SUMMARY - Background: Low magnesium (Mg) status has been implied as a factor in the development of type 2 diabetes mellitus (DM) and its complications. We therefore studied Mg-status in identical twins, discordant for type 2 DM and in matched controls. Through correlation analysis, possible associations between Mg-status and glucose uptake were evaluated.

Methods: Plasma Mg concentration was measured in 12 monozygote twin pairs, discordant for type 2 DM and in 12 matched controls. Muscle Mg content was measured in 10 persons from each group. An oral glucose tolerance test and a euglycaemic, hyperinsulinaemic clamp were utilized.

Results: Neither muscle Mg content nor plasma Mg concentration differed among groups. Plasma Mg concentration decreased during the euglycaemic, hyperinsulinaemic clamp. In the control group, muscle Mg content correlated positively with insulin stimulated glucose disposal rate ($r = 0.77$, $p < 0.01$) and negatively with two hour plasma glucose concentration during an oral glucose tolerance test (OGTT) ($r = –0.84$, $p < 0.05$). In the control group, the two hour plasma glucose concentration during an oral glucose tolerance test correlated with the decrease in plasma Mg concentration ($r = –0.80$, $p < 0.002$) and with the change in muscle Mg content ($r = 0.90$, $p < 0.0005$) induced by the clamp. None of these associations were found in the two twin groups.

Conclusions: Normal plasma Mg concentration and muscle Mg content were found in persons with type 2 DM and in persons, who were heavily predisposed to the development of type 2 DM, indicating a normal whole-body Mg content. However, the missing associations between measures of glucose disposal and changes in both plasma Mg concentration and muscle Mg content in the two twin groups indicates, that physiological mechanisms, which partly regulates insulin sensitivity and Mg status in healthy individuals are either exhausted or fully utilized in both type 2 DM and in genetically identical twins without DM.

Key-words: genetics, insulin resistance syndrome, metabolic syndrome, syndrome X.

RÉSUMÉ - Contenu du muscle squelettique en magnésium chez des jumeaux identiques discordants pour le diabète de type 2.

Contexte : Un status déficitaire en magnésium (Mg) a été impliqué comme un facteur de développement du diabète de type 2 (DM) et de ses complications. Ainsi, nous avons étudié le status en magnésium chez des jumeaux identiques discordants pour le diabète de type 2 et chez des témoins appariés. A travers une analyse de corrélation, des associations possibles entre le status en Mg et la captation du glucose ont été évaluées.

Les concentrations plasmatiques en Mg ont été mesurées chez 12 paires de jumeaux monozygotes, discordants pour le DM de type 2 et 12 témoins appariés. Le contenu musculaire en Mg a été mesuré chez 10 sujets de chaque groupe. Un test de tolérance orale au glucose et un clamp euglycémique hyperinsulinémique ont été pratiqués.

Résultats : Les groupes ne différaient pas pour le contenu musculaire en Mg ni pour le taux plasmatique en Mg. Le taux plasmatique en Mg diminue pendant le clamp euglycémique hyperinsulinémique. Dans le groupe contrôle, le contenu musculaire en Mg est corrélé positivement avec le taux d’utilisation stimulée par l’insuline du glucose ($r = 0.77$, $p < 0.01$) et négativement avec la glycémie à 2 heures pendant le test de tolérance orale au glucose (OGTT) ($r = –0.84$, $p < 0.05$). Dans le groupe contrôle, la glycémie à 2 heures pendant le test de tolérance orale au glucose est corrélée avec la diminution du Mg plasmatique ($r = –0.80$, $p < 0.002$) et avec les modifications du contenu musculaire en Mg ($r = 0.90$, $p < 0.0005$) induites par le clamp. Aucune de ces associations n’est retrouvée dans les deux groupes de jumeaux.

Conclusions : Des taux plasmatiques en Mg et un contenu musculaire en Mg normaux ont été observés chez des diabétiques de type 2 et chez des sujets fortement prédisposés à développer un diabète de type 2, indiquant un contenu corporel normal en Mg. Cependant, l’absence d’association entre les mesures d’utilisation du glucose et les changements dans les taux plasmatiques et musculaires en Mg dans les deux groupes de jumeaux indique que les mécanismes physiologiques qui régulent en partie la sensibilité à l’insuline et le status en Mg d’individus sains sont soit épuisés soit totalement utilisés à la fois chez le diabétique de type 2 et chez le jumeau génétiquement identique non diabétique.

Mots-clés : génétique, syndrome d’insulinorésistance, syndrome métabolique, syndrome X.

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Both in patients with type 1 diabetes mellitus (DM) [1-3] and in patients with type 2 DM [4], a decreased content of magnesium (Mg) has been found in skeletal muscle. At least part of the etiology for this low Mg status is an increased renal Mg excretion, partly due to imperfect metabolic control [5, 6] and partly due to hyperinsulinaemia [7]. In other categories of patients, a low Mg status has been related to atherosclerosis [8-13], hypercholesterolaemia [14], hypertriglyceridaemia [15, 16], and hypertension [16, 17]. Therefore, a low Mg status might be related to the development of diabetic late complications. Furthermore, it has been proposed that the low Mg status found in type 2 diabetic patients is important in etiology for the increased insulin resistance found in type 2 diabetic patients [16, 18, 19]. However, so far Mg supplementation has had no major effect upon the metabolic control in patients with type 2 DM [20-25]. In a recent study, we actually found a reduction in insulin sensitivity after Mg supplementation to patients with type 1 DM [26].

The aim of the present study was to evaluate the Mg content of skeletal muscle and plasma Mg concentration in patients with type 2 DM. In order to identify a possible familial component, a twin study design was used. Furthermore, a control group consisting of supposedly healthy individuals was included, and associations with glucose uptake metabolism were evaluated using correlation analysis.

### SUBJECTS AND METHODS

#### Subjects

12 pairs of identical twins discordant for type 2 DM were identified, primarily through the Danish Twin Register. A detailed description of the selection of cases and their characteristics has been presented elsewhere [27]. In brief, questionnaires were sent to twin pairs, born between 1918 and 1940, who were recorded as being monozygotic. A total of 626 twin pairs were asked whether they suffered from diabetes and their age at diagnosis. Potential participants were twin pairs discordant for DM, in whom type 2 DM was diagnosed after the age of 40 years. A total of 12 discordant twin pairs were found to be eligible for the study [27]. For each twin pair, one healthy control subject was studied. The clinical characteristics of the twin pairs and their controls during the study have been described in detail elsewhere [27] and can be seen in Table I. Plasma Mg concentration was analysed in all 12 twin pairs and in their matched controls. In two of the twin pairs, no muscle biopsy specimens were available for analysis. Excluding the two sets of cases and controls, in whom no muscle biopsy specimens were obtained did not change the basal parameters. Compared with the control group, the waist/hip ratios were higher in both the « healthy » twins and the twins with type 2 DM. The individual values of 2-hour plasma glucose concentration during the oral glucose tolerance test in the healthy subjects and in the « healthy » twins can be seen from Figures 1 and 2.

Of the 10 patients with type 2 DM, from whom a muscle biopsy specimen were obtained, 5 were treated with sulfonylurea, two patients were treated with metformin, one patient was treated with insulin,

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Diabetes duration (years)</th>
<th>BMI (kg/m²)</th>
<th>Waist/hip ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>62.5</td>
<td>25.4</td>
<td>0.89</td>
</tr>
<tr>
<td>(45 to 71)</td>
<td></td>
<td>(23.7 to 27.1)</td>
<td>(0.82 to 0.96)</td>
</tr>
<tr>
<td>«Healthy» twins</td>
<td>65</td>
<td>27.5</td>
<td>0.99</td>
</tr>
<tr>
<td>(44 to 72)</td>
<td></td>
<td>(24.7 to 30.3)</td>
<td>(0.94 to 1.05)</td>
</tr>
<tr>
<td>Type 2 diabetic</td>
<td>65</td>
<td>30.1</td>
<td>1.00 a</td>
</tr>
<tr>
<td>(44 to 72)</td>
<td></td>
<td>(27.3 to 32.8)</td>
<td>(0.95 to 1.04)</td>
</tr>
</tbody>
</table>

N = 12. aANOVA : p < 0.01.
and two were treated with diet alone. The remaining two patients, from whom only plasma samples were available, were treated with diet alone. The study was approved by the local Ethical Committee. Informed consent was obtained from all subjects.

Design and methods

The study design is described in detail elsewhere [27]. All medications were withdrawn at least 72 hours prior to studies, and the subjects were studied after a 10-hour overnight fast. Plasma glucose concentration was normalised in the twins with type 2 DM by the infusion of insulin. Following the latter, a baseline period lasting 120 min was utilized, at the end of which plasma sampling was performed 4 times with 10 min intervals. Then insulin (Actrapid, Novo-Nordisk, Bagsværd, Denmark) was infused for 180 min at a constant rate of 40 mU/m^2/min^-1 in all subjects, suppressing hepatic glucose production [27]. Plasma glucose concentration was maintained constant at a euglycaemic level using a variable glucose (180 g/l) infusion rate. Plasma sampling was performed four times, with 10 min intervals, during the last 30 min of this period. An oral glucose tolerance test was performed on all subjects.

Muscle biopsy specimens were obtained at the end of the basal period and at the end of the infusion of insulin and glucose. Muscle Mg content was determined after freeze-drying and dissection of the muscle biopsy specimens as described in detail elsewhere [28]. The twin pairs and their control were measured in the same series, without knowledge of diabetic status.

Plasma Mg concentrations were means of 4 determinations before, and 4 determinations during the last 30 min of the infusion of insulin and glucose. Mg concentrations were measured by atomic absorption spectrophotometry.
The determination of baseline and clamp insulin-stimulated glucose turnover has been described in detail elsewhere [27]. Arterialized venous blood was obtained. Plasma glucose concentrations were determined using an automated glucose oxidase method (Glucose Analyzer 2, Beckman Instruments, Fullerton, CA, USA).

Statistics

Statistical analyses were performed using the SPSS/PC + package [29]. After assuring that a Gaussian distribution could not be rejected using the Kolmogorov-Smirnov test, the Mg data were examined for differences among groups and the effect of the infusion of insulin and glucose using analysis of variance (ANOVA) or repeated measures ANOVA as appropriate. Relations between variables were evaluated using correlation analysis. Differences between correlation coefficients were evaluated as described in the Geigy Scientific Tables [30], significance limit $\chi^2_0$, $p$-values less than 0.05 were considered significant.

RESULTS

No difference was found among groups in muscle Mg content, and the infusion of insulin and glucose did not affect muscle Mg content (Table II). There were no overall differences in plasma Mg concentration among groups (Table II). The infusion of insulin and glucose induced a slight decrease in plasma Mg concentration (Table II). The insulin-stimulated glucose disposal rate in skeletal muscles correlated positively with muscle Mg content in the control group, but not in the twin groups (Table III). No association was found between muscle Mg content and basal glucose disposal rate (Table III). In the control group, the 2-hour plasma glucose concentration, during an OGTT, correlated negatively with basal muscle Mg content (Table III).

As plasma Mg concentration decreased, at least in the control group due to the infusion of insulin and glucose, the associations between the decrease in plasma Mg concentration and the various parameters related to glucose tolerance, were evaluated statistically. A negative correlation coefficient was found between the change in plasma Mg concentration induced by the infusion of insulin and glucose and the two hour plasma glucose concentration during the oral glucose tolerance test in the control group (Fig. 1). This relation was not present in the two groups formed by the twins (Fig. 1). This prompted the statistical evaluation of the two hour plasma glucose concentration during oral glucose tolerance testing as a function of the change in muscle Mg content due to the infu-

| Table II. Muscle magnesium (Mg) content and plasma Mg concentrations. |
|---------------------------------|-----------------|-----------------|-----------------|
|                                  | Control         | “Healthy” twins | Type 2 twins    |
| Muscle Mg content before intravenous | 32.5            | 30.8            | 30.9            |
| insulin/glucose, mmol/kg dry weight | (30.0 to 35.0) | (28.8 to 32.8)  | (29.0 to 32.9)  |
| Muscle Mg content after intravenous | 30.7            | 30.8            | 30.7            |
| insulin/glucose, mmol/kg dry weight | (28.0 to 33.4)  | (28.7 to 32.9)  | (28.1 to 33.2)  |
| Plasma Mg concentration before intravenous | 0.93            | 0.92            | 0.87            |
| insulin/glucose, mmol/l | (0.89 to 0.96)  | (0.88 to 0.95)  | (0.83 to 0.91)  |
| Plasma Mg concentration after intravenous | 0.90            | 0.89            | 0.86a |
| insulin/glucose, mmol/l | (0.85 to 0.94)  | (0.85 to 0.93)  | (0.83 to 0.90)  |

Means and their 95% CI. Regarding muscle Mg content n = 10 and regarding plasma Mg concentration n = 12.

*Repeated measures ANOVA: No difference among groups, plasma Mg concentration decreased due to the infusion of insulin and glucose, $p < 0.005.$
Table III. Correlation coefficients between muscle magnesium content before intravenous infusion of insulin/glucose and metabolic variables.

<table>
<thead>
<tr>
<th>Muscle magnesium content</th>
<th>Controls</th>
<th>&quot;Healthy&quot; twins</th>
<th>Type 2 twins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal glucose disposal</td>
<td>−0.54</td>
<td>−0.45</td>
<td>−0.40</td>
</tr>
<tr>
<td>rate</td>
<td>(−0.87 to 0.13)</td>
<td>(−0.84 to 0.25)</td>
<td>(−0.82 to 0.30)</td>
</tr>
<tr>
<td>Insulin-stimulated</td>
<td>0.77ab</td>
<td>−0.44</td>
<td>0.35</td>
</tr>
<tr>
<td>glucose disposal rate</td>
<td>(0.26 to 0.94)</td>
<td>(−0.84 to 0.27)</td>
<td>(−0.35 to 0.80)</td>
</tr>
<tr>
<td>2-hour plasma glucose</td>
<td>−0.64c</td>
<td>−0.19</td>
<td>-0.25</td>
</tr>
<tr>
<td>concentration during an oral glucose tolerance test</td>
<td>(−0.91 to −2)</td>
<td>(−0.73 to 0.50)</td>
<td>(−0.76 to 0.45)</td>
</tr>
</tbody>
</table>

95% CI are indicated, n = 10.

*p < 0.01. aDiffered from "healthy" twins (p < 0.01). c P < 0.05.

sion of insulin and glucose. It can be seen, from figure 2, that a positive correlation was found in the control group, and that this response differed totally from the response in the other two groups.

**DISCUSSION**

Muscle Mg content in patients with type 2 DM has previously been reported in only two studies [4, 31]. In one of these studies, a 12% reduction in muscle total Mg content was found [4], and in the other no difference could be found between patients with maturity onset diabetes and controls [31]. That muscle total Mg content did not differ between patients with type 2 DM and controls in our study might indeed be correct. After all, the predisposition to develop type 2 DM was so low, that their genetically identical twin had not developed type 2 DM. However, as both hyperinsulinaemia and hyperglycaemia lead to an increased renal Mg excretion [6, 7], muscle Mg content could be decreased in type 2 diabetic patients, although hyperinsulinaemia is not a constant finding in type 2 DM. Patients with type 1 DM are insulin-resistant and on the average, they have higher blood glucose concentrations than patients with type 2 DM. This should lead to a higher renal Mg excretion in patients with type 1 DM compared to patients with type 2 DM. In accordance with this, results regarding muscle Mg content in type 2 DM seems to be rather blurred, compared to the reduced muscle Mg content in patients with type 1 DM [32]. Furthermore, serum total and ionized Mg concentration seems to be lower in patients with type 1 DM compared to patients with type 2 DM [5].

A Mg deficit has been proposed to compromise glucose tolerance in type 2 diabetic patients [16, 18]. In accordance with this, Mg supplementation to patients with type 2 DM has been found to improve insulin-stimulated glucose uptake [18]. However, no improvement in metabolic control has been found so far in clinical studies with Mg supplementation to patients with type 2 DM [20-24], except for a single study, where a high dose of Mg induced a slight decrease in serum fructosamine [25]. In a recent study we found, that Mg seems to be a factor regulating insulin resistance, so that an increase in whole body Mg reduces insulin sensitivity in patients with type 1 DM, where the source of insulin is exogenous. As a reduction in insulin sensitivity should lead to an increase in both plasma glucose and plasma insulin concentrations, this should tend to increase the renal excretion of Mg [5-7], thereby decreasing whole body Mg content and increasing insulin sensitivity [26]. In this way, we propose a feedback regulation of both Mg homeostasis and insulin sensitivity [26]. It is doubtful as to whether one would expect Mg to be the most important factor in determining insulin sensitivity. The results of the present study seems to confirm such a feedback mechanism in the control group, where the highest insulin stimulated glucose uptake rate and lowest 2-hour plasma glucose concentration were associated with the highest basal muscle Mg content. This could indicate, that muscle Mg content is reduced when insulin sensitivity decreases due to other factors regulating insulin sensitivity. This would also explain the observed relations between 2-hour plasma glucose concentration during the OGTT and the decrease in both muscle Mg content and plasma Mg concentration in the control group.
Regarding muscle Mg content, persons with the lowest 2-hour plasma glucose concentrations were the subjects with the largest decrease in muscle Mg content, that is those, that had utilized this manner of increasing insulin sensitivity the least. The present study indicates, that the Mg lost from skeletal muscle cells are cleared elsewhere. Since subjects with the highest 2-hour plasma glucose concentration had the least decrease in muscle Mg content and the largest decrease in plasma Mg concentration, the mechanism involved in the clearance of Mg from plasma seems to be independent of glucose tolerance in healthy subjects, without any predisposition to type 2 DM. These regulatory mechanisms seems to be either exhausted or fully utilized in the prediabetic and diabetic states. Alternatively, it might also be a genetic defect in these mechanisms in subjects, predisposed to the development of type 2 DM, at least at a relatively late stage of their disease. In younger persons, predisposed to type 2 DM, we have found a decrease in muscle Mg content both after infusion of glucose and after the induction of insulin resistance (unpublished). Interestingly, the effect of glucose infusion upon muscle Mg content was abolished after the induction of insulin resistance, both in the group of younger persons, predisposed to the development of type 2 DM and in a matched control group (unpublished). This could indicate, that the feed-back mechanism is fully utilized in persons with insulin resistance.

It has been shown previously that differences in the responses to oral glucose loading exists in plasma Mg among healthy persons, persons with impaired glucose tolerance and persons with type 2 DM [33]. These latter results could argue in favour of a Mg deficit acting as a factor which increases insulin resistance, but they might equally well be the result of an increased renal excretion of Mg in controls with higher insulin [7] and glucose [6] levels. No other group, to our knowledge, has investigated, as yet, the relation between glucose uptake and muscle Mg content in type 2 diabetic patients.

All of our non-significant results should nevertheless be regarded with some skepticism, as the number of observations is small, increasing the risk of making a type 2 error. However, regarding the significant results, the risk of making a type 1 error is still less than 5%. Another drawback to the present study is the fact that the study population was selected, so that the genetic predisposition to the development of type 2 DM isn’t strong enough to induce frank diabetes in the healthy twins. A further reservation, herein, is that correlations between variables only indicate an association between the investigated variables and not necessarily a causal relationship.

In conclusion, normal plasma Mg concentration and muscle Mg content were found both in persons with type 2 DM and in persons, who were heavily predisposed to the development of type 2 DM, indicating a normal whole-body Mg content. In supposedly healthy individuals, associations were found between measures of glucose disposal and both plasma Mg concentration and muscle Mg content, which seems to confirm a physiological feed-back mechanism that partly regulates both whole body Mg content and glucose homeostasis. As none of these associations were found in the two twin groups, this feed-back mechanism seems to be either exhausted or fully utilized in persons with type 2 DM and in genetically identical twins without DM.

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