Myopathy in patients with Hashimoto´s Disease.

Jaqueline Villar¹, Héctor J. Finol¹, Sonia H. Torres² and Antonio Roschman-González¹.

¹Centro de Microscopía Electrónica, Facultad de Ciencias, Universidad Central de Venezuela. Caracas, Venezuela.
²Instituto de Medicina Experimental, Facultad de Medicina, Universidad Central de Venezuela. Caracas, Venezuela.

Keywords: Hashimoto thyroiditis, hypothyroidism, myopathy, autoimmune disease, ultrastructure, fiber types.

Abstract. Hashimoto thyroiditis (HT) is an autoimmune disease of the thyroid gland. Patients may present or not a hypothyroid state, and frequently have manifestations of myopathy. The present work was aimed to assess the clinical symptoms and signs of skeletal muscle alterations in HT, describe the muscular pathological changes and relate them to the functional thyroid status and to the autoimmune condition of the patient. Clinical and laboratory studies were performed in ten HT patients and three control subjects (hormonal levels and electromyography). Biopsies from their vastus lateralis of quadriceps femoris muscle were analyzed under light (histochemistry and immunofluorescence) and electron microscopy. All patients showed muscle focal alterations, ranging from moderate to severe atrophy, necrosis, activation of satellite cells, presence of autophagosomes, capillary alterations and macrophage and mast cell infiltration, common to autoimmune diseases. The intensity of clinical signs and symptoms was not related to the morphological muscle findings, the electromyography results, or to the state of the thyroid function. Reactions for immunoglobulin in muscle fibers were positive in 80% of the patients. Fiber type II proportion was increased in all patients, with the exception of those treated with L-thyroxine. In conclusion, autoimmune processes in several of the patients may be associated to the skeletal muscle alterations, independently of the functional state of the thyroid gland; however, fiber II type proportion could have been normalized by L-thyroxine treatment.
Miopatía en pacientes con enfermedad de Hashimoto.
Invest Clin 2015; 56(1): 33 - 46

Palabras clave: tiroiditis de Hashimoto, hipotiroidismo, miopatía, enfermedad autoinmune, ultraestructura, tipos de fibra.

Resumen. La tiroiditis de Hashimoto (TH) es una enfermedad autoinmune de la glándula tiroides. Los pacientes pueden tener o no un estado hipotiroido y suelen presentar manifestaciones de miopatía. Este trabajo estudia los síntomas y signos clínicos de alteración muscular esquelética que puedan estar presentes en pacientes con TH, describe los cambios patológicos musculares y los relaciona con el estado funcional de la glándula tiroides y la condición autoinmune del paciente. Diez pacientes y tres sujetos controles fueron examinados clínicamente, se midieron los niveles de hormonas tiroideas, se practicó electromiografía y se tomó biopsia del vasto lateral del músculo cuádriceps crural para microscopía de luz (histoquímica e inmunofluorescencia) y microscopía electrónica. Todos los pacientes mostraron alteraciones musculares locales, atrofia moderada a severa, presencia de autofagosomes (glucogénosomas), necrosis, activación de las células satélites, infiltración de macrófagos y mastocitos, así como alteraciones en los capilares, similares a las de las enfermedades autoinmunes. La intensidad de los signos y síntomas no estuvo relacionada con los hallazgos morfológicos en músculo, los resultados de la electromiografía ni con el estado funcional tiroideo. La reacción a las inmunoglobulinas fue positiva en el músculo de 80% de los pacientes. La proporción de fibras musculares tipo II estuvo incrementada en los pacientes excepto en aquellos que recibieron tratamiento con L-tiroxina. En conclusión, el proceso autoinmune hacia el músculo parece asociarse a las alteraciones en éste, independientemente del estado funcional tiroideo, sin embargo, la proporción de las fibras tipo II puede haber sido normalizada por el tratamiento con L-tiroxina.

Recibido: 14-01-2014 Aceptado: 06-11-2014

INTRODUCTION
Hashimoto´s Disease (Chronic Lymphocytic Thyroiditis) is one of two classical autoimmune conditions of the thyroid-directed autoimmunity. It may result in euthyroidism, subclinical hypothyroidism or overt hypothyroidism. The other autoimmune condition, Grave´s Disease, results in hyperthyroidism (1). In both diseases the thyroid gland is infiltrated by T and B-cells reactive to thyroid antigens. The enlargement of the thyroid gland (goitre) in Hashimoto´s thyroiditis (HT) is due to the lymphoeytic infiltration, presence of oncocyes, previously named Hurthle cells, Askanazy cells or oxyphilic cells, and fibrosis, that increases in the final atrophic stage of the disease (2). In HT, several autoantibodies are produced against thyroid peroxide (TPO), thyrogblobulin, and thyroid stimulant hormone (TSH) receptors. However, antibody-dependent, cell-mediated cytotoxicity, is necessary for the de-
struction of thyroid cells, which is produced by cytotoxic T CD8+ cells and the collaboration of helper CD4+ T lymphocytes. The TSH receptor antibodies have been classified as stimulating (Graves´ Disease), blocking (ITI) and neutral (3). Disorders of the endocrine glands are associated to muscle dysfunction. Most of the endocrine myopathies affect proximal limb muscles in the upper and lower limbs. Autoimmune thyroid disease patients may present a myopathy related to hyper or hypothyroidism, but also euthyroid patients eventually show signs of muscle disease, as proximal weakness and easy fatigue. The symptoms and signs may be subtle and missed, if the patient is not carefully interrogated and examined. The endocrine dysfunction may affect skeletal muscle, due to the deficient or augmented metabolic effects of the respective hormones. On the other hand, autoimmune mechanisms may be involved in the endocrine disorder. This would explain the increased susceptibility within individuals and families to other endocrine and non-endocrine autoimmune conditions. HIT is frequently associated to diabetes mellitus type I, vitiligo, pernicious anemia, Addison’s disease, celiac disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, polymyositis, psoriasis and Sjögren’s syndrome (4-9). There are several studies on myopathy in hypothyroid patients, some of them did not discriminate if they were due to autoimmune Hashimoto thyroiditis (10-12). Dunn et al. (13) described myopathy in four patients with subclinical hypothyroidism. Another study of 53 HIT patients found only seven subjects with myopathy showing high antithyroid antibodies (14); Rodolico et al. (15) reported 10 HIT patients, only with myopathic and not thyroid symptoms, and another study was aimed to describe the muscle capillary alterations in six HIT and five hypothyroid patients (16).

The purpose of the present study was to examine 10 goitrous patients diagnosed as HIT by thyroid biopsy, in order to relate the clinical study with laboratory results and the histological and ultrastructural findings in the quadriceps femoris muscle and to try to disclose if muscle alterations can be related to hormonal action and/or to autoimmunity.

PATIENTS AND METHODS

Patients

Ten patients of the Endocrine Unit of the Domingo Luciani Hospital, Caracas, Venezuela, and three volunteer control subjects were selected for the study after signing an informed consent. The patients were previously diagnosed as HIT by the histological examination of the enlarged gland biopsies, or as euthyroid by clinical examination (no symptoms of hyper or hypothyroidism). The characteristics of the patients are shown in Table I. Nine of the 10 patients were female and their mean age was 37.6 (range 21-54 years). The control subjects were two females, ages 39 and 58, and one 48 year-old-male. They were selected from the surgical ward in the same Hospital, with processes not related to endocrine, muscular or other general diseases.

Patients were interrogated for dysesthesia, muscle cramps, myalgia, weakness, arthralgia or arthritis. Physical examination included exploration of tone, strength, superficial and profound sensibility, osteotendinous reflexes, joint examination, walking characteristics and muscular fatigue. A scale I to V was used to evaluate muscular force, V was defined as normal force, both in upper and lower limbs. Fatigue was evaluated with a protocol of three exercises repeated 10 times each (rising and lowering arms, stepping up and down a 25 cm stool and crouching with arms ex-
tended up). All evaluations were done by the same subject.

Laboratory
The hormones free 3-iodotironine (fT3), free thyroxin (fT4) and thyroid stimulating hormone (TSH) were measured in serum. The enzymes serum glutamate-pyruvate transaminase (SGPT) and serum glutamate-oxaloacetate transaminase (SGOT), lactic dehydrogenase (LDH) and creatine kinase (CK) were determined. Antithyroid antibodies: 1) Antinuclear antibodies (ANA) were analyzed by immune-fluorescence and 2) Antimicrosomal antibodies (AMA) by passive haemaglutination.

Electrophysiological studies
Electromyography was performed at rest, with voluntary contraction at minimum and maximum effort. The muscles studied were: deltoid, braquial biceps, triceps, quadriceps femoris, gastrocnemius and tibialis anterior. Electroneuronography was practiced to measure motor nervous conduction in the external sciatic popliteal nerve.

Muscle biopsy
Samples were taken from the vastus lateralis of the quadriceps femoris muscle of the 10 patients and control subjects, with the Bergström needle. Briefly, after local anesthesia with 2 mL of 2% lidocaine, a 5-mm skin incision was performed and muscle samples were obtained. The muscle sample was divided in two. One piece was frozen in isopentane cooled with liquid nitrogen. Transverse 10 µm serial sections were cut in a cryostat at −20°C and stained for adenosine triphatase ATPase, after alkaline (pH:10.3) and acid (pH: 4.37 and 4.6) pre-incubation (17); other sections were stained with hematoxylin-eosin (H-E). The comparison of serial sections stained with different pH preincubation of ATPase reaction, allowed the classification of fiber types: 250 fibers were classified by two different subjects and the percentage of fiber type was calculated.

Immunofluorescence of muscle
Direct determination of immunoglobulins in muscle was done with polyvalent antibodies. Frozen sections of 5 and 10 µm. were air dried on a glass slide during 1 h, washed twice with phosphate buffer pH 7.2. They were covered with antihuman goat Polyvalent Anti-immunoglobulin marked with fluorescein (FITC), (G:A:D:E: and M; ATAB ATLANTIC ANTIBODIES trademark by ATLANTIC ANTIBODIES, INC. in SCARBOROUGH, 04074., UK) in a 1:40 dilution for 30 min in darkness. Washing was repeated and they were mounted in glycerine/ phosphate buffer 1:1.

Electromicroscopy
The samples were stretched between two pins, covered for 5 min with 3% glutaraldehyde in a 320 mOsmol phosphate buffer solution, pH 7.4. After that, the samples were diced into small blocks (2-mm length × 1-mm diameter), fixed in glutaraldehyde for 40 min and postfixed in 1% OsO4, dehydrated in ethanol and embedded in LX-122 resin (LADD Res. Inc., Burlington). Sections were cut with diamond knife in a Porter-Blum MT2-B ultramicrotome and stained with uranyl acetate and lead citrate. Sections were observed in a Hitachi H-500 transmission electron microscope at an accelerating voltage of 100 kV.
Statistics

Spearman Rank Order Correlations were calculated for the numerical results. The statistics program used was Statistica (Statsoft Inc. Tulsa Oklahoma, USA). Statistical significance was established at a P value of less than 0.05.

RESULTS

Patient characteristics are shown in Table I. Patient No 3 was the only male. The duration of the disease was between 4 and 16 years. In all patients rheumatic disease was discarded according to the American Rheumatic Association criteria (18).

The patients, that initially were assumed to have normal thyroid function (no clinical symptoms of hyper o hypothyroidism), were reclassified after the determination of the hormonal levels of TSH and fT4 (Table II), according to the definition of “subclinical thyroid disease” (19). The reference normal levels for TSH, fT4 and fT3 were respectively 0.45-4.5 mIU/L, 0.73-1.95 ng/dL and 2.14-5.34 pg/mL, therefore, the functional state of the thyroid gland was: Euthyroidism in patients 1-5, Subclinical Hypothyroidism (serum concentration of TSH above the statistically defined upper limit of the reference range, with serum fT4 concentration within its reference range) in patients 6-9; and hypothyroidism (increased TSH and decreased fT4) in patient 10.

The levels of fT3 were normal in all patients (Table II). The levels of fT4 correlated inversely with the levels of TSH (r= -0.92, p<0.005) and also, the levels of fT3 with TSH (r= -063, p<0.05). LDH was normal in all patients; TGO was only slightly elevated in patient 8 (35 U/L, normal 10-30 U/L), as well as TGP (42 U/L, normal 6-37 U/L). CK levels were elevated in patients 3 and 10 (Table II).

Important history data were found in some subjects: Patient 2 had a euthyroid nodule surgically removed, and a slight hyperplasia of the remaining tissue. Patient 10 had a toxic diffuse goitre partially removed two years before the present study. Only patients 3 and 5 were presently treated with L-thyroxine. Patient 3 had a previous diagnosis of hypothyroidism (Table I).

The interrogation for muscular symptoms was positive for cramps in nine patients and for weakness in seven; six reported muscle pain mainly associated to cold, and five suffered occasionally joint pain. At examination, walking, muscular tone and proximal force were normal in all subjects. Distal force in upper and lower extremities was slightly decreased in five and moderately decreased in two patients. Fatigue was slight in two patients and evident in five; reflexes were exaggerated in five patients and decreased in two (Table I).

Antinuclear antibodies (ANA) were negative in all patients. The presence of antimicrosomal antibodies (AMA) was found in two of five euthyroid patients (patients 3 and 5). It was also found high AMA titers in three of four patients with subclinical hypothyroidism (patients 7-9) and in patient 10, who was diagnosed with overt hypothyroidism (Table II): In synthesis, six out of ten HT patients showed presence of AMA.

Nerve velocity conduction was normal in all patients. Electromyography at rest was also normal in all patients. In half of the subjects (1, 3, 5, 6, and 8, (Table II) minimal and maximal voluntary contractions showed low amplitude and short duration polyphasic motor unit potentials; this was more frequently found in deltoid and quadriceps femoris muscles.

Results of muscle optical and electron microscopy examination are shown in
## TABLA I
PATIENTS CHARACTERISTICS AND CLINICAL SYMPTOMS AND SIGNS

<table>
<thead>
<tr>
<th>Pac. N°</th>
<th>Age (ys)</th>
<th>Sex</th>
<th>Disease duration (ys)</th>
<th>Cramps</th>
<th>Dysesthesia</th>
<th>Myalgia</th>
<th>Weakness</th>
<th>Joint pain</th>
<th>Distal force</th>
<th>Fatigue</th>
<th>Reflexes</th>
<th>Joint signs</th>
<th>Other relevant data</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>F</td>
<td>15</td>
<td>+</td>
<td>paresthesia</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>IV/V</td>
<td>++</td>
<td>↓</td>
<td>-</td>
<td>Operated 2 years before, euthyroid nodular goitre</td>
</tr>
<tr>
<td>2</td>
<td>38</td>
<td>F</td>
<td>10</td>
<td>+</td>
<td>paresthesia</td>
<td>-</td>
<td>+</td>
<td>+/-</td>
<td>IV/V</td>
<td>+</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>37</td>
<td>M</td>
<td>4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>IV/V</td>
<td>++</td>
<td>↓</td>
<td>-</td>
<td>Clinical diagnosis of hypothyroidism treated with levothyroxine</td>
</tr>
<tr>
<td>4</td>
<td>53</td>
<td>F</td>
<td>5</td>
<td>+</td>
<td>hypersthesia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IV/V</td>
<td>++</td>
<td>↑</td>
<td>Heberden nodules</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>F</td>
<td>4</td>
<td>+</td>
<td>paresthesia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>IV/V</td>
<td>++</td>
<td>↑</td>
<td>Heberden nodules</td>
<td>In treatment with levothyroxine</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>F</td>
<td>14</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>III/V</td>
<td>++</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>21</td>
<td>F</td>
<td>13</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>III/V</td>
<td>++</td>
<td>N</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>F</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>V/V</td>
<td>+</td>
<td>↑</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>F</td>
<td>6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V/V</td>
<td>-</td>
<td>N</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>F</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>V/V</td>
<td>-</td>
<td>N</td>
<td>-</td>
<td>Operated 2 years before, toxic diffuse goitre</td>
</tr>
</tbody>
</table>

Table III. Control patients did not show any abnormalities in muscle sample examination, and their type II fiber proportions were 49%, 46% and 55%. The histochemical examination was performed in seven patients; H-E stained sections showed infiltration of mononuclear cells (Figs. 1A and B) and in patient 5 hyaline degeneration and hemorrhage was found (Fig. 1A). ATP-ase sections allowed for classification of fiber types. In five patients an increased proportion of type II fibers (67%-79%) was found (Fig. 1D). Patients 3 and 5 showed 56% and 46% of type II fibers, which was similar to the normal controls (Fig. 1C); these two patients were receiving treatment with L-thyroxine.

Eight of the ten patients showed different intensities of fluorescence in skeletal muscle with the polyvalent anti-immunoglobulin; the fluorescence had a linear or granular aspect in the sarcolemma (Fig. 1E), and in patient 5 it was also present as granules inside the muscle fibers (Fig. 1F).

The transmission electron microscopy showed different alterations in skeletal muscle; in muscle fibers abundant mitochondria, lipid droplets, autophagosomes of glycogenosome type, multivesicular bodies and lipofuscin granules were present (Fig. 2). Most organelles were seen in subsarcolemmal and intermyofibrillar spaces. Numerous muscle fibers exhibited evident atrophy (Fig. 3) and segmental necrosis also was found (Fig. 4). Capillaries presented wide (Fig. 3) and occluded lumens (Fig. 5) with endothelial cell cytoplasm prolongations (Figs. 3 and 5). The basement membrane was usually thickened (Figs. 3, 5, and 7) and the mononuclear cell infiltrate was represented by macrophages and mast cells surrounded by numerous collagen fibrils (Figs. 6 and 7). Activated satellite cells were observed separating from muscle fibers (Fig. 3).

**DISCUSSION**

The main results in the preset work are: 1) there were muscle alterations in all

### TABLE II

**ELECTROMYOGRAM; FT3, FT4 AND THYROTROPIN (TSH) LEVELS; CREATINE KINASE ENZYME (CK) LEVELS, AND ANTITHYROID ANTIBODIES (ANTIMICROSOMAL ANTIBODIES, AMA)**

<table>
<thead>
<tr>
<th>Patient N°</th>
<th>Electro myogram</th>
<th>1° uptake (Iuptake)</th>
<th>Free T3 (pg/mL)</th>
<th>Free T4 (ng/dL)</th>
<th>TSH (mIU/L)</th>
<th>CK (U/L)</th>
<th>AMA (titre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myopathy</td>
<td>37</td>
<td>3.18</td>
<td>1.32</td>
<td>1.46</td>
<td>57</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>28</td>
<td>3.79</td>
<td>1.60</td>
<td>1.20</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Myopathy</td>
<td>3</td>
<td>5.19</td>
<td>1.29</td>
<td>1.36</td>
<td>147</td>
<td>1:1600</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>NA</td>
<td>5.28</td>
<td>1.70</td>
<td>1.04</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Myopathy</td>
<td>14</td>
<td>4.01</td>
<td>1.28</td>
<td>1.33</td>
<td>30</td>
<td>1:1600</td>
</tr>
<tr>
<td>6</td>
<td>Myopathy</td>
<td>24</td>
<td>3.47</td>
<td>0.80</td>
<td>10.51</td>
<td>86</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>NA</td>
<td>3.76</td>
<td>1.21</td>
<td>7.74</td>
<td>21</td>
<td>1:100</td>
</tr>
<tr>
<td>8</td>
<td>Myopathy</td>
<td>NA</td>
<td>4.39</td>
<td>0.89</td>
<td>16.31</td>
<td>49</td>
<td>1:102400</td>
</tr>
<tr>
<td>9</td>
<td>Normal</td>
<td>38</td>
<td>3.55</td>
<td>0.83</td>
<td>22.59</td>
<td>33</td>
<td>1:1600</td>
</tr>
<tr>
<td>10</td>
<td>Normal</td>
<td>NA</td>
<td>2.83</td>
<td>0.69</td>
<td>&gt;54</td>
<td>123</td>
<td>1:1600</td>
</tr>
</tbody>
</table>

NA = not available.
### TABLE III

HISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF VASTUS LATERALIS MUSCLE

<table>
<thead>
<tr>
<th>Patient Nº</th>
<th>Type II fiber (%)</th>
<th>Optic microscopy</th>
<th>Electron microscopy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>Hypercontraction. Fragmentation of the sarcotubular system. Necrosis.</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td>Normal sarcomeric organization. In subsarcolemmnie region abundant mitochondria, lipofuscin, lipid droplets, glycogenosomes, multivesicular bodies, multifilamentous body, fibrosis, satellite cells with swollen mitochondria, polysomes and abundant RER. Macrophages. Mast cells with degranulation.</td>
</tr>
<tr>
<td>3</td>
<td>56</td>
<td>Atrophy of fiber type I and II. Necrosis. Separation of satellite cells. Capillaries with partial lumen occlusion.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>Zones of hyaline degeneration. Zones of hemorrhage. Atrophy of type II fibers.</td>
<td>Capillaries with marked thickening of basement membrane</td>
</tr>
<tr>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td>Atrophy</td>
</tr>
<tr>
<td>7</td>
<td>79</td>
<td>Zones with rounded cells. Infiltration by mononuclear cells.</td>
<td>Normal fibers. Fibers with mild to severe atrophy. Abundant lipofuscin and lipid drops.</td>
</tr>
<tr>
<td>9</td>
<td>76</td>
<td>Atrophy of type I and type II fibers</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>70</td>
<td>Atrophy of type II fibers. Rounded cells. Moderate infiltration by mononuclear cells.</td>
<td>Moderate atrophy.</td>
</tr>
</tbody>
</table>

NA: not available.
Fig. 1. Transversal sections of vastus lateralis of quadriceps femoris. 1A. Patient N° 5. H-E. Hyaline degeneration (open arrow). Haemorrhage (arrow), slight infiltration of mononuclear cells. 1B. Patient N° 4. H-E. Moderate infiltration of mononuclear cells. 1C. Control subject. ATPase preincubation pH 10.3. Clear fibers are type II, dark fibres are type I. Horizontal bar 100 μm (applies to 1 A,B,C and D). 1D. Patient N° 4. ATPase preincubation pH 10.3. Note increased proportion of dark fibers (type II). 1E: Patient N° 2. Immunofluorescence (antiglobulines). Linear fluorescence in the sarcolemma (arrows). Horizontal bar 50 μm (applies to 1 E and F). 1F: Patient N° 5. Linear fluorescence in the sarcolemma (thin arrows). Granular fluorescence inside the muscle fiber (thick arrows).
the studied patients, including moderate to severe atrophy, necrosis, activation of satellite cells, presence of autophagosomes and macrophage and mast cell infiltration. 2) Muscle capillary alterations, common to autoimmune diseases, were found in several patients. 3) All patients, with the exception of the two which were receiving treatment with L-thyroxine, showed an increased proportion of type II muscle fibers. 4) Most patients (8/10) were positive for immunoglobulins in muscle fibers. 5) The clinical symptoms and/or signs of myopathy were present in all patients, except in patient 10. Their intensity was not related to the morphological findings in muscle, the results of electromyography or the state of thyroid function (euthyroid, subclinical or overt hypothyroidism).

The patients were selected by the presence of goitre, without clinical symptoms and signs of hypothyroidism. The diagnosis of Hashimoto disease was confirmed by thyroid gland biopsy. However, when serum TSH and fT4 levels were measured, four patients showed subclinical hypothyroidism and one, overt hypothyroidism. This last patient suffered, originally, from a non-treatable hyperthyroidism; the thyroid gland was removed two years before the present study, leaving some remnants of thyroid tissue. She did not show clinical symptoms or signs

---

**Fig. 2.** Lipid droplets (triangle), mitochondria (Mit), several glycogenosomes (asterisk), multivesicular body (arrow), lipofuscin granules (square) and abundant glycogen particles (arrowhead) are seen in the subsarcolemmal space.

**Fig. 3.** A wide space (square) is located between an atrophied skeletal muscle fiber (arrows) and a satellite cell showing an irregular shaped nucleus (N) and swollen mitochondria (Mit). The capillary presents endothelial cell cytoplasm prolongations into the lumen (arrowheads) and is covered by a thickened basement membrane (Bm).
of either hypothyroidism or myopathy; however, her TSH was increased and the fT4 was below the reference level. The muscle biopsy showed moderate atrophy and scarce mononuclear infiltration. It is possible that the remaining thyroid gland tissue was not sufficient to maintain a normal function.

The muscular alterations found in the patients were similar to those described by other authors in hypothyroid subjects. Lin et al. (20) refer to fiber atrophy, mitochondrial abnormalities and abnormal glycogen accumulation; McKeran et al. (10) also mention glycogen inclusions, glycogenosomes, excess lipid and vesicular abnormalities. But fiber atrophy, necrosis and mononuclear infiltration are also seen in some autoimmune diseases (21-24). In relation to the fiber type proportion, Ono et al. (12) describe the higher percentage of type II fibers, as it was found in the present work; however, McKeran et al. (10) report just the opposite. The normal proportion of type II fiber in the two patients that were receiving L-thyroxine suggests that increased proportion of II type fibers may be produced by the fall of fT4 and can be corrected by the hormonal treatment. However, it would be important to corroborate this assumption with a higher number of patients. An increased proportion of type II fibers is also a feature of hyperthyroidism.
In our laboratory, the study of 8 patients (7 female) with hyperthyroidism, showed a type II fiber proportion of 62 ± 4% (25). In a control group of 17 women, from a different study performed in our laboratory, the fiber II proportion was 46 ± 7% (26). The increase of type II fiber proportion, both in hyperthyroidism and hypothyroidism, may be interpreted as a modulatory effect of the level of thyroid hormones.

Satellite cells are pivotal in muscle regeneration and may be considered as dormant myoblasts (27). In the present work they showed to be activated, because they were partially separated from the muscle cells, although conserving the basement membrane. However, they were not seen being transformed into myoblasts or undergoing an apoptotic process (28). It seems likely that, as shown two months after denervation, satellite cells return into the initial position in relation to the muscle cell (29).

The abnormalities in muscle were focalized, with normal zones of muscle; this may explain why not all of the patients showed a pattern of myopathy in the electromyography. High levels of CK are a sign of necrosis. Although necrosis was seen in the muscle of some patients, it may have not been sufficiently widespread to increase CK levels.

HT is considered an autoimmune disease, and five patients showed thyroid hormonal levels considered to be normal. It is not easy to separate the cause of muscle abnormalities in these patients. It is possible that complete muscle recovery takes a long time after normalization of the hormonal levels. Other possibility is that subjects with genetic susceptibility to autoimmune disease may develop complex diseases due to numerous genes which interact with each other and with environmental factors (30). The presence of myopathy in patients with an euthyroid state, points to the contribu-
tion of an autoimmune component to muscle aggression; this assumption is stressed by the muscle capillary alterations, similar to those found in autoimmune conditions (16). In a study of 53 cases with chronic lymphocytic thyroiditis, Bai (14) found neurological alterations in 29 patients, seven with myopathy; neuropathy occurred more often in cases with both chronic lymphocytic thyroiditis and some autoimmune disorders, suggesting that abnormal immune function might be the common background in those patients. In agreement with this suggestion is the frequent association of hypothyroidism with autoimmune disease (4-9).

In conclusion, the 10 patients with HT showed morphological muscle alterations, and some symptoms and signs of myopathy. It is possible that the increase of type II muscle fiber proportion found in the IIT patients was corrected with L-thyroxin treatment used in two of these patients. The autoimmune process in several of these patients, favored by a genetic background, may be associated to the skeletal muscle alterations, independently of the functional state of the thyroid gland.

ACKNOWLEDGMENTS

Our thanks to Dr. Saverio Russo (Hospital Domingo Luciani, Caracas, Venezuela) for the clinical study of the patients and Dr. Marian Ulrich, (Institute of Biomedicine, Faculty of Medicine, Central University of Venezuela) for processing the samples of immunofluorescence in muscle.

REFERENCES


19. Surks, MI, Ortiz, E, Daniells, GH, Sawin CT, Col NF, Cobin RH, Franklyn JA, Hershman JM, Burman KD, Denke MA, Gorman C, Cooper RS, Weissman NJ. Subclinical thyroid disease. Scientific Review and guidelines for diagnosis and management. JAMA 2004; 291(2); 228-238.