ORIGINAL RESEARCH

Investigation of insulin resistance in narcoleptic patients: dependent or independent of body mass index?

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Background: Narcolepsy is a severe sleep-wake cycle disorder resulting in most cases from a lack of orexin, the energy balance-regulating hormone. Narcoleptic patients have been reported to suffer from an excess morbidity of Type 2 diabetes, even after correction for their often elevated body mass index.

Methods: To explore whether narcolepsy is specifically associated with a propensity to develop insulin resistance, we measured fasting glucose, insulin, and intact proinsulin levels in 43 narcoleptic patients and 47 controls matched for body mass index and age. The proinsulin-to-insulin ratio was calculated. Insulin resistance was determined using the homeostatic model assessment method.

Results: Narcoleptic patients did not show elevated insulin resistance parameters.

Conclusion: In contrast with earlier reports, we found no evidence that narcolepsy specifically elevates the risk of insulin resistance (and consequently of type 2 diabetes) independently of body mass index.

Keywords: fasting glucose, insulin, intact proinsulin, narcolepsy, obesity

Introduction

Narcolepsy is a rare disease that affects approximately 0.05% of the population in Western countries. It is typically characterized by excessive daytime sleepiness, cataplexy, sleep onset rapid eye movement sleep periods, and the presence of the human leukocyte antigen (HLA) DQB1*0602 allele.[1] The association between the DQA1*0102/DQB1*0602 alleles and narcolepsy is among the strongest HLA associations in medicine, and suggests an autoimmune pathogenesis of the disorder.[2] There is evidence that the hypocretin/orexin neurotransmission system is involved in the pathophysiology of narcolepsy. Orexin, a neurotransmitter which is produced by a restricted set of cells located in the hypothalamus, is not only involved in the regulation of sleep but also plays a role in energy expenditure and body weight regulation. It promotes feeding behavior. Food intake leads to decreased activity of orexinergic neurons and lower orexin levels, and fasting leads to an increased orexin level.[3–8] The role of orexin suggests that hypocretin deficiency in narcoleptic patients should lead to reduced food intake and loss of body weight.[5,6] Surprisingly, it has been found that narcoleptic patients have higher body mass indices than community-based samples.[9–11] Moreover, Honda et al reported a prevalence of type 2 diabetes mellitus in six of 48 narcoleptic patients (12.5%). They concluded that the tendency towards

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diabetes might be independent of body mass index (BMI) because there were no differences in the prevalence between the obese and nonobese narcoleptic patients. Ever since then, it is commonly understood that narcoleptic patients have an increased risk of developing type 2 diabetes.[11]

Recently a research group from Italy conducted a very detailed study on metabolic alterations in narcoleptic patients, including measurement of BMI, waist circumference, blood pressure, daily calorie intake (three-day diary), and biochemical and hormonal parameters (cholesterol, insulin, and leptin, among others).[12] They found that nine of 14 patients displayed the metabolic syndrome compared with 14 age- and gender-matched patients with idiopathic hypersomnia, of whom none were affected. Although the study was carefully performed, there were several limitations, including a small sample size and especially the fact that both groups were not BMI-matched (patients with narcolepsy 28 ± 4.4, patients with idiopathic hypersomnia 24.2 ± 2.8, $P = 0.012$), because BMI is one of the greatest influencing factors of the metabolic syndrome besides age. Even individuals in the upper normal weight and slightly overweight BMI range are at increased risk of having the metabolic syndrome.[13–16]

In order to increase the sensitivity and power of our own study we focused on insulin resistance, one of the pathophysiological components of type 2 diabetes and metabolic syndrome.[17] Many studies have shown that insulin resistance will progress to overt diabetes or metabolic syndrome within only a few years.[18–20] In insulin resistance, the pancreas compensates for the increased insulin to maintain normal blood glucose levels. However, the intracellular processing capacities may soon become exhausted and incomplete processing of the insulin leads to increased release of intact or partly processed proinsulin.[21] Once the beta cell secretion fails to maintain glucose control, impaired glucose tolerance and diabetes mellitus are the consequences.[22] The hyperinsulinemic-euglycemic clamp technique and the intravenous glucose tolerance test are the gold standard methods for measuring insulin sensitivity.[23,24] Both are very time- and resource-consuming and not suitable for routine clinical use. Therefore, we used a composite of surrogate parameters to assess insulin resistance in our patients by measuring insulin and glucose for calculation of the homeostasis assessment model of insulin resistance (HOMA-IR) score[25] in narcoleptic patients and controls matched for age, gender, and BMI. We also determined intact proinsulin to calculate the proinsulin-to-insulin ratio and to determine the prevalence of insulin resistance as described by Pfützner et al.[21]

Methods

Patients

The study design was approved by the ethics committee of the Medical Association of Rhineland-Palatinate that is responsible for all clinical studies performed at the University of Mainz. The study was conducted in accordance with all relevant ethical and legal regulations. All participants gave their written informed consent prior to study entry. A total of 43 patients (22 males and 21 females) with a diagnosis of narcolepsy according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and the International Classification of Sleep Disorders (ICSD) criteria were enrolled into this cross-sectional investigation.[26] Patients were all unrelated and were either referred from the Department of Psychiatry, University Hospital Mainz, or recruited with the help of the Deutsche Narkolepsie-Gesellschaft, a nationwide German patient organization. Narcolepsy symptoms, severity and frequency of symptoms, and total duration of various aspects of the symptomatology were assessed by unstructured and structured clinical interviews, including the Stanford Center for Narcolepsy Sleep Inventory (www.med.stanford.edu/school/Psychiatry/narcolepsy/sleepinventory.pdf). In accordance with the ICSD II for the diagnosis of narcolepsy, patients who had not undergone polysomnographic examination were only admitted to the study when unambiguous cataplexies, additional rapid eye movement-associated symptoms, and severe daytime sleepiness with recurrent daytime naps or lapses into sleep for at least three months were reported. To exclude symptomatic narcolepsy, the medical history was assessed and a neurologic examination was performed.

As a control group, 47 healthy unrelated volunteers (22 males and 25 females) matched for age and gender were recruited. All controls had undergone an oral glucose tolerance test to exclude diabetes mellitus. Further exclusion criteria were pregnancy, multiple allergies, drug abuse, or any other psychiatric disorders limiting the capacity to consent. All participants were of Caucasian origin.

Measurements

Glucose levels were determined using a standard reference laboratory method (glucose oxidase method) according to the manufacturer's instructions (Super GL, RLT, Möhnesee, Germany). Insulin and intact proinsulin were determined as previously described[27] by means of specific immunoassays (MLT insulin [intra-assay and interassay coefficients of variation 3.8% and 2.3%, respectively, reference range <30 μU/mL] and LINCO Intact Proinsulin [intra-assay and interassay

coefficients of variation 5.2% and 8.6%, respectively, reference range <10 pmol/L], LINCO Research Inc, St Charles, MO).

The proinsulin-to-insulin ratio was calculated by dividing the concentration of proinsulin (pmol/L) by insulin (converted from μU/mL into pmol/L). Insulin resistance was diagnosed based on prevalence of elevated fasting intact proinsulin[21,28] or elevated fasting HOMA-IR score calculation.[25] The estimate of insulin resistance by HOMA-IR score was calculated as fasting serum insulin (μU/mL) × fasting plasma glucose (mg/dL)/405. As described by Hedblad et al patients with HOMA scores exceeding the 75th percentile of a nondiabetic patient population (ie, 2.0) were considered to have insulin resistance.[29]

Statistical analysis

Statistical analysis was performed using descriptive statistics and appropriate parametric and nonparametric tests. The Kolmogorov–Smirnov test was used to ensure that the studied variables did not significantly differ from a normal distribution. The Mann–Whitney U test was used to compare rank sums. For the comparison of proportions, the Chi-square test was used. Descriptive results of continuous variables were expressed as the median and interquartile range for the non-Gaussian variables. All tests were two-sided. Linear regression was used to analyze the impact of BMI and age on the parameters of insulin resistance. Results with P values <0.05 were considered to be statistically significant. All calculations were conducted with SPSS software (version 15.0 for Windows, SPSS Inc, Chicago, IL).

Results

A general characterization of patients and controls is given in Table 1.

Fasting glucose

Median fasting plasma glucose was significantly lower in the narcoleptic patients as compared with the healthy controls (narcoleptic patients 85.0 [76.0–95.0] mg/dL, controls 96.5 [87.5–102.8] mg/dL, $P < 0.001$). The differences were also conserved in the gender subgroups (male narcoleptic patients 86.0 [81.3–103.5] mg/dL; controls 101.0 [93.8–108.3] mg/dL, $P = 0.031$; female narcoleptic patients 78.0 [72.5–88.0] mg/dL; controls 93.3 [85.3–93.3] mg/dL, $P = 0.001$). One control and one narcoleptic patient had fasting glucose values above >126 mg/dL ($P = 0.987$). Fasting glucose correlated with BMI in narcoleptic patients (r = 0.384, $P = 0.011$) and controls (r = 0.349, $P = 0.020$) and with age in the narcoleptic group (r = 0.339, $P = 0.026$), but not in the control group (r = 0.247, $P = 0.106$).

Table 1 Characterization of patients and controls

Characteristics	Narcoleptic patients	Controls	P value
n	43	47	
Males	22 (51.2%)	22 (46.8%)	
Females	21 (48.9%)	25 (53.2%)	
Age (years)	49.2 ± 16.6	50.6 ± 16.3	0.678
(Males)	54.5 ± 15.3	55.1 ± 15.3	0.891
(Females)	43.7 ± 16.5	46.7 ± 16.5	0.535
BMI (kg/m²)	30.2 ± 6.1	31.0 ± 5.2	0.505
(Males)	31.4 ± 5.2	32.4 ± 4.0	0.481
(Females)	28.8 ± 6.8	29.7 ± 5.8	0.655
HLA DR15 positive	38 (88.4%)	n.a.	
(Males)	20 (90.9%)		
(Females)	18 (85.7%)		
HLA DR15 negative	5 (11.6%)		
(Males)	2 (9.1%)		
(Females)	3 (14.3%)		
Duration of narcolepsy (years)	29.8 ± 17.2		
Age of onset (years)	16.0 (12.0–23.0)		
ESS score	18.14 ± 3.68		

Abbreviations: BMI, body mass index; HLA, human leukocyte antigen; ESS, Epworth Sleepiness score; n.a., not asked.

Insulin

Median insulin levels were 7.58 (5.6–11.6) μU/mL in narcoleptic patients and 10.14 (6.9–13.7) μU/mL in controls ($P = 0.185$). Accordingly, median insulin levels in narcoleptic females (6.50 [5.5–10.0] μU/mL) were not different from female controls (8.06 [4.5–10.3] μU/mL, $P = 0.680$) and median insulin levels in male narcoleptic patients were similar to those in male controls (narcoleptic patients 9.40 [6.1–17.0] μU/mL; controls 11.20 [10.1–15.9] μU/mL, $P = 0.205$). Five narcoleptic patients and four controls had values above 17 μU/mL ($P = 0.698$). Insulin levels did correlate with BMI in both diagnostic groups (narcoleptic patients: r = 0.413, $P = 0.006$; controls r = 0.522, $P < 0.001$) and did not correlate with age (narcoleptic patients: r = 0.077, $P = 0.622$; controls r = 0.172, $P = 0.266$).

Intact proinsulin

Median intact proinsulin values were slightly higher in controls than in narcoleptic patients (narcoleptic patients 2.46 [1.3–6.3] pmol/L versus controls 3.82 [2.4–7.1] pmol/L, $P = 0.046$). Intact proinsulin medians were 2.83 (1.9–9.4) pmol/L in narcoleptic men and 1.6 (1.1–3.4) pmol/L in narcoleptic women. The corresponding values for the male controls were 5.32 (3.8–9.9) pmol/L ($P = 0.060$) and 2.4 (1.6–2.4) for the female controls ($P = 0.172$). Five narcoleptic patients and three controls showed intact proinsulin values above 11 pmol/L ($P = 0.382$), ie, the reference value for stage III

beta cell dysfunction and insulin resistance.[16] In both groups, there was a significant correlation between intact proinsulin and BMI (narcoleptic patients: r = 0.464, P = 0.002; controls r = 0.485, P = 0.001). Correlations of proinsulin with age were r = 0.243 (narcoleptic patients, P = 0.116) and r = 0.308 (controls, P = 0.035). The proinsulin-to-insulin ratio was marginally lower in narcoleptic patients than in the controls (narcoleptic patients 4.31 versus controls 5.35, P = 0.033).

HOMA-IR

Median HOMA values were 1.51 (1.1–3.1) for narcoleptic patients and 2.68 (1.6–3.5) for controls (P = 0.048). HOMA > 2.5 were found in 12 patients (27.9%) and 23 controls (52.3%, P = 0.020). Two patients and two controls had HOMA values >5 (P = 0.981). HOMA correlated with BMI in both diagnostic groups (narcoleptic patients: r = 0.489, P = 0.001; controls r = 0.474, P = 0.001) but not with age (narcoleptic patients: r = 0.195, P = 0.209; controls r = 0.236, P = 0.123).

Insulin resistance

Insulin resistance, defined as fasting glucose levels >126 mg/dL, insulin levels above 17 μU/mL, intact proinsulin values above 11 pmol/L, and HOMA values >5, was prevalent in one narcoleptic patient (2.3%) and not in any controls (P = 0.478). Insulin resistance, as defined by a HOMA-IR score >2.0 and increased fasting intact proinsulin levels,[21] was present in four narcoleptic patients (9.3%) and three of the healthy control subjects (6.4%, P = 0.450). A summary of the results is also given in Table 2. Linear regression analysis with fasting glucose, insulin, intact proinsulin, and HOMA-IR as dependent variables and BMI and age as independent variables showed no impact of BMI on intact proinsulin (P = 0.557) but did show an impact on fasting glucose (P = 0.015), insulin (P < 0.001), and HOMA-IR (P < 0.001). No impact of age was found for insulin (P = 0.400), intact proinsulin (P = 0.869), and HOMA-IR (P = 0.908) but did show an impact for fasting glucose (P = 0.016). None of the markers were correlated with disease duration (data not shown).

Discussion

In our study, narcoleptic patients did not show higher fasting blood glucose levels than age- and BMI-matched controls. Also, insulin and intact proinsulin levels were not elevated in the narcoleptic group, nor was the proinsulin-to-insulin ratio, HOMA-IR-levels, or prevalence of insulin resistance. This result is in contrast with the clinical belief that narcolepsy per se and independently from BMI is associated with an increased

Table 2 Glucose levels, insulin, intact proinsulin, proinsulin-to-insulin ratio, and homeostasis assessment model of insulin resistance of narcoleptic patients and control subjects, values are given as median (quartiles). Additionally, both groups were split for gender

	Narcoleptics	Controls	P value
Fasting glucose (mg/dL)	85.0 (76.0–95.0)	96.5 (87.5–102.8)	<0.001
Males	86.0 (81.3–103.5)	101.0 (93.8–108.3)	0.031
Females	78.0 (72.5–88.0)	93.3 (85.3–93.3)	0.001
Insulin (μU/mL)	7.58 (5.6–11.6)	10.14 (6.9–13.7)	0.185
Males	9.40 (6.1–17.0)	11.20 (10.1–15.9)	0.205
Females	6.50 (5.5–10.0)	8.06 (4.5–10.3)	0.680
Intact proinsulin (pmol/L)	2.46 (1.3–6.3)	3.82 (2.4–7.1)	0.046
Males	2.83 (1.9–9.4)	5.23 (3.8–9.9)	0.060
Females	1.63 (1.1–3.4)	2.38 (1.6–2.4)	0.172
Proinsulin-to-insulin ratio (%)	4.31 (3.0–7.0)	5.35 (4.4–8.1)	0.033
Males	4.99 (3.1–8.8)	6.16 (4.8–10.6)	0.250
Females	3.52 (2.4–6.0)	5.07 (3.6–6.1)	0.055
HOMA index	1.51 (1.1–3.1)	2.68 (1.6–3.5)	0.048
Males	2.01 (1.3–3.6)	2.96 (2.6–3.9)	0.096
Females	1.3 (1.0–2.0)	1.96 (1.0–2.0)	0.264

Abbreviation: HOMA, homeostatic model assessment.

(pre) diabetic metabolic state. Our conclusion is supported by the fact that all controls were examined by an oral glucose tolerance test prior to inclusion in the study and in this way manifest diabetes was excluded from the control group. Therefore, the only potential bias was in favor of an overestimation of potential markers of prediabetes in the narcoleptic patients.

One limitation of the study is that because of the low prevalence of narcolepsy, it was not possible to recruit a strictly representative population sample. Instead, patients were recruited with the support of a patient organization. It has been estimated that a large fraction of narcoleptic patients in the general population are undiagnosed.[30] This problem is unavoidable when studying rare disorders and is true for most, if not all, published narcolepsy studies. However, we took care not to introduce additional bias by embedding our study in a study of overweight and metabolic parameters with no explicit reference to type 2 diabetes.

A second limitation is the choice of the observation parameters. In particular, no oral glucose tolerance test, intravenous glucose tolerance test, or hyperinsulinemic-euglycemic clamp test were performed in the narcoleptic patients. Although being a very simple, cheap, and convenient parameter (and recommended by the American Diabetes Association),[31] fasting glucose alone is poorly predictive of insulin resistance and a very crude parameter

for assessing beta cell function.[32–34] Fasting glucose also has a poor sensitivity for diabetes diagnosis (40%–60%) and may delay the diagnosis of dysglycemia.[35] Waugh et al found that the majority of patients with impaired glucose tolerance would be missed by assessing only fasting plasma glucose levels.[36] Nevertheless, elevated fasting glucose levels are predictive for manifest diabetes.[22,31]

Although fasting insulin is considered to be a relatively good indicator of insulin resistance among nondiabetic persons,[37] other studies have revealed that the relationship between insulin resistance measured by an euglycemic clamp and fasting serum insulin level is weak. Insulin levels are less suited to yield a diagnosis in the single patient than to study metabolic tendencies in groups.[38,39] Intact proinsulin has been shown to be a highly specific indirect marker for insulin resistance. Elevation of intact proinsulin values above the reference range ($>$11 pmol/L) shows a very high specificity and a moderate sensitivity as a marker for insulin resistance.[21] Plasma intact proinsulin concentrations are usually highest in patients with type 2 diabetes, lower in those with normal glucose tolerance, and moderately elevated in patients with impaired glucose tolerance. The results reported by Reaven show that ambient plasma proinsulin concentrations increase as glucose tolerance declines.[13]

In addition, impaired glucose tolerance in type 2 diabetes is often characterized by an increased ratio of proinsulin to insulin. The results reported by Mykkanen et al suggest that an increased intact proinsulin-to-insulin ratio is a marker of defective insulin secretion.[40,41] While this group considered the proinsulin-to-insulin ratio to be a good marker for impaired beta cell function, Vezzosi et al found that the proinsulin-to-insulin ratio did not result in better diagnostic accuracy than proinsulin levels alone. Because there are no commonly accepted reference ranges set for the ratio, we compared our results with other research papers and found values of 1%–16% described to be physiological.[41–44]

The HOMA method is derived from a mathematical assessment of the interaction between beta cell function and insulin resistance in an idealized model, which is then used to compute steady-state insulin and glucose concentrations. The output of the model is calibrated to give normal beta cell function of 100% and a normal insulin resistance of 1. Once this interrelationship is calculated, one can estimate beta cell function and insulin resistance for any pair of fasting plasma glucose and insulin concentrations. The HOMA model has proved to be a reliable clinical and epidemiological instrument to describe the pathophysiology of diabetes, except in the stage of severe beta cell dysfunction leading to proinsulin secretion.[25]

Taken together, the combined measurement of fasting glucose, insulin, and intact proinsulin is a practical and robust way to not only diagnose manifest diabetics but also to characterize prediabetic metabolic states.[28]

In summary, we found no evidence that narcolepsy elevates the risk for prediabetic states of insulin resistance independently of BMI. The fact that the narcoleptic patients had even lower insulin resistance parameters than the controls might give the wrong impression that narcoleptic patients have a smaller risk of developing type 2 diabetes. This effect still needs to be elucidated. Clinically, type 2 diabetes still remains a challenge in narcoleptic patients because of the association of narcolepsy with an elevated BMI.

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Disclosure

The authors report no conflicts of interest in this work.

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