Controls (N=15)	Narcolepsy (N=15)	P-value
male/ female	15/0	15/0	
Age (years)	36.3 ± 13.8	35.6 ± 13.8	0.89
BMI (kg/m^2)	26.0 ± 2.8	26.8 ± 2.3	0.42
VO2 (ml/min)	250.9 ± 34.2	252.4 ± 33.4	0.91
VCO2 (ml/min)	225.9 ± 24.6	220.3 ± 31.0	0.58
RQ	0.88 ± 0.06	0.86 ± 0.07	0.45
RMR (kcal/24h)	1767.1 ± 226.5	1766.5 ± 226.5	0.99
RMR / kg	19.9 ± 2.0	20.1 ± 2.2	0.77
C (Carbohydrate) (g/min)	222.5 ± 56.9	191.9 ± 95.0	0.29
C / kg	2.5 ± 0.6	2.1 ± 1.0	0.34
C in % of RMR	72.5 ± 18.5	62.6 ± 31.0	0.29
F (Fat) (g/min)	41.7 ± 29.5	53.7 ± 38.9	0.35
F / kg	0.5 ± 0.3	0.6 ± 0.5	0.35
F in % of RMR	30.6 ± 21.6	39.4 ± 28.5	0.35

Values in the table are means ± standard deviation. T-tests were used to assess group differences; no significant differences were found. BMI, body mass index; kcal, kilocalories; 24h, per 24 hours; RMR, resting metabolic rate; kg, kilograms; RQ, respiratory quotient; VO2, oxygen consumption; VCO2, carbon dioxide consumption; g, grams; min, minute.

Discussion

The pathogenesis of obesity in narcoleptic patients remains unexplained. Obviously, eating more or moving less are potential explanations. Hypocretin neuron-ablated narcoleptic mice[12] and human patients[17] were shown to eat less than normal controls (total daily food intake, narcoleptic humans: 8,756 ± 2,312 kilojoules; controls: 10,640 ± 3,129 kJ; p<0.001, data from Lammers et al.),[17] which is in accordance with the orexigenic qualities of hypocretin peptides. Actigraphy studies showed that although periods of activity and inactivity were more scattered in narcoleptic subjects versus controls, the total intensity of physical activity did not differ.[35, 36] Furthermore, narcoleptic subjects are more obese than equally active subjects suffering from idiopathic hypersomnia.[11] Thus, hypocretin deficiency must have other metabolic consequences to explain why narcoleptic animals and humans are obese. Since hypocretin peptides were shown to activate the sympathetic nervous system and increase oxygen consumption in rat,[18-21] we hypothesized that hypocretin deficiency would lead to reduction of sympathetic tone and resting metabolic rate in patients with narcolepsy.

In the present study, spectral power analysis of heart rate and blood pressure variability revealed an increase in the power across all frequency domains in narcoleptic patients, but no differences in the LF/HF ratio for heart rate. These results could point to a reduced sympathetic tone in narcoleptic patients. The HF peak is effected almost

Table 8.2 Autonomic Measures

	Controls (N=9)	Narcolepsy (N=9)	P-value
male/ female	9/0	9/0	
Age (years)	29.2 ± 4.1)	32.6 ± 16.2	0.55
BMI (kg/m2)	24.9 ± 2.6)	26.2 ± 2.1	0.31
Total Power in HRV spectrum x $10^4 s^2$/Hz	27.8 ± 14.1	77.4 ± 52.0	0.01*
- VLF (0-0.04 Hz) x 104 s2/Hz	12.6 ± 8.2	30.7 ± 22.0	0.03*
- LF (0.04-0.15 Hz) x 104 s2/Hz	9.7 ± 5.5	30.6 ± 24.2	0.02*
- HF (0.15-0.4 Hz) x 104 s2/Hz	5.6 ± 2.9	16.2 ± 14.9	0.05
LF/HF Ratio	1.9 ± 1.1	2.3 ± 1.2	0.48
Mean Heart Rate (BPM)	59.6 ± 8.8	56.7 ± 5.4	0.42
Mean Diastolic Blood Pressure (DBP, mmHg)	55.8 ± 9.0	52.8 ± 6.9	0.44
Mean Systolic Blood Pressure (SBP, mmHg)	104.0 ± 13.7	99.5 ± 17.6	0.55
Total Power in SBP spectrum x 10^4 mmHg^2/Hz	27.5 ± 11.1	47.7 ± 19.7	0.02*
Total Power in DBP spectrum x 10^4 mmHg^2/Hz	6.8 ± 3.5	16.7 ± 7.9	<0.00*

Values in the table are means ± standard deviation. T-tests were used to assess group differences. yrs, years; BMI, body mass index; HRV, heart rate variability; s, seconds; Hz, hertz, VLF, very low frequency; LF, low frequency; HF, high frequency; BMP, beats per minute.

exclusively by the parasympathetic system, so an alteration in sympathetic tone may be expected to leave it unchanged. Somewhat unexpectedly, however, an increase in power in both the LF and the HF band can result from an exclusive reduction of sympathetic tone, as proven by selective sympathetic blockade studies.[30, 31] A possible explanation could be that diminished sympathetic control may lead to higher fluctuations in blood pressure, induced by respiratory or other influences that in turn cause parasympathetic heart rate responses. The lack of differences in the LF/HF ratio in our study may in part be due to the fact that sympathetic tone contributes to both the LF and HF peaks, affecting both elements of the ratio. Sympathetic tone is already low in the supine position, so any further decreases are not likely to affect the ratio under these circumstances.[30, 31] This hampers straightforward interpretation of this ratio.[33] The only finding that is not readily compatible with decreased sympathetic tone is that mean heart rate was not lower in the narcolepsy group.[30, 31]

Surprisingly, although sympathetic activity drives resting energy expenditure, at least in rodents, resting metabolic rate was similar in narcoleptic patients and controls. Accordingly, hypocretin knockout mice have normal RMRs (C.M. Sinton, personal communication). However, large cohorts of patients and controls are needed to detect small differences in energy expenditure by indirect calorimetry, and even subtle reductions of RMR may lead to body weight gain in the long term.[37]

What are the potential explanations for the increased variability in blood pressure and heart rate in our narcoleptic patients? Firstly, narcoleptic subjects may not have been as awake as the control subjects, although special care was taken to keep patients alert
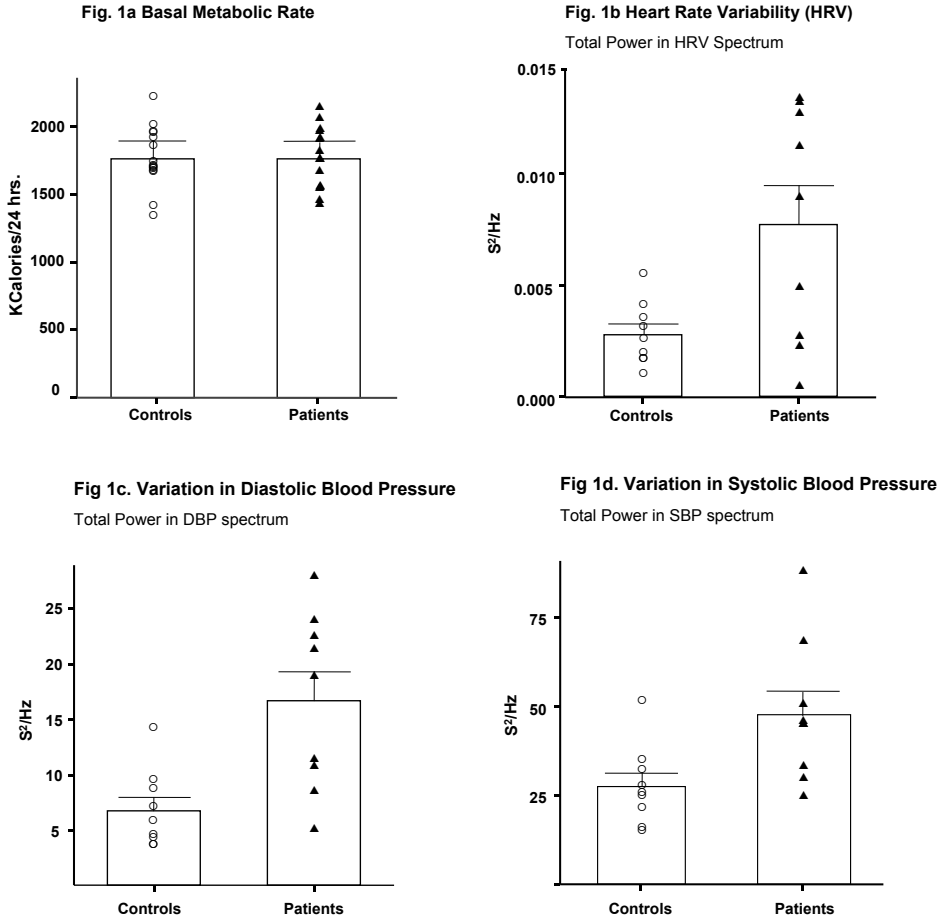
Fig. 1a Basal Metabolic Rate

Fig. 1b Heart Rate Variability (HRV)

Total Power in HRV Spectrum

Fig 1c. Variation in Diastolic Blood Pressure

Total Power in DBP spectrum

Fig 1d. Variation in Systolic Blood Pressure

Total Power in SBP spectrum

Figure 8.1 | Results

Resting metabolic rate (a), heart rate variation (b), variation in systolic blood pressure (c) and variation in diastolic blood pressure (d) estimated by total power in the SBP and DBP frequency spectrum in narcoleptic patients and controls. Bars represent means, error bars indicate standard deviation, triangles represent patients and circles represent controls.

during the measurements. Narcolepsy is commonly seen as a loss of state boundary control, which means that patients are unable to remain awake steadily.[38] A tendency to drift into drowsiness could lead to a higher variability in autonomic parameters, as autonomic control differs in the various sleep stages. The transition between wakefulness and sleep affects the power in both the HF and the LF band.[39] A tendency to shift from waking to drowsiness frequently might therefore show up as increased HRV. None of the subjects was visibly asleep during any test, but we cannot exclude a contribution of drowsiness to the higher variability in blood pressure and heart rate in our narcoleptic patients. Drowsiness would not only affect autonomic parameters, but might also have lead to an underestimation of the RMR in narcoleptic subjects, since RMR is lower during sleep.[40] However, this would mean that the actual RMR in narcoleptic subjects
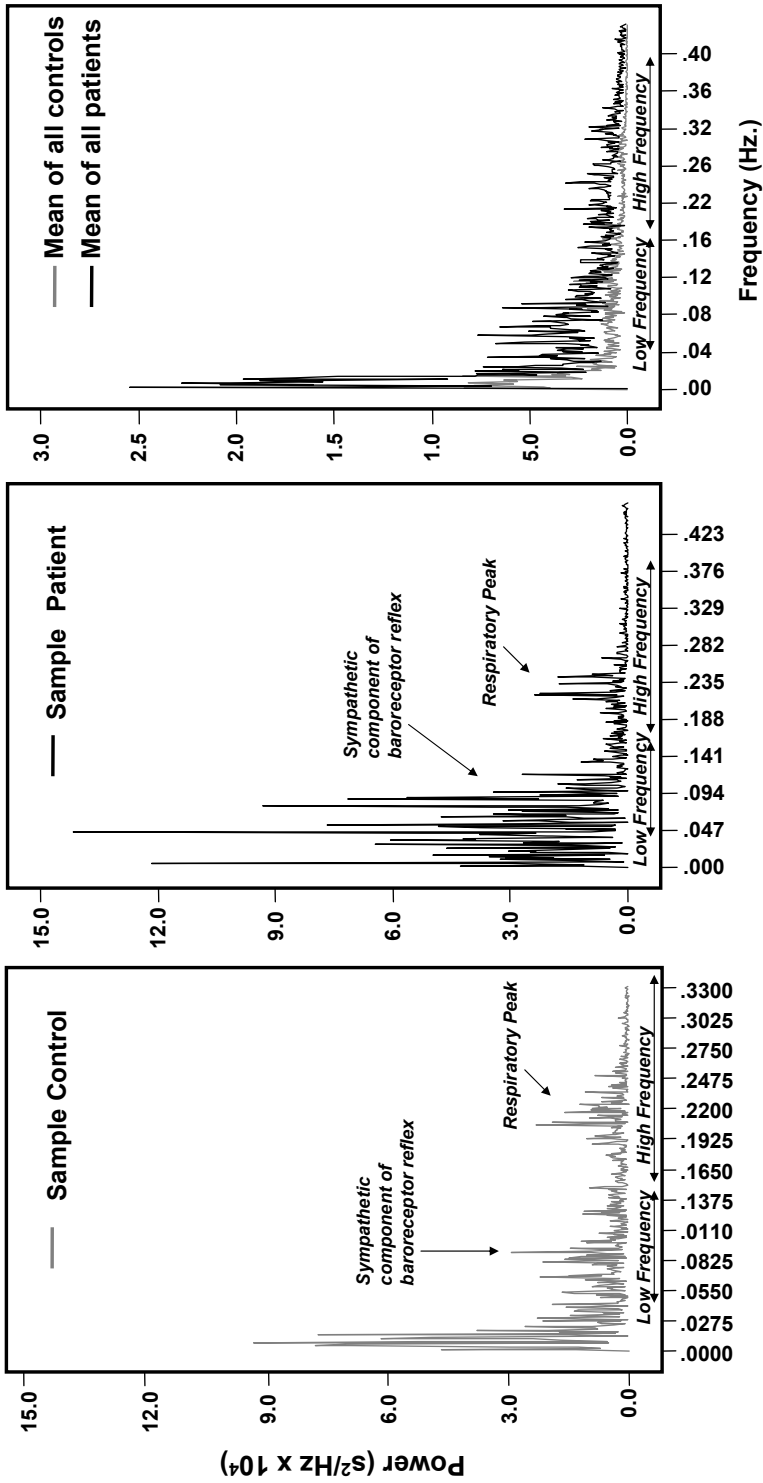
Figure 8.2 | Power Spectra
Example of typical power spectrum including respiratory peak, sympathetic component of the baroreceptor reflex and LF/HF regions for a control (left) and a narcoleptic patient (middle), and mean spectrum for both groups (right).

is higher, which does not explain their obesity. We suggest that further studies should take drowsiness into account. Interestingly, when looking at individual data points (Figure 8.1), there is a large variation in heart rate and blood pressure variability in narcoleptic patients. This was not correlated with BMI. The higher variation might explain or perhaps be explained by differences in phenotype, sleepiness or disease severity.

Alternatively, hypocretin deficiency may directly inhibit sympathetic activity. Various studies have shown that the hypocretin system is heavily involved in autonomic control and that hypocretins stimulate sympathetic activity.[41] Indeed, orexin neuron-ablated mice, which grow obese, have lower sympathetic vasoconstrictor outflow.[42] There is direct innervation of adipose tissue by sympathetic (catabolic) and parasympathetic (anabolic) pathways,[22] implying that a low sympathetic tone can directly promote fat accrual.[23]

Other authors who have looked at autonomic nervous function in narcoleptic patients found no abnormalities during provocations[43] and no primary disturbance between 6 and 8 PM or during sleep.[44] An increased LF/HF ratio compared with controls was found just before sleep onset, but this was thought to be related to the impairment of the sleep-wake cycle in narcolepsy and not to a primary disturbance.[44]

In conclusion, we did not find abnormalities in resting metabolic rate in narcoleptic humans when measured by indirect calorimetry. However, there are signs of reduced sympathetic activity, which may lead to fat accrual through direct effects on adipocytes. Future studies should directly measure sympathetic tone, for example using microneurography.

Acknowledgements

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