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Highlights

- Insulin resistance was found in 33/64 patients with multiple sclerosis
- Insulin resistance was associated with the degree of disability of patients
- Patients with the secondary progressive form showed the highest prevalence

Metabolomic profile of insulin resistance in patients with multiple sclerosis is associated to the severity of the disease

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Abstract

Background. Dysglycemia and adiposity have been related to disability in patients with multiple sclerosis. The objective of this work was to determine the prevalence and characteristics of insulin resistance in patients with multiple sclerosis using the metabolomics Quantose score.

Methods. A total of 64 patients were accrued in the study. A blood sample was drawn to estimate the Quantose score, which is derived from fasting measurements of insulin, α-hydroxybutyrate, linoleoyl-glycerophosphocholine, and oleate, three nonglucose metabolites shown to correlate with insulin-stimulated glucose disposal.

Results. Insulin resistance was documented in 33 out of 64 patients and it was found in association with the degree of disability and the time from diagnosis. Patients with the secondary progressive form of the disease showed the highest prevalence.

Conclusion. Insulin resistance is frequent in patients with multiple sclerosis and might contribute to metabolic complications and general disability. Early markers of dysglycemia should be sought for in these patients to avoid additional deterioration of their quality of life.

Key Words: Insulin sensitivity, insulin resistance, metabolism, dysglycemia, multiple sclerosis

1. Introduction.

Insulin resistance (IR) seems to be associated with chronic inflammatory process and oxidative stress in patients with multiple sclerosis (MS) and with disease disability as measured by the expanded disability status scale (EDSS) score (1, 2). Hyperinsulinemia by itself has not been associated to chronic inflammation or physical disability (3) and hence, it is possible that factors involved in the complex metabolic network of insulin resistance, other than hypersecretion of insulin, participate in the central nervous system function impairment in this disease. Metabolic syndrome, also associated to IR, has been found to have a higher prevalence in patients with MS and it has been proposed that this and other comorbidities should be part of the standard care of MS patients (4)

A serious limitation of previous studies is that IR has been defined through the homeostasis model assessment (HOMA) formula (5) which depends on the fasting levels of plasma glucose and insulin. For the HOMA index to detect IR, either glucose or insulin levels –or both- must be abnormally high, which indicates an advanced stage in the continuum progression of dysglycemia towards impaired glucose tolerance (IGT) and diabetes. Individuals in the upper tertile of impaired IGT are believed to have lost approximately 70–80% of their pancreatic β-cell function and may progress to type 2 diabetes mellitus with rates over 15% per year (6-8).

Using fasting plasma samples from healthy individuals, metabolomic biomarkers that correlate strongly with the rate of whole body insulin-mediated glucose disposal derived from the euglycemic insulin clamp technique have been identified (9). Individually, α-hydroxybutyrate (α-HB), oleate, and insulin were found to correlate inversely with insulin-stimulated glucose metabolism, while L-linoleoyl-glycerophosphocholine (L-GPC) correlated positively. These four analytes are used to estimate a score (called Quantose) that is able to predict the 3-year progression from normal glucose tolerance to IGT and to overt diabetes (9). To the

best of our knowledge, the Quantose score is nowadays the best non-invasive test to estimate IR at a very early stage.

The objective of this study was to determine the prevalence of IR as evaluated by the Quantose metabolomics score in patients with MS, and analyze the association of this comorbidity with the degree of disability and other clinical parameters.

2. Material and Methods

2.1 Patients.

A total of 64 patients (45 females and 19 males) with MS were included in this study. All patients were participants in a clinical trial (ClinicalTrials.gov identifier NCT02674217) aimed to evaluate the efficacy and safety of autologous hematopoietic stem cell transplantation (AHSCT) in MS (10). Their median age was 46 and ranged from 21 to 73 years. The time elapsed from diagnosis to the time of the study ranged from 0.3 to 37 years with a median of 10. Their EDSS score ranged from 1 to 7.5 with a median of 5.5. Twenty-nine patients were classified as having the relapsing-remitting form of the disease (RRMS), 12 had the primary progressive (PPMS) variant and the remaining 23 showed the secondary progressive (SPMS) form. Insulin resistance was measured prior to the administration of any drugs and all patients had been deprived of immunosuppressant or cytotoxic drugs at least three months before. Routine laboratory examination such as hemoglobin, whole blood cell counts, blood chemistry, urine analysis, chest x rays and electrocardiogram were normal or negative.

The study protocol was approved by the Ethics Committee of Laboratorios Clínicos de Puebla and Centro de Hematología y Medicina Interna in accord with the Helsinki Declaration of 1975 (CONBIOETICA 21CEI 001 201 30605, Reg.13 CEI 21114126).

2.2 Estimation of the Quantose score.

Fasting blood samples were drawn by venipuncture into EDTA containing vacuum tubes. Insulin levels were measured by means of an automated micro particle chemiluminescent immunoassay (Abbott Architect Insulin, Abbott Park, IL) whereas the metabolites were determined by an analytically and clinically validated ultra-high performance liquid chromatography and tandem mass spectrometry (UPLC-MS-MS) assay developed and carried out in an accredited laboratory (6). Briefly, 50 µL of EDTA plasma samples were spiked with internal standards and subjected to protein precipitation with 250 µL of methanol. After centrifugation, aliquots of supernatant were injected onto an UPLC-MS-MS system, consisting of an Exevo TQ-S Mass Spectrometer coupled to a Waters Acquity UHPLC system (Waters Corporation, Milford, MA) equipped with a column manager module in 2.5-minute assay. α-HB, L-GPC, and oleic acid were eluted with a gradient on a Waters Acquity single RP C-18 column (2.1 mm × 50 mm, 1.7-mm particle size) at a flow rate of 0.8 mL/min at 40°C. Ionization was achieved by heated electrospray ionization source. Quantitation was based on the area ratios of analyte and internal standard peaks using a weighted linear least-squares regression analysis generated from fortified calibration standards in an artificial matrix, prepared prior to each analytical run. Stable isotope-labeled compounds (α-HB-D3, L-GPC-D9, and oleic acid-13C18) were used as internal standards. The inter-run CVs for α-HB, L-GPC, and oleic acid were 2.08, 2.64, and 2.40%, respectively.

Inasmuch as the algorithm to determine the Quantose score is proprietary and patent-protected, the results of all 4 analytes were sent to Metabolon Inc. in North Carolina for the calculation of the score.

2.3 Statistical analysis.

Chi squared test was used to estimate non parametric associations whereas regression analysis was used to establish parametric associations. Analysis were performed with the aid of the MedCalc® software package.

3. Results

Table 1 depicts individual values of plasma concentrations of insulin, α-OH-butyric (a-HB), L-linoleoyl-glycerophosphocholine (L-GPC), oleic acid, and the Quantose score of the 64 patients included in the study. Reference values for insulin are 3.13-21.3 μg/mL, for α-HB 1.92-7.37 μg/mL, for L-GPC 7.60-25.4 μg/mL and for oleic acid 25.9-114.0 μg/mL. Values of the Quantose score ≤ 63 are referred to as insulin sensitive whereas values above this cutoff are consistent with insulin resistance. Accordingly, 33 patients had insulin resistance while 31 were insulin sensitive. Five of the 64 patients had abnormal (≥100 mg/dL) fasting glucose plasma concentrations but they never exceeded 115 mg/dL.

3.1 Insulin resistance and type of MS.

As shown in table 2, the different analytes that compose the Quantose score were not significantly different in patients with RRMS, PPMS or SPMS, however, the Quantose score was significantly higher in patients with SPMS than in patients with RRMS and PPMS. Moreover, as shown in figure 1, the proportion of patients with SPMS that showed insulin resitance (17/23) was significantly different than that of patients with RRMS and PPMS (16/41) (Chi squared = 7.181, p = 0.0007). When analyzing other differences among patients with SPSS, PPMS and RRMS, we found that the time elapsed from diagnosis was significantly longer (Table 3) in patients with SPMS than patients with the other two variants of the disease. Regression analysis of the Quantose score value and the time elapsed from the diagnosis, confirmed that there is a significant relationship of this feature with insulin resistance (Figure 2). The effect of age was ruled out as responsible for this correlation, for regression of patients' age and the Quantose score was not significant (r = 0.06, p = 0.621). Moreover, the proportion of patients bearing insulin resistance was not different in those with ages below or above the median value (Chi-squared = 0.2767, p = 0.598).

3.2 Insulin resistance and severity of disability

When patients were clustered according to their EDSS score, it was found that insulin resistance was significantly more frequent in patients with higher EDSS values (Table 4 and figure 3). Moreover, linear regression analysis of the EDSS score and the Quantose score further proved that they are statistically correlated (Figure 4). Regression analysis of each of the analytes that compose the Quantose score with the EDSS value, proved that none of the isolated analytes, by themselves, correlated as strongly as the Quantose score. In fact, as summarized in table 5, insulin and oleic acid did not correlate significantly with the EDSS score, and, although αHb and LGPC did show some correlation with the EDSS score, the regression and determination coefficients were lower, whereas the α vaues were higher than those observed between the Quantose and the EDSS scores.

3.3 Association of insulin resistance with other parameters

Insulin resistance was not related to sex, age, weight and body mass index. Neither were differences observed in the prevalence of insulin resistance and prior treatment, country of residence, ethnicity or other comorbidities such as allergies, arterial hypertension, fibromyalgia, and thyroid disease.

3.4 Correlation of Quantose score and HOMA index.

Figure 5 depicts the correlation between the Quantose score values and the HOMA index of the 64 patients included in the study. Despite the significant correlation between the two values, the determination coefficient r^2 was only 0.4096.

This finding is consonant with previous indications that the Quantose score shows a more robust correlation with the hyperinsulinemic-euglycemic clamp test to assess insulin sensitivity than the HOMA index (6, 9). In our own experience, when both approaches were used to classify 134 normoglycemic normoinsulinemic

adults as insulin resistant or sensitive, the contingency coefficient was 0.429 (Chi-squared 30.143, $p < 0.0001$).

Table 6 compares the rate of detection of insulin resistance by the Quantose score (≥63) and the HOMA index (≥3.0) in the 64 patients with MS. As shown, whilst 33 patients were classified as insulin resistant by the Quantose score, only 2 them were detected as such by the HOMA index. On the other hand, there were 3 patients that were tagged as insulin resistant by the HOMA index but had normal Quantose score. The correlation of the results obtained by the two methods in these patients was practically null.

4. Discussion

Insulin resistance, oxidative stress and adiposity have been described as comorbidities in multiple sclerosis, and is has been suggested that these might contribute to the progression and disability (1-3). In the study of Oliveira and co-workers (1), IR was estimated through the HOMA index, and found in 40% of the patients with MS, as compared to 21% in a control population. The HOMA index is a ratio of fasting plasma glucose and insulin levels and hence, it will reveal changes when either or both such analytes are altered.

The Quantose insulin resistance score which is estimated from fasting measurements of insulin, α-hydroxybutyrate, linoleoyl-glycerophosphocholine, and oleate, three nonglucose metabolites that have been shown to correlate closely with insulin-stimulated glucose disposal. It has been shown to detect patients with insulin resistance at an earlier stage of the dysglycemic process, before any alterations of the glucose or insulin plasma levels are present (8-9).

Using the Quantose score, we could document insulin resistance in 51% of 64 patients with various clinical types of MS and distinct degrees of disability. We found a clear association with the Secondary Progressive variant of the disease

(SPMS), as well as with the degree of disability as measured by the EDSS score, and also with the time elapsed from diagnosis to the time of the study. Inasmuch as all these three variables correlate positively with each other, it is difficult to propose a pathophysiological role of insulin resistance in the progression of the disease. Insulin resistance along with adiposity and oxidative stress has been proposed to play a pathogenetic role in the progression of MS (1) however, IR could result from the prolonged evolution that characterizes patients with SPMS. Association with higher EDSS values might also reflect the longer evolution time of these patients. The fact that we found no association of IR with BMI or bodyweight seems to indicate that obesity due to sedentary lifestyle is not the underlying cause of insulin resistance (11).

Whether or not insulin resistance plays a pathogenetic role in MS aggravation and disability, it surely contributes to complications, mainly when it progresses to impaired fasting glucose and diabetes. Several studies have shown that IR as detected by metabolomic biomarkers bears a high risk of conversion to overt forms of dysglycemia such as prediabetes and diabetes (12-17), and that timely intervention with lifestyle or medications might reverse the progression of the dysglycemic process.

Recent epidemiological data suggest that excessive caloric intake and metabolic stress might modulate immune regulation and lead to self tolerance breakdown and hence, autoimmune diseases in general might be related to obesity (18); moreover, in the particular case of multiple sclerosis, it has been proved that genetically determined increase in body mass index is a risk factor for developing the disease (19).

Our results seems sufficiently robust as to propose that insulin resistance should be sought for in patients with MS, using metabolomic biomarkers and, when considered appropriate, intervene to reverse it. Complications of dysglycemia such as retinopathy, neuropathy and nephropathy might appear early in the progression of the metabolic continuum (20) and certainly will contribute to further deterioration of the sense of well being of patients with multiple sclerosis. If prevention of

complications of dysglycemia is opportunely implemented in patients with MS, they will be offered a better quality of life and a considerable reduction of the their treatment cost.

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

5. Conclusion

Our results seems sufficiently robust as to propose that insulin resistance should be sought for in patients with MS, using metabolomic biomarkers and, when considered appropriate, intervene to reverse it.

6. Contribution statement

Alejandro Ruiz-Argüelles.Contributed substantially to the conception and design of the work, data analysis and interpretation and drafting the article

Mariana A Méndez-Huerta. Contributed to the design of the work, data acqusition and analysis, and review and approval of the manuscript

Claudia D Lozano. Contributed substantially with laboratory data acquisition and analysis as well as review and approval of the manuscript.

Guillermo J Ruiz-Argüelles, Contributed to the conception of the work, provided all the clinical data from the patients and reviewed and approved the final manuscript.

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Table 1. Individual results of the four analytes used to calculate the Quantose IR score and its value in 64 patients with MS.

Insulin	αHB	L-GPC	Oleic acid	Quantose score
5,3	5,64	18,3	113	66
8,6	7,81	8,99	125	85
5,3	6,26	19,6	53,9	62
3,6	2,85	21,6	49,7	31
5,5	3,89	16,9	74,7	58
20,9	6,64	10,6	118	93
7,9	8,59	16,4	93,3	79
3,8	6,39	22,5	89	56
9,6	4,01	15,4	65,3	71
5,2	2,3	16,7	69,4	45
6	4,75	17,4	56,4	61
7,3	5,01	20,4	45,9	64
4,9	3,27	19,6	39,5	44
5,9	3,68	22,7	52,1	52
7,8	2,94	19,2	28,7	52
8,7	2,07	17,6	59,5	55
5	2,54	17,8	32,1	38
6,5	4,31	16,4	59,5	63
11,9	6,17	11,8	62,3	82
5,1	3,84	22,7	39,3	47
6,6	4,08	18,3	73,5	62
5,1	3,47	17,6	26,1	44
3,9	4,34	18,1	151	56
17	5,1	12	45	84
4,9	2,11	15,8	47,8	39
6,2	4,48	9,42	67,4	69

5,8	6,13	11,8	86,3	72
5,8	5,1	7,28	48,4	70
10,1	2,59	10,2	35	65
11,4	3,21	12	62,2	73
21,4	5,37	10,4	49,9	89
7,1	2,69	18	87,2	58
7,2	5,5	13	53,6	70
10,5	2,54	19,8	45,6	61
6,2	5,06	11,2	29,4	63
10,8	3,83	18,8	34,9	67
10,2	3,89	15,6	33,7	67
5,5	2,84	19,2	50,4	47
12	3,09	12	64,9	74
5,8	1,94	27,3	7,92	15
3,6	3,01	12,8	58,4	43
12,7	2,23	13,9	71,6	69
7,9	4,19	13,4	66,8	69
8,2	5,09	16,1	53,1	70
5,9	3,68	13,8	57	59
14,1	15,49	10,2	72,3	85
5,1	6,52	8,02	67,4	72
5,9	5,05	12,2	56,5	66
5	3,66	16,9	56,6	52
3,1	2,91	9,58	91,2	46
9,8	3,38	5,91	78,2	78
9,1	4,8	12	72,9	75
7,6	4,88	15,9	57	68
3,5	4,81	24,1	52,8	43
5,4	6,82	10,3	93,6	73
16,9	3,34	29,8	28,9	68
4,3	1,91	17,3	37,9	28
4	3,6	26,9	19,4	27
8,8	3,59	20,5	17,6	55
6,9	4,29	6	51,2	72
10,8	2,14	5,47	50	72
7,2	4,66	7,55	120	77
6,2	4,91	2,73	37,4	77
5,1	3,36	12,1	73,4	57

αHB = α-OH-butyrate; L-GPC = L-linoleoyl-glycerophosphocholine. All results are expressed in mg/mL except for the Quantose score which is expressed in an arbitrary scale ranging from 0-100.

Table 2. Plasma concentrations of insulin,α-hydroxy-butyrate (αHB), L-linoleoyl-glycerophosphocholine (L-GPC). oleic acid, and the estimated Quantose insulin resistance score in MS patients according to the clinical progression type.

Type of MS	Insulin, μg /mL	αHB, μg/mL	L-GPC, μg/mL	Oleic acid, μg/mL	Quantose
RRMS	7.06 ± 0.63*	3.90 ± 0.26	15.62 ± .12	58.3 ± 5.4	57.9 ± 2.8
PPMS	6.84 ± 0.55	4.53 ± 0.55	17.55 ±1.48	60.1 ± 7.9	58.6 ± 5.4
SPMS	9.06 ± 1.01	4.82 ± 0.55	13.47 ± 0.99[a]	63.3 ± 5.2	68.1 ± 2.8
p_{RR-PP} ¶	NS	NS	NS	NS	NS
p_{SP-RR} ¶	NS	NS	NS	NS	0.0149

RRMS = relapsing-remitting multiple sclerosis; PPMS = primary progressive multiple sclerosis; SPMS = secondary progressive multiple sclerosis. * Results are expressed as arithmetic mean ± standard error of the mean. ¶ Means were compared by the t test for independent observations.

Table 3. Time elapsed from diagnosis in MS patients according to the clinical progression type

Type of MS	Time from diagnosis, years	Difference, p value¶		
RRMS	9.45 ± 1.58*	NS	0.004	
PPMS	6.20 ± 1.53			0.0001
SPMS	17.08 ± 2.01			

RRMS = relapsing-remitting multiple sclerosis; PPMS = primary progressive multiple sclerosis; SPMS = secondary progressive multiple sclerosis. * Results are expressed as arithmetic mean ± standard error of the mean. ¶ Means were compared by the t test for independent observations.

Table 4. Association of insulin resistance as defined by the Quantose score with the EDSS score of 64 patients with MS.

	EDSS Score		
	≤4.5	5.0 – 6.0	≥6.5
Insulin Resistant	7	9	18
Insulin Sensitive	18	7	5

Contingency coefficient = 0.401, Chi-squared 12.236, p = 0.0022

Table 5. Summary of the regression values between of the EDSS score and the Quantose score, as well as the 4 measurements that are used to estimate the latter.

	m	r	r^2	p
Quantose	2.47	0.311	0.097	0.0121
Insulin	0.29	0.148	0.022	NS
αHB	0.26	0.256	0.066	0.039
LGPC	-0.76	0.273	0.075	0.027
Oleic Acid	1.85	0.134	0.018	NS

αHB = α-OH-butyrate; L-GPC = L-linoleoyl-glycerophosphocholine.

Table 6. Classification of 64 MS patients as Insulin Resistant or Sensitive according to the Quantose score and the HOMA Index.

		Insulin status according to Quantose	
		Sensitive	Resistant*
Insulin status according to HOMA	Sensitive	28	31
	Resistant¶	3	2

* Quantose score ≥ 63; ¶ HOMA index ≥ 3. Contingency coefficient = 0.0667, Fisher's p = 0.667252

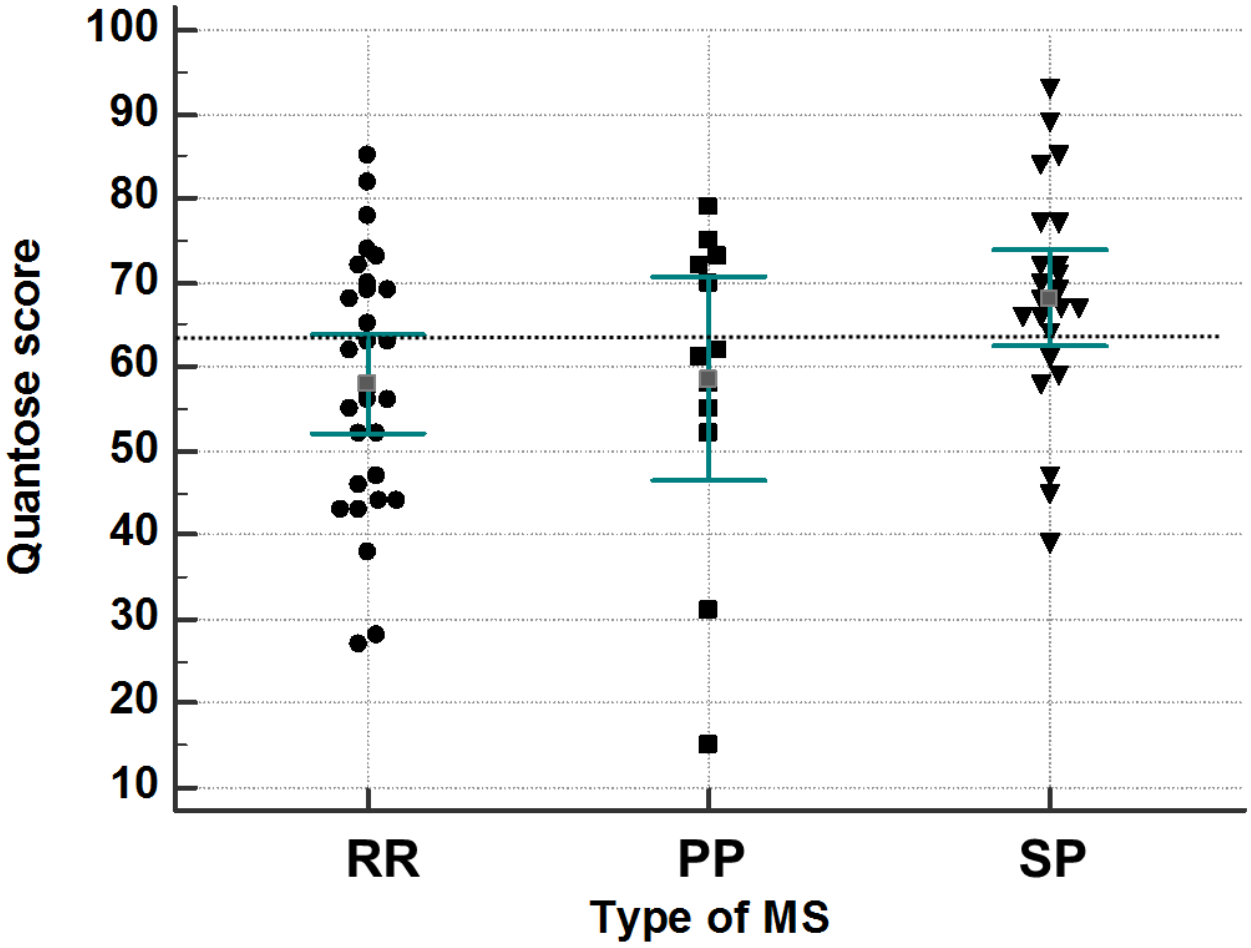

Figure 1. Individual values of the Quantose score in patients with different types of MS. The horizontal dotted line is the cutoff value to define insulin resistance

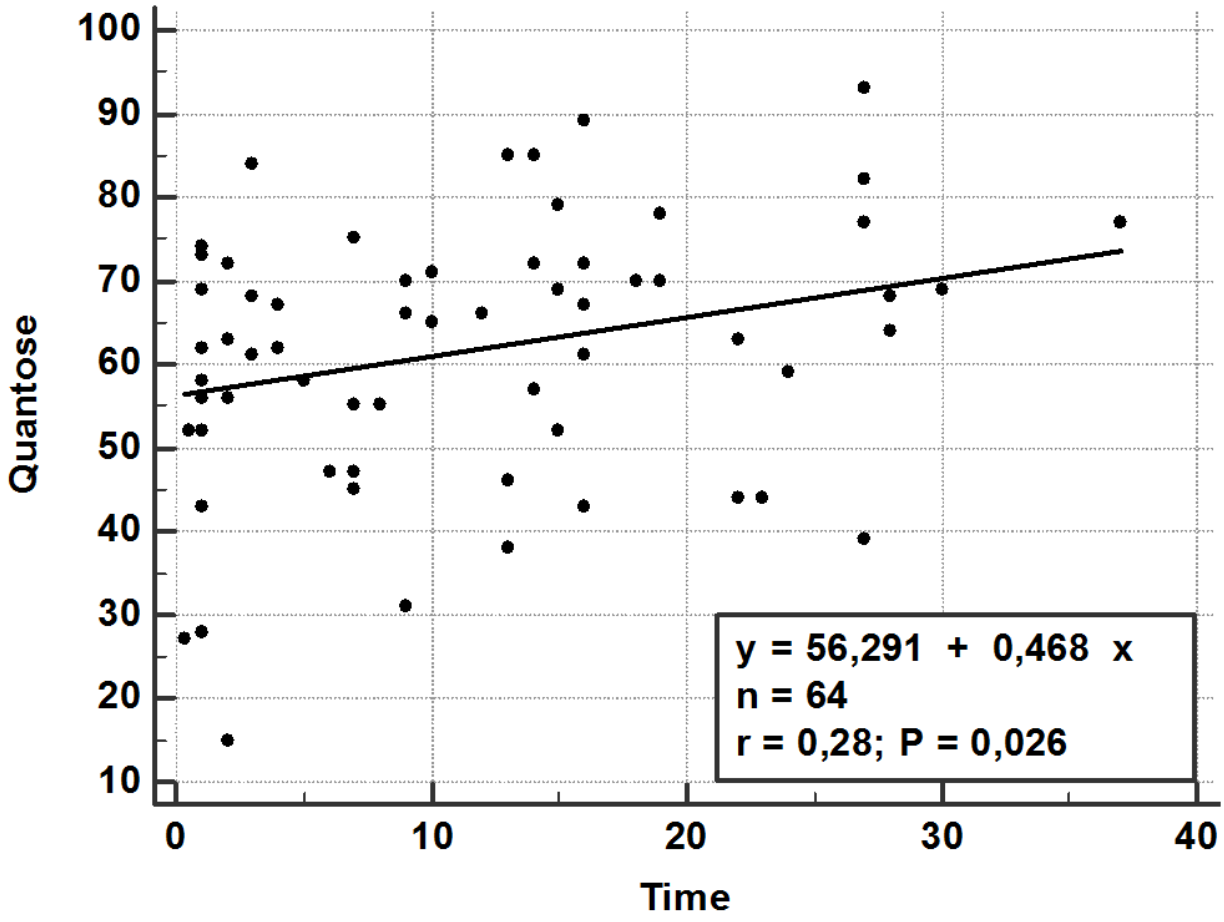

Figure 2. Correlation of the Quantose score with the time elapsed from diagnosis in 64 patients with MS.

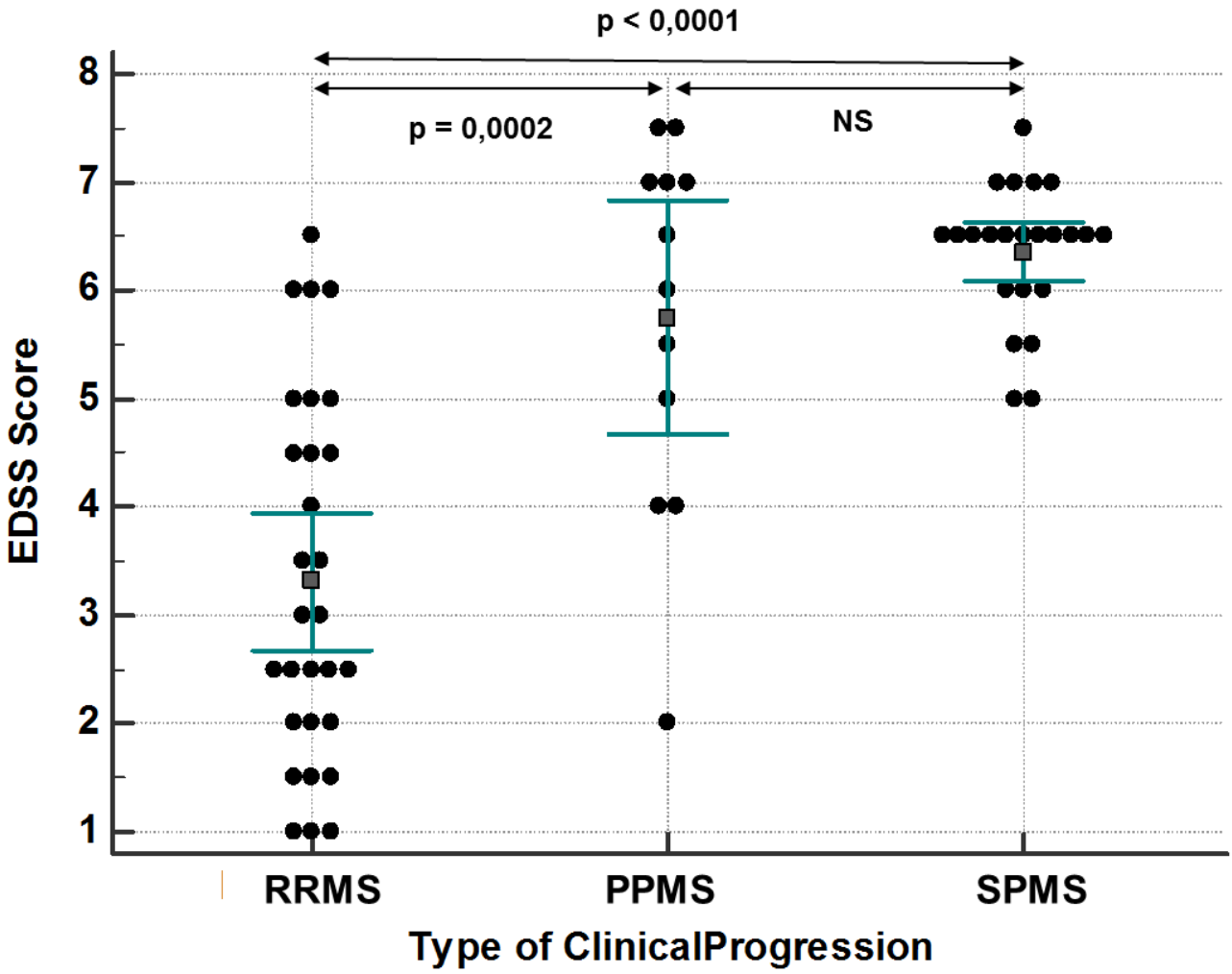

Figure 3. Individual values of the EDSS score in patients with different types of MS. The values from the RRMS patients were significantly lower than those of the PPMS and SPMS groups. The difference between these two groups was not statistically significant.

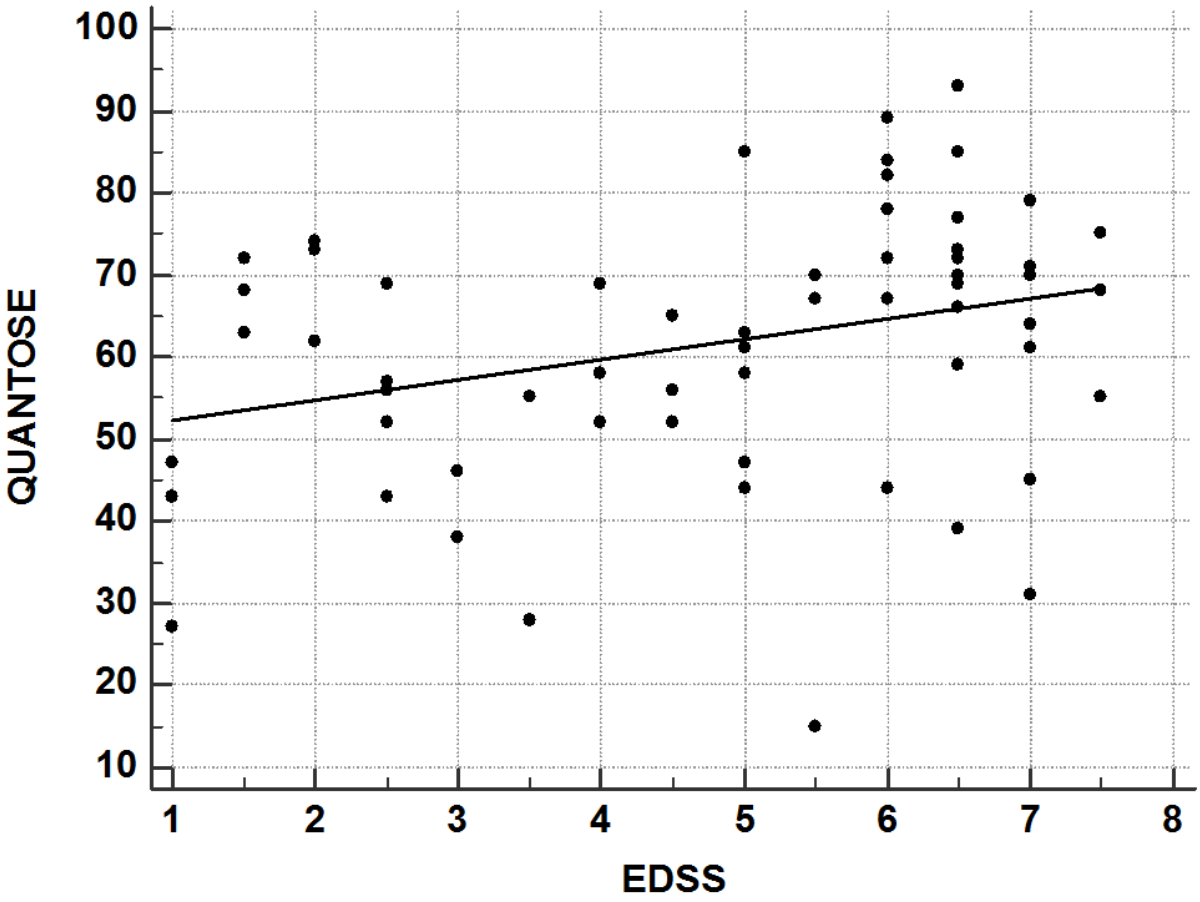

Figure 4. Regression line and scattergram of the Quantose and EDSS score in all 64 patients with MS. See table 5 for regression values.

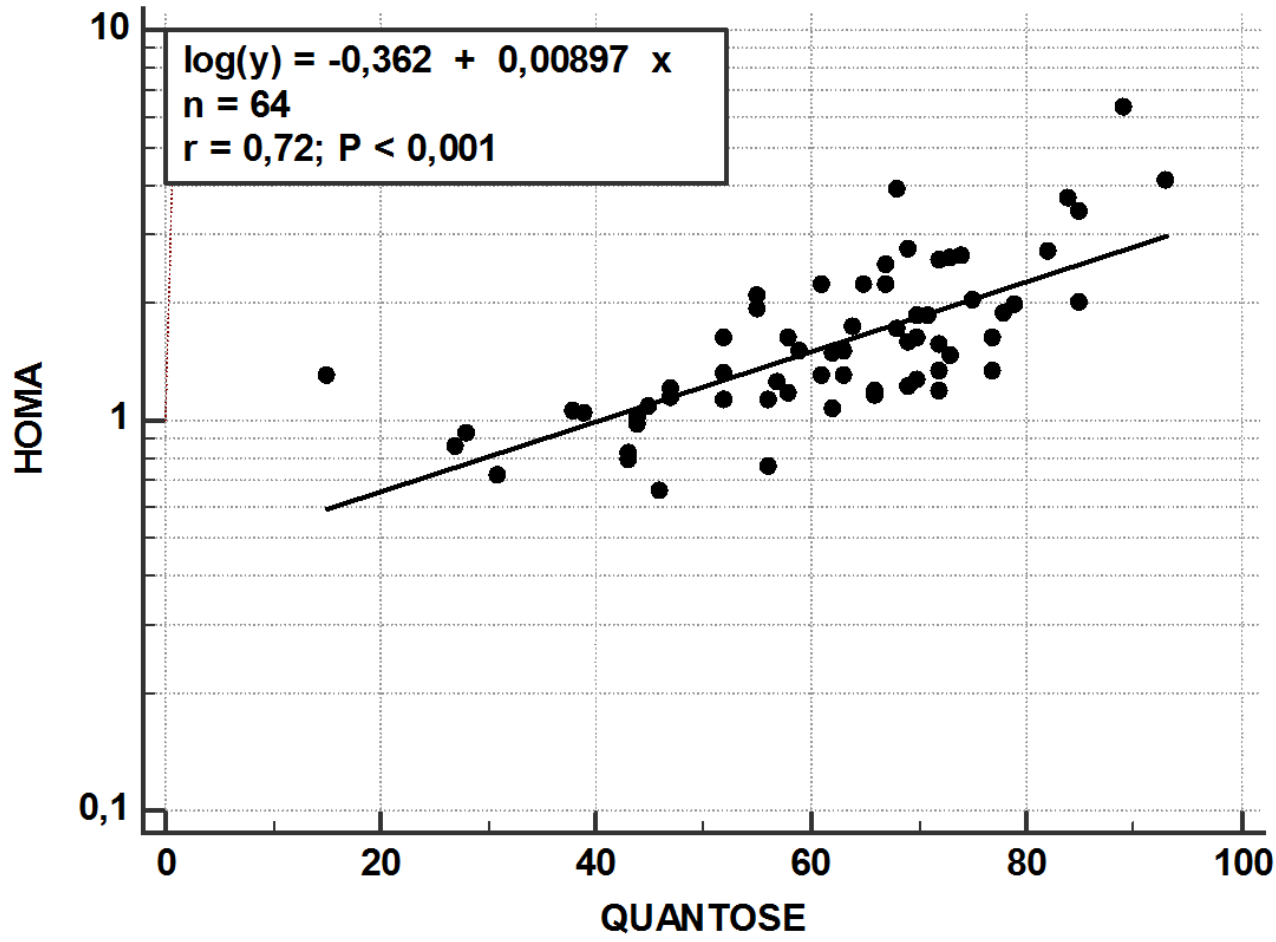

Figure 5. Regression analysis of the Quantose score and HOMA index of the 64 patients with MS.