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Predictors of Insulin Resistance in Obesity and Type 2 Diabetes Mellitus – The Role of Magnesium

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Abstract

Objectives: Hypomagnesaemia and insulin resistance are two major clinical problems, with intertwining pathophysiology. We aimed to explore this association in obese patients and in non-insulin-treated patients with type 2 diabetes mellitus (T2DM).

Methods: Subjects were recruited from the outpatient diabetes/obesity clinic of the Antwerp University Hospital. The population (N=2731) consists of 2 subject groups with different degrees of insulin resistance and insulin secretory potential: 1) overweight (Body Mass index (BMI) ≥ 25 kg/m² and <30 kg/m²) and obese (BMI ≥ 30 kg/m²) subjects, 2) adult T2DM patients. Hypomagnesaemia was defined as serum magnesium <1.7 mg/dl. Insulin resistance was estimated using the Homeostasis model assessment (HOMA-IR; cut-off point 2.82).

Results: Hypomagnesaemia was present in 6.1% of the entire population. Patients with hypomagnesaemia had more visceral adipose tissue (VAT), and a higher HOMA-IR. They suffered more from the metabolic syndrome and T2DM. Patients with a HOMA-IR<2.82 were younger, had lower BMI and less VAT. They suffered less from hypomagnesaemia. Hypomagnesaemia was more prevalent in T2DM patients than in obese subjects without T2DM. Although serum magnesium and HOMA-IR were negatively correlated, logistic regression analysis showed that magnesium was not a significant predictor for HOMA-IR.

Conclusions: Despite a significant negative correlation between magnesium and HOMA-IR, magnesium was not retained as a significant determinant of insulin resistance compared to the other predictors in our population of obese subjects and T2DM patients.

Keywords Hypomagnesaemia; Insulin resistance; Visceral adipose tissue; Obesity; Diabetes mellitus; HOMA-IR; Predictors

Introduction

Insulin resistance (IR) and hypomagnesaemia are two major clinical problems. Worldwide 200 million people suffer from IR and 40% of these patients will develop type 2 diabetes mellitus (T2DM) [1]. It is estimated that by 2030, diabetes mellitus will be the 7th most important cause of death worldwide. Moreover, the treatment of T2DM and its complications amount to 5.8% of the total expenditure of health care costs in Europe [2-4]. Research is therefore essential to investigate how the evolution of IR to full-blown T2DM can be interrupted. Supplementation of magnesium has been suggested as one potential approach [5].

The extent of hypomagnesaemia is often underestimated. The incidence of hypomagnesaemia in in-hospital patients amounts to 12%, and even rises to 60-65% in patients at the intensive care unit, mostly due to a poor nutritional status, medication or hypoalbuminemia [6-8]. Also in the healthy population there seems to be a tendency of magnesium depletion due to the increased consumption of refined foods and the decreased consumption of green vegetables [9,10]. Most cases of hypomagnesaemia in clinical practice are asymptomatic. The clinical manifestation may depend more on the total body the Mg^{2+} deficit rather than on the actual serum Mg level [11]. Personality changes, muscle weakness, tremor and dysphagia may occur at concentrations of <1.45 mg/dl, while confusion and a decreased consciousness develop at concentrations of ≤ 1.00 mg/dl [12].

There are important interfaces between magnesium and IR as magnesium is an essential co-factor in the ATP-associated reactions on which the mechanism of the insulin receptor depends [13]. Small-scale fundamental studies have shown that Mg^{2+} is essential for the insulin receptor phosphorylation, but the effect of Mg^{2+} on the downstream targets in the muscle, liver, and adipocytes is largely unknown. Moreover, a hypomagnesaemic state leads to an increase in pro-inflammatory cytokines that induce on their turn inflammation of the visceral adipose tissue (VAT) in obese patients [14-16]. This inflammation might contribute to the onset of IR [14,17]. The prevalence of hypomagnesemia in T2DM has been reported to range

between 14 and 48%, meaning that millions of people worldwide are affected [18-20]. In DM patients, magnesium deficiency is associated with the development of micro- and macrovascular complications [21-24]. A few papers have been published that specifically investigated the role of magnesium deficiency in the emergence of insulin resistance. Some papers confirmed a causative role for magnesium deficiency [25,26] and others did not find any associations [23,27]. Most studies were performed in rather small populations.

The aim of the present study was first to investigate the association between hypomagnesaemia and IR in a large group of well-characterized subjects with different degrees of IR and insulin secretory potential. Second, we aimed to investigate by means of a multivariate analysis if hypomagnesemia might serve as an independent risk factor for IR and third, to define a cut-off value for the homeostatic model assessment for insulin resistance (HOMA-IR) in a Western population as there is no consensus to define a cut-off value worldwide and HOMA-IR among others depends on race [28-30].

Materials and Methods

Subjects

The population consists of two prospectively recruited subject groups with different degrees of IR and insulin secretory potential from the department of Endocrinology, Diabetology and Metabolic diseases in the Antwerp University Hospital (a tertiary referral facility). For the current study, the collected data were retrospectively analyzed. All procedures followed were approved by the Ethics Committee of the Antwerp University Hospital [1,10,32] and were conducted in accordance with the Helsinki Declaration of 1975, as revised in 2008. Informed consent was obtained from all patients for being included in the study.

The first database contains data of patients presenting with overweight (body mass index (BMI) 25-30 kg/m^2) or obesity (BMI ≥ 30 kg/m^2) at an outpatient obesity clinic.

Patients consulted the clinic at their own initiative or they were referred by their treating family physician. Every patient underwent a standard metabolic work-up. Patients were ≥ 18 years. At their enrolment, patients were not involved in a weight management program. Glucose tolerance status was defined based on the criteria of the American Diabetes Association [31].

The second database contains data of adult T2DM subjects (>18 years old) with disease duration of at least 2 years. As glucose lowering therapy they were only treated with metformin and/or sulfonylurea.

A total of 2731 obese and/or T2DM patients were included. The flowchart for patient inclusion is shown in Figure 1.

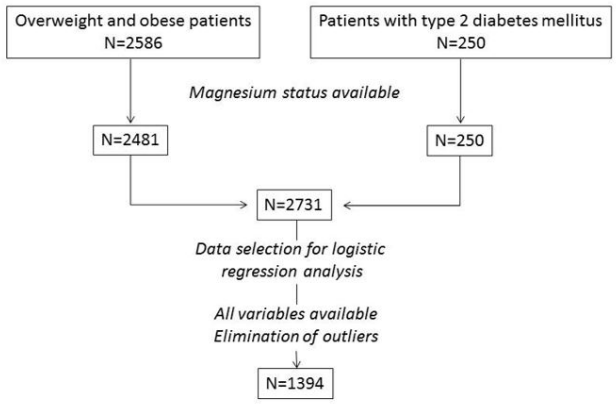

Figure 1: Flowchart of the included patients. Data selection for logistic regression analysis was based 4 on following criteria: factors had to be determined in at least 50% of the population and only 5 parameters that significantly differed between both groups were withheld.

Patient parameters

The following parameters were retrieved from the medical records in our study design: ethnicity, gender, age, anthropometric measures including measurement of visceral and subcutaneous adipose tissue by computed tomography (CT), plasma and urine standard biochemical tests, hormonal assessment, glucose status using oral glucose tolerance test (OGTT) and glycated hemoglobin (HbA1c).

Definitions

Magnesium: Hypomagnesaemia was defined as a serum magnesium concentration <1.7 mg/dl [32-35]. The fractional excretion of magnesium (FE Mg^{2+}) was computed using the following equation: (100 × [Magnesium in urine (mg/d) × serum creatinine (mg/dl)]/ [(0.7 × serum magnesium (mg/dl)) × creatinine in urine (mg/d)]] [6,36,37].

Metabolic syndrome: Metabolic syndrome was defined by the criteria from the HARMONIZING study by Alberti et al.: 1) elevated triglycerides (>150 mg/dL) or drug treatment for elevated triglycerides, 2) reduced high density lipoprotein (HDL) (<40 mg/dL in males or <50 mg/dL in females) or drug treatment for reduced HDL, 3) elevated blood pressure (BP) (>130/85 mmHg) or antihypertensive drug treatment, 4) elevated fasting glucose (>100 mg/dL) or drug treatment for elevated fasting glucose and 5) elevated waist circumference (>94 cm in Caucasian males or >80 cm in Caucasian females)[38] Patients with an elevated waist circumference and >2 other criteria were considered to have metabolic syndrome [39].

Diabetes Mellitus: T2DM was defined by the criteria from the American Diabetes Association Classification, more precisely a fasting glucose >126 mg/dL and/or a HbA1c >6.5% and/or a 2-hour plasma glucose >200 mg/dL using OGTT [31] Prediabetes was defined as having a fasting glucose between 100-125 mg/dL (impaired fasting glucose) and/or a 2-hour plasma glucose between 140-199 mg/dL using OGTT (impaired glucose tolerance) and/or an HbA1c between 5.7-6.4% [31].

IR was determined using HOMA-IR, calculated as follows: [insulin 0' (mU/l) × glucose 0' (mmol/l)]/22.5 [39].

Statistical analysis

Distributions of continuous data were tested for normality by histograms and by the Kolmogorov-Smirnov test. Variables with a skewed distribution were transformed to a logarithmic scale and re-evaluated for normality.

The unpaired Student's t-test or Mann-Whitney-U-test was used to determine differences between 2 groups, with Bonferroni adjustments for multiple comparisons. Data are expressed as mean ± standard deviation (SD) or median [minimum-maximum]. Differences in distributions of categorical data were evaluated by χ^2 or Fisher Exact test and expressed as percentages. Pearson correlation analysis was performed to test the link between serum magnesium and HOMA-IR. Stepwise logistic regression analysis was performed to assess the strength and independency of associations. First, the regression analysis was performed using a backward logistic regression method with and without serum magnesium included. Since serum magnesium was not included in this model for the prediction of HOMA-IR but we aimed at investigating its predictive capacities, the predictive variables were included in a new regression analysis together with serum magnesium using an ENTER method. To evaluate the influence of magnesium, the probabilities of both models were compared using ROC analysis. A two-tailed $p<0.05$ was considered significant.

Ultimately, the probabilities that were obtained after the LR regression analyses were used to perform a partial correlation analysis between HOMA-IR and magnesium serum, corrected for confounding factors.

HOMA-IR was used as a surrogate variable for IR. A cut-off point was defined to divide the population in patients with a higher versus a lower insulin sensitivity.

There is no consensus for a cut-off point for HOMA-IR worldwide and this cut-off value differs between the different races. A cut-off point for a Belgian population has not been defined yet [28] Receiver-operating characteristics curves (ROC) were used to determine the HOMA-IR value with the highest combined sensitivity and specificity for metabolic syndrome (featured by IR) as state variable. This value was used as a cut-off point for IR in our Belgian population.

Data were analyzed using SPSS (SPSS Inc., Chicago, USA) version 22.

Results

Population

The population predominantly existed of Caucasians (86%) with a mean age of 44.6 ± 13.5 years. Most subjects were female (70.7%) amongst whom 23.2% were in a postmenopausal state.

Patients had a mean BMI of 36.1 ± 7.0 kg/cm² and 82% were obese. The mean waist circumference was 107 ± 15 cm in women and 117 ± 17 cm in men. The total mean total cholesterol was 210 ± 40 mg/dl and 65% of the patients had hypertension or were treated with antihypertensive therapy. Fifty-one percent of the patients were diagnosed with metabolic syndrome. Seventy-one percent of the patients met the criteria for prediabetes. Eighteen percent of the population had T2DM. The mean HbA1c in the entire population was 6.0 ± 1.5%. The mean HOMA-IR value was 4.2 ± 3.8.

In the entire population, serum magnesium averaged 1.9 ± 0.2 mg/dl and 6.1% of the patients were diagnosed with hypomagnesaemia. When analyzing both groups separately, 3.4% of the obese patients had hypomagnesemia and 18.2% of the individuals with diabetes mellitus.

Cut-off point for HOMA-IR

The HOMA-IR value of 2.82 had the highest combined specificity (0.609) and sensitivity (0.749) and was used as cut-off point in our study population (Figure 2). This cut-off value is in line with previously reported HOMA-IR values pointing to insulin resistance so from now on used to define IR throughout this paper. Fifty-seven per cent of the population had a HOMA-IR value above the cut-off point.

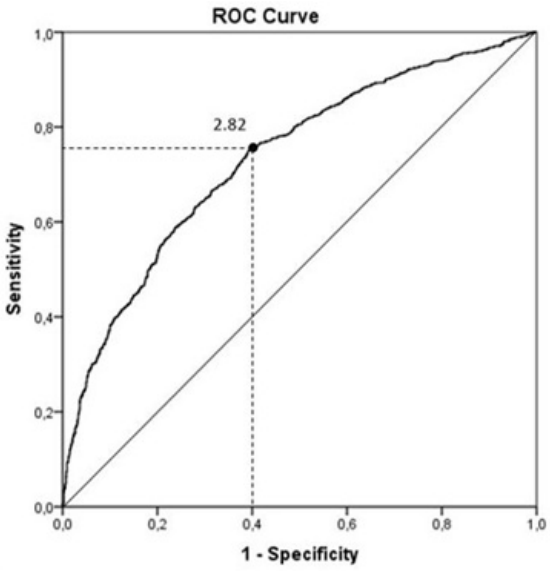

Figure 2: ROC-curve to define the HOMA-IR cut-off point for insulin resistance in Western population 2 of obese patients and patients with T2DM. HOMA-IR was plotted against metabolic syndrome as a 3 state variable of insulin resistance. The best results were obtained with a HOMA-IR of 2.82 (sensitivity 4 of 0.749 and specificity of 0.609). The area under the curve (AUC) was 0.733.

Characterization of insulin resistant patients

Patients with IR were compared to those with normal insulin sensitivity. Amongst others, the following parameters significantly differed between the insulin resistant and the non-insulin resistant group (Table 1): gender ($p<0.001$), age ($p<0.001$), BMI ($p<0.001$), waist ($p<0.001$), hypertension ($p<0.001$), metabolic syndrome ($p<0.001$), serum Mg^{2+} ($p<0.001$), fasting glucose ($p<0.001$), glucose 120' in OGTT ($p<0.001$), HbA1c ($p<0.001$) and T2DM ($p<0.001$). Factors that were determined in at least 50% of the population and significantly differed between both groups were selected for further logistic regression analysis.

Variables	Mean/Median*/Percentage		p-value
	HOMA-IR < 2.82(n=1161)	HOMA-IR ≥ 2.82 (n=1561)	
Gender (M)	214 (18.4%)	382 (37.3%)	p<0.001
Age (years)	43.29 (± 12.73)	45.40 (± 13.93)	p<0.001
Anthropometry			
Weight (kg)*	93.20 (47.00-213.60)	104.40 (54.10-226.60)	p<0.001
BMI (kg/m²)*	33.50 (17.80-60.90)	36.80 (17.30-82.50)	p<0.001
Waist (cm)*	104.35 ± 13.61	115.34 ± 15.63	p<0.001
Waist hip ratio*	0.89 (0.63-1.39)	0.99 (0.64-1.73)	p<0.001
VAT on CT (cm²)*	129 (15-532)	184 (30-590)	p<0.001
SAT on CT (cm²)	510 ± 154.51	537 ± 173.34	p<0.001
Obesity	884 (76.1%)	1339 (85.8%)	p<0.001
Systolic BP (mmHg)	128.66 ± 14.26	134.93 ± 15.75	0.021
Hypertension	577 (48%)	867 (56.8%)	p<0.001
Metabolic syndrome	349 (30.1%)	1040 (66.6%)	p<0.001
Standard biochemical tests			
Leucocytes (x10^9/l)*	6.50 (2.60-14.50)	7.10 (2.10-20.30)	p<0.001
HDL (mg/dl)*	56 (22-128)	46 (14-114)	p<0.001
Triglycerides (mg/dl)*	114 (33-851)	152 (31-845)	p<0.001
Adiponectin (ng/ml)*	10679 (2870-28182)	8606 (2090-30955)	0.004
Leptin (ng/ml)*	22.18 (1.53-103.75)	17.99 (1.00-110.20)	0.035
Serum creatinine (mg/dl)*	0.81 ± 0.14	0.86 ± 0.19	p<0.001
Serum sodium (mmol/l)	140.66 ± 2.03	140.57 ± 2.18	0.009
Serum potassium (mmol/l)	4.17 ± 0.29	4.19 ± 0.33	0.02
Serum chloride (mmol/l)	104.66 ± 2.39	103.78 ± 2.70	p<0.001
Serum calcium (mg/dl)*	9.10 (3.40-11.00)	9.20 (2.90-11.90)	p<0.001
Vitamin D (ng/ml)*	26 (5-150)	20 (3-120)	p<0.001
Magnesium status			
Serum magnesium (mg/dl)*	2.00 (1.00-2.50)	1.90 (0.70-3.70)	p<0.001
Hypomagnesaemia	40 (3.4%)	125 (8%)	p<0.001
FE Mg^{2+} (%)	3.61 (0.05-13.18)	3.50 (0.03-17.21)	
24h urine collection			
Creatinine 24h urine (mg/d)*	1228 (80-4128)	1383 (73-4042)	p<0.001
Calcium in 24h urine (mg/d)*	146.50 (5.50-677.60)	162.50 (3.27-785.00)	p<0.001
Proteins in 24h urine (mg/d)*	73 (0-1095)	59 (0-3465)	0.002
Anorganic phosphate in 24h urine (g/d)	0.90 ± 0.34	0.97 ± 0.39	p<0.001

Free cortisol 24h urine (mg/d)*	58 (6-740)	64 (4-664)	p<0.001
Hormonal assessment			
TSH (µu/ml)*	1.41 (0.01-27.30)	1.50 (0.01-35.31)	0.028
Estradiol (pg/ml)	40.31 ± 44.77	33.51 ± 36.11	p<0.001
Progesterone (ng/ml)	1.18 ± 2.57	0.87 ± 2.14	p<0.001
Testosterone (nmol/l)	2.99 ± 4.62	4.53 ± 5.17	p<0.001
SHBG (nmol/l)*	40 (6-200)	27 (5-200)	p<0.001
Menopause	283 (30.5%)	346 (36.6%)	p< 0.001
Glucose status			
Glucose 0' in OGTT(mg/dl)	83.50 ± 17.20	107.04 ± 44.65	p<0.001
Glucose 120' in OGTT(mg/dl)	122.02 ± 48.45	168.12 ± 87.70	p<0.001
Insulin 0' in OGTT (µU/ml)	8.95 ± 3.25	23.20 ± 12.45	p<0.001
HbA1c (%)*	5.5 (4.5-13.9)	6.1 (4.7-13.5)	p<0.001
Diabetes mellitus type 2	79 (6.8%)	410 (26.2%)	p<0.001
IGF 1 (ng/ml)*	170 (28-560)	160 (29-500)	p<0.001

Variables with a normal distribution were analysed using Student's T-test and displayed as mean +/- standard deviation (SD). Variables with a skewed distribution (*) were analysed using Mann-Whitney U test and displayed as median (range (minimum-maximum)). Nominal variables were analysed using cross-tabs and Chi²-test and displayed as n (%). Mg^{2+}: magnesium; VAT: visceral adipose tissue; CT: computed tomography; SAT: subcutaneous adipose tissue; LDL: low density lipoprotein; HDL: high density lipoprotein; FE Mg^{2+}: fractional excretion of magnesium; FT4: thyroxine; HbA1c: glycosylated haemoglobin; HOMA-IR: homeostatic model assessment for insulin resistance; IGF-1: insulin like growth factor.

Table 1: Differences between patients with high and low HOMA-IR.

Characterization of hypomagnesaemic patients

The population was divided into two groups based on the presence or absence of hypomagnesaemia. In the hypomagnesaemic group, the median serum magnesium level was 1.6 mg/dl (range 0.70-1.70 mg/dl) while in the normomagnesaemic group, the median serum magnesium level was 2.0 mg/dl (range 1.71-3.70 mg/dl, p<0.001). Amongst others, following parameters significantly differed between both groups (Table 2): gender (p=0.021), age (p<0.001), hypertension (p<0.001), HOMA-IR (p<0.001), metabolic syndrome (p=0.001), fasting glucose (p<0.001), glucose 120' in OGTT (p<0.001), HbA1c (p<0.001) and T2DM (p<0.001). BMI (p=0.643) and waist (p=0.064) did not significantly differ between normomagnesaemic and hypomagnesaemic patients.

Variables	**Mean/Median*/Percentage**		**p-value**
	Mg^{2+} serum <1,7 mg/dl (n=176)	**Mg^{2+} serum ≥ 1,7 mg/dl (n=2658)**	
Gender (M)	67 (38%)	792 (30%)	0.021
Age	49.22 ± 13.69	44.35 ± 13.37	<0.001
Anthropometry			
BMI	35.47 (17.3-82.5)	35.15 (17.8-78.5)	0.78
Waist	113 (70-150)	109 (68-193)	0.056
Waist Hip Ratio	1.00 (0.70-1.36)	0.94 (0.63-1.73)	<0.001
VAT on CT (cm^2)*	186 (36-521)	157 (15-590)	0.001
SAT on CT (cm^2)	478 ± 191	528 ± 164	0.005
Metabolic syndrome	92 (57.50%)	1072 (43.44%) (1072)	0.001

Hypertension	65 (37%)	1405 (53%)	<0.001
Standard biochemical tests			
Cholesterol (mg/dl)	208.25 ± 48.71	210.18 ± 39.15	0.005
LDL (mg/dl)	128.91 ± 42.92	128.69 ± 36.54	0.007
HDL (mg/dl)*	45 (14-104)	50 (16-128)	<0.001
Triglycerides (mg/dl)*	158 (38-875)	133 (31-851)	<0.001
Leptin (ng/ml)*	11.94 (1.00-43.17)	19.84 (1.21-110.20)	0.001
Serum creatinine (mg/dl)	0.82 ± 0.21	0.84 ± 0.17	0.008
Serum chloride (mmol/l)*	102.77 ± 3.09	104.25 ± 2.55	<0.001
Serum calcium (mg/dl)*	9.20 (7.80-10.90)	9.20 (2.90-11.90)	0.019
Magnesium status			
FE Mg^{2+} (%)*	4.23 (0.05-15.27)	3.52 (0.03-17.21)	<0.001
Magnesium 24h urine (mg/d)*	67.00 (1-243)	79 (1.20-273.50)	0.002
24h urine collection			
Creatinine in 24h urine (mg/d)*	1230 (410-3064)	1309.50 (73-4128)	0.001
Free cortisol 24h urine (mg/d)*	94.50 (12-598)	60 (4-740)	<0.001
Hormonal assessment			
FT4 (pmol/l)	15.09 ± 3.36	14.31 ± 2.78	0.02
Progesterone (ng/ml)	0.47 ± 0.47	1.04 ± 2.40	<0.001
Menopause	44 (44%)	589 (33.13%)	0.035
Glucose status			
Glucose 0' in OGTT (mg/dl)*	204.50 (100-350)	167 (80-338)	<0.001
Glucose 120' in OGTT	232.28 ± 123.07	143.34 ± 70.05	<0.001
HbA1c% (%)*	8.0 (4.9-13.9)	5.70 (4.5-11.8)	<0.001
Diabetes mellitus type 2	90 (53.89%)	405 (15.80%)	<0.001
HOMA-IR*	5.30 (0.25-55.73)	3.13 (0.04-52)	<0.001
IGF 1 (ng/ml)*	140 (40-390)	160 (28-560)	<0.001

Variables with a normal distribution were analysed using Student's T-test and displayed as mean +/- standard deviation (SD). Variables with a skewed distribution (*) were analysed using Mann-Whitney U test and displayed as median (range (minimum-maximum)). Nominal variables were analysed using cross-tabs and Chi^2-test and displayed as n (%). Mg^{2+}: magnesium; VAT: visceral adipose tissue; CT: computed tomography; SAT: subcutaneous adipose tissue; LDL: low density lipoprotein; HDL: high density lipoprotein; FE Mg^{2+}: fractional excretion of magnesium; FT4: thyroxine; HbA1c: glycosylated haemoglobin; HOMA-IR: homeostatic model assessment for insulin resistance; IGF-1: insulin like growth factor.

Table 2: Differences between patients with and without hypomagnesaemia.

Correlation analysis between insulin resistance and hypomagnesaemia

We found a significant negative correlation (r=-0.157; p<0.001) between serum magnesium and HOMA-IR in a univariate analysis.

Predictors of insulin resistance

The logistic regression analysis revealed a predictive model consisting of the following variables: VAT, creatinine, triglycerides, waist, systolic blood pressure, estradiol, age, serum chloride, 24h-proteinuria, gender and HDL as presented in Table 3. The regression analysis including serum magnesium showed that serum magnesium could not be withheld as a significant predictor for HOMA-IR (p=0.593) in our population.

Variables	Exp (B)	95% confidence interval		p-value
		Lower	Upper	
VAT on CT* (cm^2)	11.154	3.878	32.081	p<0.001
HDL* (mg/dl)	3.485	1.385	8.766	p=0.008
Triglycerides* (mg/dl)	3.282	1.66	6.492	p=0.001
Serum creatinine (mg/dl)	1.04	1.027	1.054	p<0.001
Gender (M/F)	1.02	1.011	1.03	p<0.001
Protein in 24h urine* (mg/24h)	0.997	0.994	1	p=0.038
Serum chloride (mmol/l)	0.96	0.948	0.973	p<0.001
Age (years)	0.929	0.884	0.98	p=0.007
Waist (cm)	0.616	0.515	0.735	p<0.001
Systolic blood pressure (mmHg)	0.536	0.362	0.794	p=0.002
Estradiol (pg/ml)	0.091	0.026	0.319	p<0.001
Serum Mg^{2+} (mg/dl)	2.299	0.108	48.721	P=0.593

Variables with skewed distribution (*): logarithmic function was used. VAT: visceral adipose tissue; CT: computed tomography; HDL: high density lipoprotein; M/F: male/female.

Table 3: Multivariate logistic regression: predictive variables for HOMA-IR.

Partial correlation analysis

A new correlation analysis between HOMA-IR and serum magnesium was performed in which we corrected for the predictors of HOMA-IR as identified in the regression analysis. After correction for these predictors, a significant negative correlation between HOMA-IR and serum magnesium remained (r=-0.085; p<0.001) indicating that serum magnesium does play a role in IR although the effect is too small to be confirmed in a regression model.

Discussion

This study showed an association between hypomagnesaemia and IR in a large representative sample of an obesity clinic, including all obese subjects and not only those suspected of insulin resistance. However, we could not prove that hypomagnesemia served as an independent risk factor for IR.

In our population of obese patients and patients with T2DM, hypomagnesaemia was associated with higher HOMA-IR values. This association was confirmed by a significant negative correlation between serum magnesium and HOMA-IR, indicating that patients with lower serum magnesium are more insulin resistant and vice versa. Hypomagnesaemic patients also suffered more from metabolic syndrome and T2DM and had increased VAT on CT compared to patients without hypomagnesaemia. Patients with T2DM had more often hypomagnesaemia and higher HOMA-IR values compared to non-T2DM obese patients. Serum magnesium levels were significantly lower in IR patients compared to non-IR patients.

In this study, we propose a cut-off value for HOMA-IR in a Belgian population. Although HOMA-IR is not the gold standard for assessment of insulin sensitivity, it is a clinically useful index used in many studies [39]. In Western populations the HOMA-IR cut-off value for insulin resistance varies between 2.0 and 3.8 [29] and in a non-Caucasian population, the HOMA-IR cut-off value approximates 2.5 [30]. In the present study, a ROC-analysis was performed in which the presence of the metabolic syndrome was used as a proxy of IR. A HOMA-IR ≥ 2.82 indicated the presence of IR, which is in range with the values found in literature.

In the entire population of our study, only 6.1% of the patients developed hypomagnesemia, which is below reported data in literature. Recently, Guerrero-Romero et al. reported an incidence of 17.1%, 22.4% and 24% in normal-weight, overweight and obese patients respectively [40]. These higher incidences could be explained by the fact that in that paper hypomagnesemia was defined as serum magnesium level below 1.8 mg/dl, in contrast to the present study in which 1.7 mg/dl was used as a cut-off to define hypomagnesemia. And indeed, if we re-analyze our data with a cut-off serum magnesium value of 1.8mg/dl, 15,1% of the entire population is diagnosed with hypomagnesemia: 11.5% of the overweight and obese patients and 32.6% of the patients with DM2, which is more in line with the Guerrero-Romero study and to studies performed in patients with DM2 (14% to 48%) [18-20]. Also, racial differences in serum magnesium levels have been reported. It appeared that white Canadian women develop less frequently hypomagnesemia compared to South Asian women [41]. As most patients in our study were Caucasians, the incidence of hypomagnesemia might be lower compared to studies in which non-Caucasian patients were included.

In the present study, 18% of the obese and overweight patients met the criteria for T2DM, which is in line with other reports on the prevalence of T2DM in overweight and obesity. In a study of Vinci Guerra et al., T2DM was present in 14% of the patients in an obese population [42]. In the APNA study, the prevalence of T2DM in overweight and obesity was 11.5 and 25.2% respectively [43], while in the OBEDIA study, the prevalence was higher (17.8% for overweight and 34.8% for obesity) [44].

With the results from the present study, we substantiate the hypothesis that overweight and obesity are the strongest predictors for IR [45-47]. Our regression analysis showed that in our population VAT was the most important determinant of HOMA-IR in accordance with previous research [48]. Other important determinants (Table 3) of HOMA-IR identified in our population were triglycerides and waist circumference, in line with other data [49-54]. Other parameters that were predictive for IR in this study cohort include HDL, serum creatinine, female gender, urine protein level, serum chloride, age, systolic blood pressure and estradiol. In a recent paper, female gender was found to be a predictor of IR [55]. Proteinuria and increased serum creatinine may point to increased IR as hyperinsulinemia may induce glomerular hyperfiltration, endothelial dysfunction, and increased vascular permeability, leading to proteinuria. In turn, proteins in the tubular lumen may lead to tubulointerstitial injury and fibrosis, reflected by an increased serum creatinine [56].

T2DM patients had higher HOMA-IR values compared to obese patients, indicating that they were more insulin resistant as also shown in previous studies. Based on our results, hypomagnesaemia is associated with both higher HOMA-IR values and more VAT. In turn, VAT is associated with both hypomagnesaemia and higher HOMA-IR values. This raises the question whether the relation between

hypomagnesaemia and the higher HOMA-IR values exists independent of the excessive presence of VAT. In addition, hypomagnesaemia is the most frequent electrolyte disorder seen in T2DM patients as was also the case in our population [18-20,57], so the association between hypomagnesaemia and higher HOMA-IR might be purely related to the higher prevalence of T2DM in hypomagnesaemia patients. To clarify these associations, further research with a longitudinal set-up is definitely warranted.

Serum magnesium could not be withheld as an independent determinant of HOMA-IR. Nevertheless, after correction for the independent determinants of HOMA-IR, including VAT and T2DM, the negative correlation between magnesium and HOMA-IR remained significant indicating that serum magnesium is independently associated with IR. From these analyses, we can conclude that hypomagnesemia is probably a consequence of IR and not the other way around.

The most important limitation of our study is the fact that it is a cross sectional study making it impossible to investigate causality of our findings. The patients who were included into our study were all followed in a tertiary hospital. Another limitation is the fact that all patients were prospectively included as part of another trial and retrospectively analyzed for the current study. Therefore, some essential information is lacking such as detailed drug and alcohol intake, two conditions that might cause hypomagnesemia [36, 58, 59]. On the other hand, our research question was to investigate if hypomagnesemia might induce metabolic disturbances and not the causative factors of hypomagnesemia. In addition, the analyses were performed in a large group of well-characterized subjects offering a big advantage, thereby using the most suitable methodologies.

Conclusion

In conclusion, our results confirm the major role of VAT and obesity in IR. However, the role of magnesium in the development of IR as suggested in literature could not be confirmed in our cohort. Based on our results, the association between serum magnesium and HOMA-IR values could not be confirmed in the logistic regression analysis. We therefore conclude that serum magnesium cannot be appointed as a determining factor for IR in this large population of obese patients and patients with T2DM. Whether hypomagnesaemia plays a role in the development of IR can only be answered by a prospective longitudinal study.

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