Postabsorptive and insulin-stimulated energy and protein metabolism in patients with myotonic dystrophy type 1

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ABSTRACT

Background: Exaggerated insulin resistance was described as the major metabolic abnormality in myotonic dystrophy type 1 (DM1). We reported recently that the severity of the impairment in insulin-stimulated glucose metabolism in these patients was overestimated.

Objective: The aim was to dissect out insulin action with respect to whole-body energy homeostasis and glucose, protein, and lipid metabolism in patients with DM1 to assess the relevance of insulin resistance to the heterogeneous clinical manifestations of this syndrome.

Design: Ten nondiabetic patients with DM1 and 10 matched healthy control subjects were studied by means of 1) dual-energy X-ray absorptiometry; 2) a euglycemic-hyperinsulinemic clamp (40 mU · m−2 · min−1) combined with a primed, continuous infusion of [6,6-d2]glucose and [1-13C]leucine; 3) indirect calorimetry; and 4) localized 1H magnetic resonance spectroscopy of the calf muscles.

Results: Patients with DM1 had less lean body mass, greater fat mass, and greater intramyocellular lipid contents than did healthy control subjects. Energy expenditure and glucose and lipid metabolism did not differ significantly between the groups. In contrast, markers of proteolysis were higher in DM1 patients in the postabsorptive and insulin-stimulated conditions and were associated with lower plasma concentrations of insulin-like growth factor 1 (P < 0.03) and higher plasma concentrations of tumor necrosis factor α receptor 2 (P = 0.04).

Conclusions: Despite greater body fat and intramyocellular lipid contents in patients with DM1, insulin sensitivity was not significantly different between patients and control subjects. In contrast, the loss of lean body mass in patients with DM1 was associated with abnormal postabsorptive and insulin-stimulated regulation of protein breakdown. Lower plasma insulin-like growth factor 1 concentrations and higher tumor necrosis factor system activity might be involved in the muscle wasting of DM1. Am J Clin Nutr 2004; 80:357–64.

KEY WORDS Insulin sensitivity, intramyocellular lipid content, leucine metabolism, fatty acid metabolism, indirect calorimetry, 1H magnetic resonance spectroscopy

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is the most common adult form of muscular dystrophy and has an estimated prevalence of 1 in 8000 (1). It is caused by heterozygosity for a trinucleotide repeat expansion mutation in the 3′ untranslated region of a protein kinase gene (DM kinase) located on the q13.3 band of chromosome 19 (2–5). At the onset of symptoms, many organs may be involved, and patients often show cardiac conduction defects, smooth muscle involvement, hypersonnia, premature balding, testicular atrophy, and progressive muscle wasting and weakness. Insulin resistance and hyperinsulinemia were also described as metabolic abnormalities (6–11) and were considered pivotal pathogenic factors of the clinical manifestations of DM1 that could lead to type 2 diabetes and abnormal muscle protein metabolism. Recently, we performed euglycemic-hyperinsulinemic clamp procedures in patients with DM1 before the development of glucose intolerance or overt type 2 diabetes (12). Our results downplayed the relevance of the impairment of insulin-stimulated glucose metabolism and highlighted early alterations in insulin secretion. In light of these results, the present study was undertaken to further explore insulin action, not only with respect to glucose metabolism but also with respect to other insulin-dependent metabolic pathways. Using dual-energy X-ray absorptiometry (DXA), indirect calorimetry, stable-isotope infusion in the postabsorptive condition and during euglycemic insulin stimulation, and 1H magnetic resonance (MR) spectroscopy of the calf muscles, we assessed body composition, energy homeostasis, insulin action, and intramyocellular lipid (IMCL) content in 10 patients with DM1 and in 10 healthy control subjects.

SUBJECTS AND METHODS

Subjects

Ten patients with DM1 were recruited in the Division of Neurology of the Istituto Scientifico H San Raffaele, and healthy

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Euglycemic-hyperinsulinemic clamp

The subjects were admitted to the Metabolic Unit of the Division of Internal Medicine I of the Istituto Scientifico H San Raffaele at 0700 after they had fasted for 10 h overnight. A polytetrafluoroethylene catheter was inserted into an antecubital vein for infusions, and an additional one was inserted retrogradely into a wrist vein for blood sampling. The hand was kept in a heated box throughout the experiment to allow for sampling of arterialized venous blood. A bolus of [6,6-2H2]glucose (5 mg/kg body wt) and of [1,13C]leucine (0.5 mg/kg body wt) was followed by a continuous infusion ([6,6-2H2]glucose: 0.05 mg · kg body wt−1 · min−1 and [1,13C]leucine: 0.007 mg/kg body wt). The [6,6-2H2]glucose and [1,13C]leucine were from Mass Trace (Woburn, MA). Blood samples for postabsorptive plasma glucose, total cholesterol, HDL cholesterol, triacylglycerols, free fatty acids, insulin, C-peptide, leptin, growth hormone, insulin-like growth factor 1 (IGF-1), glucagon, cortisol, and tumor necrosis factor α receptor 2 (TNFR2) were performed in duplicate in the postabsorptive condition. After a 150-min tracer equilibration period, a euglycemic-hyperinsulinemic clamp was performed as previously described (13–15). Insulin was infused at a rate of 40 μU · m−2 · min−1 to reach a plasma insulin concentration of ~350 pmol/L, and the plasma glucose concentration was kept at ~5 mmol/L for an additional 150 min by means of a variable infusion of 20% dextrose. Blood samples for plasma hormones, substrates, and tracer enrichment were drawn every 15 min throughout the study.

Indirect calorimetry

After the subjects had lain quietly for 30 min, REE was measured by continuous indirect calorimetry with a ventilated hood system (SensorMedics 2900 metabolic measurement cart; SensorMedics, Yorba Linda, CA) performed for 30 min during the basal equilibration period and at the end of the euglycemic-hyperinsulinemic clamp as previously described (14, 15). The mean CVs within the session for both oxygen and carbon dioxide measurements were <2%. In our metabolic unit, the daily variability in the REE assessed in 15 healthy subjects in the same period was 2.5 ± 0.7% for oxygen consumption and 3.8 ± 0.7% for carbon dioxide production.

Body composition

DEXA was performed with a Lunar-DPX-IQ scanner (Lunar Corp, Madison, WI). A different scan mode was chosen with respect to each subject’s body size, as suggested by the manufacturer’s operating manual. For regional analysis, three-compartment processing was performed in the arms, trunk, and legs (13, 15). Fat content is expressed as kg fat mass and as % of tissues.

1H MR spectroscopy

1H MR spectroscopy was performed on a GE Signa 1.5-T scanner (General Electric Medical Systems, Milwaukee) with a conventional linear extremity coil as previously described (13, 15). Two 1H spectra were collected from a 15 × 15 × 15-mm volume within the soleus (rich in fiber type I) and tibialis anterior (richer in fiber type IIb) muscles by using a PRESS pulse sequence (repetition time = 2000 ms and echo time = 60 ms); 128 averages were accumulated for each spectrum, with a final acquisition time of 4.5 min. The water signal was suppressed during the acquisition because it would dominate the other metabolite’s

### Table 1

<table>
<thead>
<tr>
<th>Anthropometric and body-composition variables in the study groups†</th>
<th>DM1 patients (n = 8 F, 2 M)</th>
<th>Control subjects (n = 8 F, 2 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>38 ± 4</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>60 ± 4</td>
<td>65 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 3</td>
<td>173 ± 2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.3 ± 1.4</td>
<td>21.7 ± 1.2</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.77 ± 0.01</td>
<td>0.72 ± 0.03</td>
</tr>
<tr>
<td>Ideal body weight (%)</td>
<td>104 ± 4</td>
<td>102 ± 3</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>22.1 ± 2.8</td>
<td>16.4 ± 2.4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>34.1 ± 3.3</td>
<td>25.4 ± 2.6</td>
</tr>
<tr>
<td>Arm fat content (%)</td>
<td>31.4 ± 3.4</td>
<td>21.9 ± 3.4</td>
</tr>
<tr>
<td>Trunk fat content (%)</td>
<td>33.5 ± 3.2</td>
<td>21.3 ± 3.4</td>
</tr>
<tr>
<td>Leg fat content (%)</td>
<td>38.0 ± 2.2</td>
<td>32.1 ± 2.8</td>
</tr>
<tr>
<td>Soleus IMCL content (AU)</td>
<td>239 ± 64</td>
<td>71 ± 6</td>
</tr>
<tr>
<td>Tibialis anterior IMCL content (AU)</td>
<td>238 ± 54</td>
<td>11 ± 2</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>35.6 ± 2.7</td>
<td>46.4 ± 3.0</td>
</tr>
<tr>
<td>Physical activity index†</td>
<td>10.2 ± 0.9</td>
<td>8.9 ± 1.4</td>
</tr>
</tbody>
</table>

† All values are ± SEM. DM1, myotonic dystrophy type 1; IMCL, intramyocellular lipid; AU, arbitrary units.

‡,§ Significantly different from healthy control subjects (Student’s unpaired t test and Bonferroni correction): ‡ P < 0.05, § P < 0.02.

The range of possible scores is 3–15; the lowest value corresponds with the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 MJ/h; eg, bowling) and who participates in sedentary activities during leisure time. The highest value corresponds with the level of physical activity of a person who is very physically active at work (eg, a construction worker), who plays heavy sports (energy expended is ≥1.76 MJ/h; eg, boxing, basketball, football, or rugby), and who is very physically active during leisure time (eg, walking >1 h/d or biking >45 min/d).

volunteers served as control subjects. All control subjects were in good health as assessed by medical history, physical examination, hematologic and urine analysis; they had no family history of diabetes, obesity, or hypertension traced through their grandparents and had sedentary lifestyles. The clinical and laboratory characteristics of the 2 groups of subjects are summarized in Table 1. Informed consent was obtained from all subjects after the purposes, nature, and potential risks of the study were explained to them. The protocol was approved by the Ethical Committee of the Istituto Scientifico H San Raffaele.

Experimental protocol

The subjects were instructed to consume an isocaloric diet (~250 g carbohydrate/d) and to abstain from exercise activity for 3 wk before the studies. Women were studied between days 3 and 10 of the menstrual cycle. Subjects were studied by means of the euglycemic-hyperinsulinemic clamp to assess whole-body insulin sensitivity and by means of indirect calorimetry to assess resting energy expenditure (REE), insulin-stimulated energy expenditure, and glucose and lipid oxidation. Within 2–3 d, they were also studied by means of 1H MR spectroscopy of the calf muscle to assess IMCL content and by DEXA to assess body composition. The MR spectroscopy sessions were performed in the Division of Diagnostic Radiology of the Istituto Scientifico H San Raffaele. DEXA was performed in the Department of Science, Nutrition and Microbiology, Nutrition Section, Università degli Studi di Milano.
peak signals of interest. A third \textsuperscript{1}H spectrum of a triacylglycerol solution inside a glass sphere, positioned within the extremity coil next to the calf, was also obtained during the same session to have an external standard adopted in the same conditions as the subject’s spectra. Post processing of the data, which was executed with Sage/IDL software (GE Medical Systems, Milwaukee), was performed as previously described (13, 15). The T2 value of the muscle CH\textsubscript{2} signal of the IMCL compartment was measured in one DM1 patient and was not significantly different from the T2 values measured in our experimental setting in normal-weight (n = 5) and overweight or obese nondiabetic individuals (n = 2) in whom signal intensities were obtained at 6 different TEIs chosen to linearly fit a monoeponential function.

**Analytic procedures**

Plasma glucose was measured with a Beckman glucose analyzer (12–15). Plasma free fatty acids and plasma total cholesterol, HDL cholesterol, and triacylglycerol were measured as previously described (13). LDL cholesterol was calculated by using the Friedewald formula. Plasma insulin was measured with a microparticle enzyme immunoassay technology (16; IMx Insulin assay; Abbott Laboratories, Rome), and C-peptide was measured by radioimmunoassay with a double-antibody (17). Plasma growth hormone, glucagon, and cortisol concentrations were assessed as previously described (17); IGF-1 was measured with a radioimmunoassay (RIA-coated; Medgenics Diagnostics SA, Fleurus, Belgium) according to the manufacturer’s recommendations. TNFR2 was measured with an enzyme immunoassay (Immunotech; Beckman Coulter Co, Marseille, France) as previously described (13, 15). Plasma leptin concentrations were measured as previously described (13, 15) by radioimmunoassay with a double-antibody (17). Plasma growth hormone, glucagon, and cortisol concentrations were assessed as previously described (17); IGF-1 was measured with a radioimmunoassay (RIA-coated; Medgenics Diagnostics SA, Fleurus, Belgium) according to the manufacturer’s recommendations. TNFR2 was measured with an enzyme immunoassay (Immunotech; Beckman Coulter Co, Marseille, France) as previously described (13, 15). Plasma leptin concentrations were measured as previously described (13, 15) by radioimmunoassay with a human kit (Linco Research Inc, St Charles, MO). The \textsubscript{d}5-glucose and [1-\textsuperscript{13}C]\textsubscript{2}leucine enrichments were measured by gas chromatography–mass spectrometry as previously described (18). Expansion size in DM1 patients was assessed on DNA extracted from peripheral blood samples as previously described (2). All patients had an expansion size >150 triplets (minimum = 150, maximum = 1000) for an average of 524 ± 149 triplets.

**Calculations**

Glucose kinetics were calculated by using Steele’s equations for the nonsteady state (18). We did not attempt to describe intracellular leucine kinetics with equations for the nonsteady state, because this approach would imply many more assumptions than the simpler, monocompartmental approach. To define the leucine release from proteolysis (endogenous leucine flux, or ELF), the intracellular leucine enrichments were estimated by the plasma [1-\textsuperscript{13}C]ketoisocaproic acid enrichments, which are derived from the intracellular leucine reciprocal pool approach with the standard steady-state equation as previously described (18). Endogenous glucose production was calculated by subtracting the glucose infusion rate from the rate of glucose appearance measured with the isotope tracer technique. Total-body glucose uptake was determined during the clamp by adding the rate of residual endogenous glucose production to the exogenous glucose infusion rate. REE was calculated by Weir’s standard equation (19) from the rates of oxygen consumption and carbon dioxide production measured by means of indirect calorimetry (excluding the first 10 min of data acquisition) and from urinary nitrogen excretion. Predicted REE was calculated by using the Harris-Benedict equations (20). Glucose, lipid, and protein oxidation were estimated as previously described (14, 15). Nonoxidative glucose disposal was calculated by subtracting the glucose oxidation rate from the tissue glucose disposal. Protein oxidation was estimated from urinary nitrogen excretion (21).

**Statistical analysis**

All data are presented as means ± SEMs. Analyses were performed by using SAS software (version 8.2; SAS Institute Inc, Cary, NC). Steady state was defined as a nonsignificant correlation with time (P > 0.05) by standard linear regression. Independent t tests were used to compare basal values between groups for each variable. Independent t tests were also used to compare, between groups, the percentage suppression or stimulation of each variable during the insulin clamp. A two-factor repeated-measures analysis of variance was used to assess group, insulin, and group-by-insulin interactions on the parameters of energy homeostasis and glucose, lipid, and protein metabolism during the clamp when appropriate. Correlations between IGF-1, TNFR2, and ELF were reported after excluding significant interactions (the GLM procedure in SAS). Stepwise regression analysis was performed, with use of an F-ratio-to-remove of 4 and an F-ratio-to-enter of 3.996, to assess the partial R\textsuperscript{2} and model R\textsuperscript{2} of IGF-1 and TNFR2 with the use of postabsorptive ELF as a dependent variable.

**RESULTS**

**Anthropometric characteristics and body composition**

The patients and the control subjects did not differ significantly by sex, age, body weight, height, body mass index, waist-to-hip ratio, ideal body weight, or physical activity. Patients with DM1 were characterized by altered body composition: they had a smaller lean body mass (LBM; P < 0.02; Table 1) and a slightly higher fat mass, which altogether caused a higher percent body fat component (P < 0.05), particularly in the arms and trunk (P < 0.05; Table 1). In addition, patients with DM1 had larger IMCL contents in both the soleus and tibialis anterior muscles than did the healthy control subjects (Table 1). The IMCL content of the 2 muscles in the control subjects differed significantly (P = 0.02), which confirms previous results. However, this was not the case in the DM1 patients. This suggests a generalized abnormality in the skeletal muscle of these patients that affects both type I and type II fibers.

**Energy homeostasis in the postabsorptive and clamp conditions**

The REE of the DM1 patients was lower than that of the healthy control subjects (P < 0.02) and was lower than predicted values according to the Harris-Benedict equations (Table 2). When it was normalized for kg LBM, however, REE was not significantly different between the groups (Table 2).

**Glucose metabolism in the postabsorptive and clamp conditions**

As shown in Table 3, postabsorptive plasma glucose concentrations were lower in the DM1 patients than in the control subjects (P < 0.02), whereas endogenous glucose production rates did not differ significantly between the groups (P = 0.198). Glucose disposal during the last hour of the clamp, expressed per kg LBM, was
Glucose, lipid, and protein metabolism in the postabsorptive (basal) and insulin-stimulated (insulin) conditions

**TABLE 2**

Energy expenditure of the study groups in the postabsorptive (basal) and clamp (insulin) conditions

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Control subjects</th>
<th>Insulin</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>REE (kcal/d)</td>
<td>1191 ± 93</td>
<td>1560 ± 102</td>
<td>1211 ± 83</td>
<td>1602 ± 157</td>
</tr>
<tr>
<td>Measured REE/predicted REE (%)</td>
<td>87 ± 4</td>
<td>97 ± 2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>REE/kg body wt (kcal·kg⁻¹·d⁻¹)</td>
<td>20.2 ± 0.9²</td>
<td>20.6 ± 0.7</td>
<td>24.2 ± 1.2</td>
<td>24.6 ± 2.0</td>
</tr>
<tr>
<td>REE/kg LBM (kcal·kg⁻¹·d⁻¹)</td>
<td>33.4 ± 0.9</td>
<td>33.9 ± 2.0</td>
<td>33.8 ± 1.5</td>
<td>34.5 ± 2.1</td>
</tr>
</tbody>
</table>

² All values are ± SEM; n = 10 patients with myotonic dystrophy type 1 (DM1) and 10 healthy control subjects. REE, resting energy expenditure; LBM, lean body mass. No significant main effect of insulin and no group-by-insulin interaction was found (two-factor repeated-measures ANOVA).

Lipid metabolism in the postabsorptive and clamp conditions

The lipid profile (Table 3) was not significantly different between the study groups in the postabsorptive state, in terms of plasma total cholesterol (both HDL and LDL fractions), triacylglycerols, free fatty acids, glycerol, and β-hydroxybutyrate. Also, whole-body lipid oxidation, expressed per kg LBM, did not differ significantly between groups. During the insulin clamp, the suppression of the concentrations of the substrates of lipid metabolism and of lipid oxidation was not significantly different between the groups (Table 3).

**TABLE 3**

Glucose, lipid, and protein metabolism in the postabsorptive (basal) and insulin-stimulated (insulin) conditions

<table>
<thead>
<tr>
<th></th>
<th>DM1 patients</th>
<th>Control subjects</th>
<th>DM1 patients</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose metabolism</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mmol/L)</td>
<td>4.59 ± 0.23²</td>
<td>5.09 ± 0.13</td>
<td>4.56 ± 0.16</td>
<td>4.98 ± 0.15</td>
</tr>
<tr>
<td>Endogenous glucose production (mg·kg⁻¹·min⁻¹)</td>
<td>1.96 ± 0.09</td>
<td>2.22 ± 0.11</td>
<td>0.38 ± 0.15</td>
<td>0.33 ± 0.11</td>
</tr>
<tr>
<td>Glucose disposal (mg·kg LBM⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glucose oxidation (mg·kg LBM⁻¹·min⁻¹)</td>
<td>1.54 ± 0.26</td>
<td>1.82 ± 0.27</td>
<td>3.40 ± 0.55</td>
<td>3.35 ± 0.55</td>
</tr>
<tr>
<td>Nonoxidative glucose disposal (mg·kg LBM⁻¹·min⁻¹)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| **Lipid metabolism** |             |                  |             |                  |
| Plasma total cholesterol (mmol/L) | 4.81 ± 0.29 | 4.57 ± 0.29      | —           | —                |
| Plasma HDL cholesterol (mmol/L)     | 1.51 ± 0.08 | 1.34 ± 0.06      | —           | —                |
| Plasma LDL cholesterol (mmol/L)     | 2.75 ± 0.34 | 2.52 ± 0.22      | —           | —                |
| Plasma triacylglycerol (mmol/L)     | 1.22 ± 0.18 | 0.87 ± 0.32      | —           | —                |
| Plasma free fatty acids (μmol/L)    | 669 ± 92    | 586 ± 58         | 99 ± 31     | 47 ± 11          |
| Glycerol (μmol/L)                   | 49 ± 5      | 45 ± 5           | 16 ± 6      | 11 ± 4           |
| β-Hydroxybutyrate (μmol/L)          | 319 ± 79    | 172 ± 34         | 11 ± 4      | 24 ± 7           |
| Lipid oxidation (mg·kg LBM⁻¹·min⁻¹)  | 1.27 ± 0.19 | 1.27 ± 0.08      | 0.56 ± 0.12 | 0.60 ± 0.11      |

| **Protein metabolism** |             |                  |             |                  |
| Leucine (μmol/L)         | 103 ± 5     | 120 ± 10         | 66 ± 4      | 71 ± 7           |
| α-Ketoisocaproic acid (μmol/L) | 49 ± 8     | 44 ± 5           | 32 ± 8      | 29 ± 4           |
| Phenylalanine (μmol/L)   | 45 ± 2      | 48 ± 3           | 38 ± 2      | 37 ± 3           |
| Glutamine (μmol/L)       | 403 ± 47    | 547 ± 52         | 362 ± 32    | 442 ± 52         |
| Alanine (μmol/L)³        | 152 ± 8²    | 239 ± 30         | 168 ± 9     | 229 ± 17         |
| Endogenous leucine flux (μmol·kg LBM⁻¹·h⁻¹)⁴ | 203 ± 15² | 146 ± 9          | 185 ± 25    | 120 ± 8          |

² All values are ± SEM; n = 10 patients with myotonic dystrophy type 1 (DM1) and 10 healthy control subjects. A significant main effect of insulin (P < 0.05) was found for all variables (when appropriate), with the exception of plasma glucose.

² Significant different from healthy control subjects, P < 0.05 (Student’s unpaired t test and Bonferroni correction).

³ Significant main effect of group (P = 0.03) but no significant main effect of insulin or group-by-insulin interaction.

⁴ Significant main effect of group (P = 0.018) and significant main effect of insulin (P = 0.023) but no significant group-by-insulin interaction (P = 0.358).

Protein and leucine metabolism

Postabsorptive plasma leucine, α-ketoisocaproic acid, phenylalanine, and glutamine concentrations were not significantly different between the DM1 patients and the control subjects (Table 3). By contrast, plasma alanine concentrations were significantly lower in the DM1 patients (P = 0.01; Table 3). Postabsorptive ELF, which represents a parameter of whole-body proteolysis, was higher in the DM1 patients (P = 0.02; Table 3). During insulin stimulation, the suppression of plasma leucine and α-ketoisocaproic acid was not significantly different between the groups (Table 3); however, the percentage changes in plasma phenylalanine, glutamine, and ELF were lower in the DM1 patients than in the control subjects (P = 0.05; Table 3).
Hormone profile

Postabsorptive plasma insulin (47 ± 10 and 30 ± 4 pmol/L) and C-peptide (0.58 ± 0.12 and 0.46 ± 0.03 nmol/L) concentrations did not differ significantly between the DM1 patients and the control subjects, respectively. Plasma glucagon (94 ± 15 versus 99 ± 9 ng/L), cortisol (2472 ± 303 versus 2290 ± 260 nmol/L), and leptin (10.8 ± 1.5 versus 6.7 ± 1.6 ng/mL; P = 0.09) were also not significantly different between the groups. Despite a lack of difference in postabsorptive plasma growth hormone concentrations (2.1 ± 0.9 versus 1.1 ± 0.4 ng/mL; P = 0.30), plasma IGF-1 concentrations were lower in the DM1 patients than in the control subjects (125 ± 18 compared with 212 ± 8 ng/mL; P < 0.03). Postabsorptive TNFR2 concentrations were higher in the DM1 patients than in the control subjects (1.9 ± 0.2 compared with 1.4 ± 0.2 ng/mL; P = 0.04).

Postabsorptive plasma concentrations of IGF-1 and TNFR2 were inversely associated, as shown in Figure 1. There was no significant main effect of group and no significant group-by-time interaction. IGF-1 was also significantly associated with ELF (Figure 2), and ELF was associated with TNFR2. Stepwise regression analysis, with postabsorptive ELF used as the dependent variable, showed a major effect of IGF-1 (partial $R^2 = 0.51$; $P < 0.02$) and a minor effect of TNFR2 (partial $R^2 = 0.09$; $P = 0.05$), which add little predictive power (combined model $R^2$ from 0.51 to 0.61). There were no significant main effects of group and no significant group-by-time interaction.

DISCUSSION

A generalized defect in RNA metabolism (22) and an alternative splicing of the insulin receptor pre–messenger RNA (23) have been proposed to induce the reduction in insulin receptor expression in DM1 and therefore receptor insulin resistance. We showed that DM1 patients had a smaller LBM (12), which was associated with a larger body fat. The assessment of body composition allowed us to normalize indexes of insulin-dependent metabolism to LBM, showing that insulin-stimulated glucose metabolism, which seemed lower in absolute terms (12), was not significantly different in the DM1 patients and the healthy control subjects. We therefore aimed to revisit insulin control of the metabolic pathways that take place in muscle to test the features of insulin resistance in DM1.

Insulin action, energy homeostasis, and glucose and lipid metabolism

In DM1 patients, REE, which was lower in absolute terms (Table 2), was not significantly different from that in healthy control subjects when normalized to LBM. In the same set of patients (12), we observed that insulin-stimulated oxidative and nonoxidative glucose metabolism (Table 3) were comparable with that in control subjects when normalized to kg LBM. With respect to fatty acid metabolism, higher total cholesterol and triacylglycerol concentrations were reported in DM1 patients previously (24), but our cohort showed minimal alterations (Table 2). Even if the 40-mU · m$^{-2}$ · min$^{-1}$ clamp is not the ideal procedure to test insulin-dependent fatty acid metabolism, we failed to detect any alterations in postabsorptive and insulin-stimulated concentrations of FFAs and related metabolites (Table 3). Also, the whole-body lipid oxidation rate was not significantly different in the DM1 patients compared with that in the control subjects in either the postabsorptive or the insulin-stimulated state (Table 3).

Insulin action and IMCL content

The lack of insulin resistance of glucose and lipid metabolism was surprising because it was found despite a larger IMCL content. Other work suggested that the intramyocellular fat pool rather than total body fat is more strongly associated with insulin resistance in DM1.
resistance (25) as a result of acquired or inherited defects in mitochondrial fatty acid oxidation, defects in adipocyte fat metabolism, or simply increased fat delivery to muscle as the result of increased caloric intake. Therefore, we measured IMCL content by using 1H MR spectroscopy of the soleus and the tibialis anterior muscles. The IMCL content in the DM1 patients was larger, representing an example in which the IMCL accumulation was not associated with insulin resistance. In athletes, in which the larger IMCL content is paralleled by enhanced insulin sensitivity, a similar lack of association was found (26).

This paradox may have different explanations. IMCL might represent a surrogate for other factors that directly modulate insulin action (eg, long-chain fatty acyl-CoA). Alternatively, a different oxidative capacity of the muscle cells may imply an effect on insulin sensitivity regardless of IMCL content. Another explanation could be a difference in the subcellular localization of fat in DM1 patients compared with that in insulin-resistant subjects, corresponding with a different effect on insulin action. In DM1, this last explanation may be a fascinating one. In contrast with the case in other forms of muscular dystrophy, replacement of muscle by fat and fibrous tissue is not typical in DM1 and was excluded in our patients by the MR imaging scan obtained before the MR spectroscopy. Ultrastructural alterations are more typical (1); 1H MR spectroscopy may exclude the contamination of the IMCL with an extracellular source, but it cannot give insights about fat localization around the mitochondrion (rapid
disposal pool) or within lysosomes. A morphologic analysis might be conclusive, but muscle biopsy samples were not obtained.

Insulin action and protein metabolism

In contrast with glucose metabolism, leucine metabolism was impaired in both the postabsorptive and insulin-stimulated conditions. The ELF, which is an index of proteolysis, was higher in the postabsorptive condition when normalized to kg LBM, and its suppression during the clamp was impaired. Impairment of insulin-dependent suppression of plasma glutamine and phenylalanine concentrations was also evident. It is believed that in DM1, reduced muscle protein synthesis rather than increased protein degradation is a cause of muscle wasting (27). We did not measure insulin-dependent protein accretion in vivo in the present study; nevertheless, our findings of higher proteolysis reflect an impairment of insulin action in suppressing protein degradation, which may contribute to the reduced LBM observed in DM1 patients.

Protein metabolism and hormones

IGF-1 concentrations were inversely related to ELF in the DM1 patients (Figure 2). It has been noted that IGF-1 may be a more potent stimulator than insulin of muscle anabolic processes (28, 29); thus, its reduced availability could contribute to the muscle wasting in DM1. IGF-1 is involved in the age-related loss of skeletal muscle function (30) and maturation of the myogenic program (31) in animal models. In the past, administration of recombinant human IGF-1 in DM1 patients led to metabolic and muscle improvements (32). DM1 patients seem to be characterized by an increment of circulating concentrations of TNFR2 (24, 33, 34), a finding that suggests a higher activity of the TNF-α system. In the present study, the circulating concentration of TNFR2 in DM1 patients was higher than that in healthy control subjects and was correlated with ELF (Figure 2). Whether TNF is primarily involved in the muscle wasting of DM1 or is a secondary alteration was not assessed; nevertheless, its association with low IGF-1 concentrations (Figure 1) may be important. It has been shown that IGF-1 and TNF-α reciprocally influence nonmuscle tissues in vitro and in vivo cell survival (IGF-1 protecting survival and TNF-α promoting degeneration) (35). Additionally, TNF-α inhibits IGF-1–induced expression of myogenin in C2C12 myoblasts (36).

Mutation analysis and metabolic variables

Previous studies found an association between mutation size and the severity of clinical and metabolic alterations in DM1 patients (37), but we failed to observe this relation with the abnormal indexes of protein metabolism. In contrast, the data showed a relation between the soleus IMCL content and the mutation size \( R^2 = 0.62, P < 0.05 \) in DM1 patients, which is difficult to interpret. In fact, the expansion size may vary from tissue to tissue (38), and our determinations were performed in peripheral blood cells and not in skeletal muscle cells.

Limitations of the study

The pool size of the study groups was determined by the statistical objective to search for abnormal insulin-dependent responses of energy homeostasis, glucose, fatty acids, and leucine metabolism in DM1. Variables with greater variability that did not differ significantly might do so, thus increasing the pool-size. Furthermore, sex differences in endocrine variables were previously described in DM1 (39), and this aspect was not taken into account. Nevertheless, our finding of insulin resistance of protein turnover, despite the lack of insulin resistance of glucose metabolism, is interesting because it may partially explain the loss of LBM in DM1 patients. We are limited in this analysis because we did not assess leucine oxidation and consequently nonoxidative leucine disposal (a marker of protein synthesis), and we cannot exclude the possibility that the increased proteolysis observed in the DM1 patients may be compensated for by increased protein synthesis. In terms of metabolism, we conclude that normal energy homeostasis was properly sustained after correcting for kg LBM. The proper procedure to adjust the REE to normalize for LBM rather than for body weight (40); however, the heterogeneity of the heat-producing tissue that constitutes LBM prohibits us from formally concluding that the muscle metabolic rate of DM1 patients was normal. Finally, in morphologic terms, the lack of insulin resistance with respect to glucose metabolism despite the increased IMCL content is intriguing but needs to be confirmed by biopsy of the skeletal muscle in conjunction with electron microscopy to look for a difference in localization of the fat droplets in the cytoplasm of the muscle cell.

Conclusions

In conclusion, energy metabolism was preserved in DM1 patients, but the loss of LBM was severe. Abnormal regulation of protein breakdown, in which reduced circulating IGF-1 concentrations and increased TNF-α system activity may be involved, may contribute to this loss of LBM. In contrast with protein metabolism, the lack of insulin resistance of glucose and lipid metabolism does not support the concept of insulin receptor alterations as being the major cause of the metabolic disturbances in DM1.

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