

Clinical Case Report: Ultrastructural Evidence of Skeletal Muscle Mitochondrial Dysfunction in Patients With Subclinical Hypothyroidism

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Abstract. Objective: The lack of overt signs and symptoms and controversies surrounding the thyroid stimulating hormone (TSH) reference range variability make the management of subclinical hypothyroidism (sHT) a challenge. Because muscle cramps and weakness have been noted in sHT, histological skeletal muscle examination may be of diagnostic significance as the presence of abnormalities would substantiate a significant consequence of the mild thyroid failure presumed to be present in the individual with sHT. The objective of this study was to investigate the ultrastructural and histological changes of skeletal muscle associated with sHT.

Design: Skeletal muscle biopsies from the vastus lateralis were obtained from four subjects with sHT. Samples were fixed, sectioned, and stained for quantitative and qualitative electron and light microscopic analysis.

Main Outcome: Analyses revealed characterizable morphological and ultrastructural alterations and quantitative mitochondrial variations between subjects, indicative of skeletal muscle mitochondrial dysfunction in sHT patients. For the 4 subjects, mean mitochondrial perimeter (MP) was $1.09 \pm 0.312 \mu$, mean mitochondrial area (MA) was $0.10 \pm 0.05 \mu^2$, and mean mitochondrial volume density was 1.92 ± 0.95 .

Conclusions: The observed and quantified mitochondrial alterations and the noted morphological and ultrastructural alterations identify previously undocumented pathological skeletal muscle alterations associated with sHT. The observed morphological and ultrastructural alterations lend support to a trend of progression of sHT into overt hypothyroidism as a result of mitochondrial dysfunction and associated metabolic shift. The identification of these skeletal muscle alterations as sequelae of sHT may lend convincing objective evidence of a pathophysiologically significant abnormality in patients with sHT. If so, this should diminish the substantial resistance to treatment of these patients at an early stage of disease and attenuate the progression to overt hypothyroidism.

Keywords. Subclinical hypothyroidism • Mitochondria • Morphology • Skeletal muscle • Ultrastructure

Introduction

Thyroid dysfunction affects more than 21 million Americans.^[1] Untreated thyroid dysfunction has negative health consequences, including coronary heart disease, osteoporosis, atrial fibrillation, cognitive impairment, and depression. When analyzed as a whole, these negative health consequences resulting directly from untreated thyroid dysfunction are among the nation's largest causes of morbidity, mor-

tality, and diminished quality of daily life in older adults.^[1] The incidence of diagnosed hypothyroidism ranges from 4% to 8.5%.^[2] Subclinical hypothyroidism is more common in women over the age of 60, (20%)^[2] and males ≥ 65 years.^[2,3]

Subclinical hypothyroidism represents the earliest stage of thyroid hypofunction.^[4] It is diagnosed by an elevation of serum thyroid-stimulating hor-

ference range serum free T₄ (FT₄) level of 0.8-2.3 ng/dL.^[4] The rate of progression from subclinical hypothyroidism to overt hypothyroidism directly correlates with the patient's initial TSH value,^[2] and most experts recommend treatment of subclinical hypothyroidism when TSH values exceed 10 mIU/L. However, 75% of those diagnosed with subclinical hypothyroidism have TSH values in the 5-10 mIU/L range,^[5] thereby creating an imprecise barometer regarding the need to and recommendations for the initiation of thyroid hormone treatment.

Typical signs and symptoms of overt hypothyroidism include cold intolerance, dry skin, constipation, depression, myalgia, arthralgia, cramps, and weakness as well as altered metabolic parameters including increased total and LDL cholesterol.^[1] Adding to the difficulty in diagnosis, subclinical hypothyroid patients may not present with consistent symptoms. Since myalgia, muscle cramps, and weakness have been reported in subclinical hypothyroidism,^[6] a histological examination of skeletal muscle in patients with subclinical hypothyroidism would be important in confirming a physical consequence of subclinical hypothyroidism. However, no literature exists describing the ultrastructural morphological characteristics of skeletal muscle in subclinical hypothyroid patients.

The purpose of this study was to examine biopsied skeletal muscle of 4 clinically diagnosed subclinical hypothyroid patients and identify ultrastructural and morphological characteristics, specifically mitochondrial alterations, found in the subclinical hypothyroid patients.

Materials and Methods

Subjects. Four patients served as study subjects. Each patient's informed consent for these procedures was obtained as part of the Institutional Review Board (Lifespan Academic Medical Center) approval process. A comprehensive physical examination and medical history review were included in the patients screening. Patients were included if they were ages 21-80 years, had no prior history of hormone supplements in the preceding six months, and had elevated TSH levels with FT₄ levels within reference range.

Patient 1. A 34 year old female weighing 76.7 kg (169.1 lbs) with no family history of thyroid dysfunction presented with a TSH level of 14.33 mIU/L and a FT₄ level of 0.84 ng/dL (Table 1). The patient presented with a weight gain \geq 6.8 kg (14.99

lbs) over the course of the previous year, cold intolerance and low energy levels characterized by muscle weakness, myalgia, and muscle spasms exacerbated by cold exposure and alopecia. Her sleep pattern and bowel function were normal. At the time of her presentation, she was treated only with an oral contraceptive.

Table 1. TSH, FT₄ Levels and Clinical Symptoms of 4 Patients with Subclinical Hypothyroidism.

Patient	TSH (mIU/L)	FT ₄ (ng/dL)	Clinical Symptoms*
1	14.3	0.84	E, W, MW, P, MP, MS, CI, H
2	15.2	0.86	
3	5.5	1.03	E, P
4	11	0.8	E, S, W, MW, P, MP, MS, H

*Decreased energy level (E), abnormal sleep pattern (S), increased weight (W), muscle weakness (MW), decreased physical performance (P), muscle pain (MP), muscle spasm (MS), cold intolerance (CI), and hair loss (H).

Patient 2. A 46 year old male weighing 78.0 kg (172.0 lbs) with no family history of thyroid dysfunction, presented with a TSH level of 15.2 mIU/L and a FT₄ level of 0.86 ng/dL (Table 1). He was not on medication at the time of evaluation. Review of systems revealed no decrease in energy level as measured by physical performance, normal sleeping patterns, no weight change within the last year, no reported muscle weakness, spasm or myalgia and no perceptible intolerance to cold. Bowel movements were normal and there was no evidence of alopecia.

Patient 3. A 49 year old male weighing 83.5 kg (184.1 lbs) with a positive family history of thyroid dysfunction presented with a TSH of 5.5 mIU/L and FT₄ level of 1.03 ng/dL (Table 1). His medications at presentation included Lovastatin, niacin, Metoprolol, Clopidogrel, ASA, and Ramipril. He reported a severe lack of energy characterized by a decrease in physical performance. He reported no weight gain, no muscle weakness, spasm or pain, and no perceptible increase in cold intolerance. His bowel function was normal and no alopecia was noted.

Patient 4. A 44 year old male weighing 70.8 kg (156.1 lbs) with a history of Graves' Disease. He was not on medication and presented with a TSH level of 11.01 mIU/L and a FT₄ level of 0.80 ng/dL (Table 1). He reported a loss of energy, a significant weight

Table 2. Mitochondrial morphometric data of 4 subclinical hypothyroidism patients.

Patient	Mitochondria perimeter (MP) size range (i)	Mean MP (i) ± SD	Mitochondrial area (MA) (i ²)	Mitochondrial volume density (MVD)(%)
1	0.40-0.97	0.65 ± 0.14	0.03	1.95
2	0.56-2.25	1.25 ± 0.44	0.12	0.57
3	0.57-3.34	1.11 ± 0.36	0.10	2.55
4	0.53-4.57	1.36 ± 0.52	0.15	2.6

*Mean Patient MP = 1.09 ± 0.312μ; Mean Patient MA = 0.10 ± 0.05 μ²; Mean Patient MVD = 1.92 ± 0.95%.

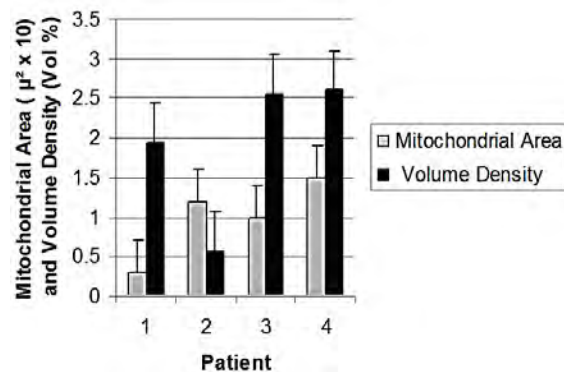
gain of ≥ 6.8 kg (15 lbs) within the previous year, alopecia, and generalized muscular weakness in his arms, legs, and back. He also complained of recurrent and localized back pain.

Blood Measurements. Blood samples were then taken to identify TSH and FT₄ levels to confirm the presence of subclinical hypothyroidism (Table 1). Patients were instructed to refrain from nonsteroidal anti-inflammatory drugs (NSAIDS) for 5 days prior to biopsy.

Muscle Biopsy Procedure. Biopsied samples were taken from the vastus lateralis muscle, 25 cm proximal to the tibial tuberosity and 5 cm lateral to the midline of the femur from each of the 4 patients.^[7] The tissue was immediately placed in cold (4°C [39.2°F]) paraformaldehyde and 1.4% gluteraldehyde in 0.1M sodium cacodylate buffer. The tissue was cubed for light and electron microscopic evaluation and post fixed in osmium tetroxide in sodium cacodylate buffer. Following buffer rinses and dehydration in ethanol and propylene oxide, the tissues were flat embedded in Epon 812 and placed in a 70°C (158°F) oven to polymerize for 48-hours. Thick and ultrathin sections were cut using a Dupont Sorval MT2B ultramicrotome and prepared for light and electron microscopic analysis using standard procedures.^[8]

Morphological Analyses. Qualitative light microscopy was performed using an Olympus BX51 light microscope with an Olympus DP11 digital camera. Digital images were analyzed using Image J and NIH Image analysis software. Electron microscopic examination was performed using a Philips 301 transmission electron microscope (TEM) equipped with an Advanced Microscopy Techniques image capturing system at magnifications from 4,500x to 45,000x, allowing for a more expansive examination of the muscle fiber.

Quantitative mitochondrial analysis was performed using Media Cybernetics, Inc. (Silver Springs, MD) Image-Pro Express image analysis software. Digital images were captured using a 1024 x 768 capture resolution setting using the Lumenera Scientific Infinity 2 side-column mounted digital camera. Each digital micrograph was then analyzed using the Image-Pro Express software.

Figure 1. Comparison of mitochondrial area and volume density of four subclinical hypothyroid patients.

A grating replica calibration grid (Electron Microscopy Services, Hatfield, PA) in conjunction with a Photomicrographic Scale Marker (Dunn and Reidman, Pacific Palisades, CA) were used to calibrate the determined perimeter of each mitochondrion and to convert measurements from pixels to micrometers (i). Ten captured electron micrographs per subject, each taken from different fibers, were analyzed to determine mitochondrial perimeter (MP). Mitochondrial area (MA) was calculated as a function of perimeter. Mitochondrial volume density was

Mitochondrial area (MA) was calculated as a function of perimeter. Mitochondrial volume density was measured using a 110 point lattice grid and applying the stereological procedures of Weibel.^[9]

Results

Morphological Analysis. Microscopic analysis of skeletal muscle from these patients revealed focal areas of marked fiber size variation and atrophy, regions of myofibrillar disarray, and degeneration as well as widened intermyofibrillar regions. Areas of sarcolemmal blebbing, indicating a loss in membrane integrity, thickening, and lamination of the basement membrane were observed. Regions with occluded capillaries and areas with distended perivascular spaces filled with material of low electron density containing an infiltrate of red blood cells, macrophages, and lymphocytes were observed. Pyknotic nuclei as well as central nuclear migration, particularly in type II atrophic fibers, as well as Z-band streaming and disintegration, and areas devoid of myofilaments were observed. An increased concentration of glycogen aggregates and lipid deposits was obvious, particularly in type II fibers. Reduced numbers of mitochondria, many with disorganized membranes and cristae of unusual configurations, were observed (Figure 2). Vesicular structures adjacent to Z bands, intracytoplasmic inclusions, and lipofuscin granules were observed throughout but predominantly within subsarcolemmal and perinuclear regions.

Clinical and Morphometric Analyses. TSH, FT₄, and clinical symptoms of the 4 patients are shown in Table 1. Patients 1 and 2 had higher blood TSH values when compared to patients 3 and 4. Also, the skeletal muscle mitochondrial densities of patients 1 and 2 (Table 2) were lower when compared with patients 3 and 4.

Mitochondrial mass per unit of muscle fiber area is a morphological indicator of muscle oxidative capacity and can be expressed by measuring mitochondrial area and mitochondrial density in the same micrograph. Figure 1 shows graphic illustrations of mitochondrial mass per unit area of skeletal muscle mass. This was determined by measuring mitochondrial volume density in combination with mean mitochondrial area for the 4 patients. Patients 3 and 4 had a greater mitochondrial mass per unit area of muscle mass than did patients 1 and 2. Smaller sized mitochondria were evident in the skeletal muscle of patient 1 in comparison to the other three.

Discussion

The relationship between TSH levels and clinical symptoms in patients with subclinical hypothyroidism is variable and is thus of restricted diagnostic utility. This is illustrated by the cases reported in this study. For example, patient 2 had the highest TSH level (15.2 m IU/L) but did not complain of myalgia and reported no other hypothyroid symptoms (Table 1). In contrast, patient 4 complained of myalgia, reported symptoms compatible with overt hypothyroidism (Table 1), but had a lower TSH level (11.01m IU/L). This represents an example where morphological analysis offers a more objective measurement of disease progression.

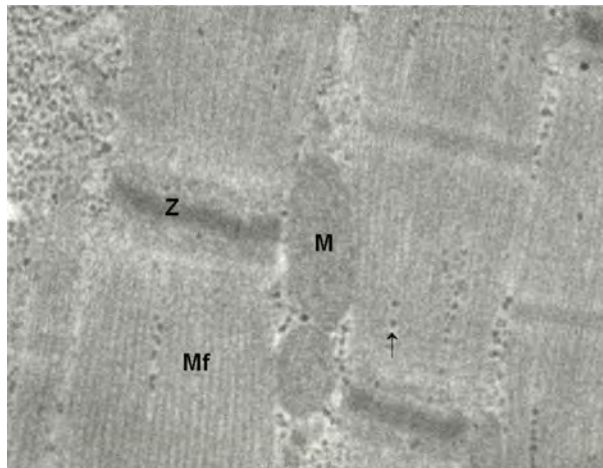
Myalgia is a consistent clinical hypothyroid symptom and often is a precursor to a decline in physical activity and exercise tolerance.^[10,11,12] An early investigation of hypothyroid skeletal muscle identified areas of normal muscle that were interrupted by segments of complete structural disorganization characterized by myofilament loss.^[13] Subsequent studies confirmed these results and thus established a relationship between myalgia due to overt hypothyroidism and skeletal muscle morphological changes.

Recognizing that specific skeletal muscle alterations are typical of hypothyroid patients, we examined skeletal muscle for histological changes that occur during subclinical hypothyroidism prior to its progression to overt hypothyroidism. We propose that the appearance of characteristic skeletal muscle “markers” would provide support for earlier intervention and perhaps a more efficacious treatment regimen.

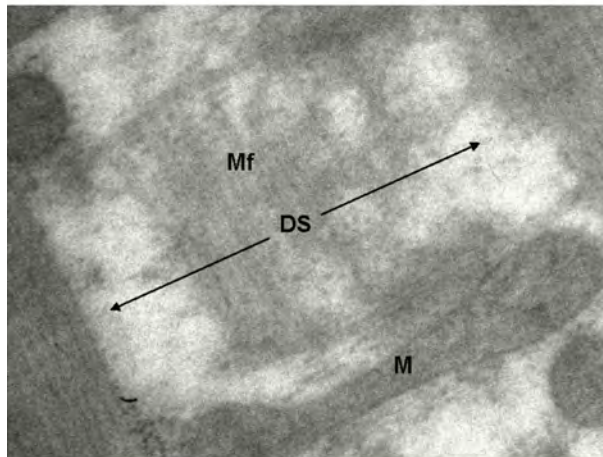
Table 2 indicates that the mitochondrial mass (mitochondrial area or mitochondrial volume density) in 2 of our 4 patients is smaller compared to normal skeletal muscle (mitochondrial area = $0.1\text{ }\mu\text{m}^2$).^[14] The number, size, and volume fraction of mitochondria closely reflect the metabolic capacity of the organism. Therefore, our data suggest that altered skeletal muscle metabolism is a prominent feature in the subclinical hypothyroid patient.

It is interesting to note that the 2 patients with elevated TSH levels (1 and 2) exhibited a low mitochondrial area per muscle fiber area (Figure 1) due to either an unusually low mean mitochondrial area (Patient 1 in Table 2) or a low mitochondrial volume density (Patient 2 in Table 2). In contrast, the 2 patients with lower TSH levels had mean mitochondrial

Figure 2. Ultrastructural comparison of a control (a) and a subclinical hypothyroid patient (sHT) (b).



a. Control Patient 25,000X



b. sHT Patient 4 25,000X

Fig. 2a. Control biopsy taken from an earlier unpublished study. Z bands (Z), mitochondria (M) and myofilaments (Mf) show good integrity. Small black dots are glycogen (t) particles.

Fig. 2b. Skeletal muscle taken from patient 4. Note the damaged sarcomere (DS) displaying sparse myofilaments (Mf) with elongated mitochondria (M) spanning across the sarcomere space, once occupied by Z bands.

areas and densities closer to those for healthy adults (range is $0.088 \mu^2 - 0.155 \mu^2$).^[15]

In an earlier study, we measured mitochondrial area in patients with heart failure prior to (mitochondria area = $0.036 \mu^2$) and following (mitochondria area = $0.046 \mu^2$) combined aerobic and resistance training.^[16] We also measured mitochondrial area in postmenopausal women at baseline ($0.039 \mu^2$) (un-

published data). Other authors reported a mitochondrial area of $0.076 \mu^2$ (obese), $0.063 \mu^2$ (diabetic), and $0.114 \mu^2$ (lean adults) in muscle biopsies.^[17] The mean mitochondrial area from the subclinical hypothyroid patients in this study ($0.10 \pm 0.05 \mu^2$) was within a range of $0.03 \mu^2$ to $0.15 \mu^2$ (Table 2). This indicates that although mitochondrial area in subclinical hypothyroid patients did not differ greatly

from healthy adults, it did demonstrate a tendency to decrease as TSH levels increased. The larger than expected mean mitochondrial area in our patients may be due to the high frequency of enlarged and distorted mitochondria (Figure 2 and Figure 3) often referred to as megamitochondria.^[16,17] These very large mitochondria with low inner membrane density may be undergoing apoptosis. However, mitochondrial area in the subclinical hypothyroid patients did demonstrate a tendency to decrease as TSH levels increased (Table 1 and Table 2), suggesting that specific mitochondrial changes in skeletal muscle are reflective of increased TSH levels. These data are in contrast to another investigation that reported the presence of mitochondrial alterations only in prolonged clinical cases.^[18]

The skeletal muscle histological profile of subclinical hypothyroidism was found to include focal areas of marked fiber variation and atrophy, regions of myofibrillar disarray, degeneration, and central nuclear migration, predominantly in type II fibers, regions devoid of myofilaments, Z band streaming and disintegration. Other studies involving overt hypothyroid patients reported “cores” of myofibrillar

thyroidism.^[19,20,21] Increased concentrations of glycogen aggregates was observed in type II fibers occupying up to $\frac{1}{3}$ rd of the cross-sectional area.^[20] The aggregates are thought to result from derangements of glycogen metabolism and to be related to the severity of subclinical hypothyroidism.^[22] These observations are consistent with other reported findings.^[20,22] The skeletal muscle changes that characterize subclinical hypothyroidism in our report are consistent with a previous study reporting a disease-related metabolic shift toward anaerobic ATP production in subclinical hypothyroidism.^[24]

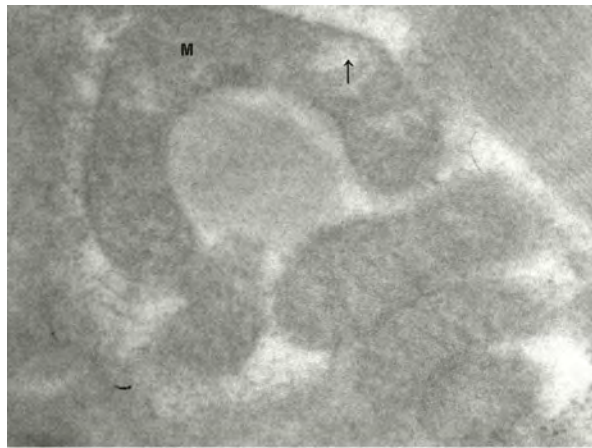
Mitochondrial plasticity is a response to changing metabolic conditions.^[3,8,23,24] We observed reduced numbers of mitochondria, many with loss of membrane integrity, containing dense lipid inclusions and cristae of unusual configurations. These observations are consistent with previous studies of hypothyroid patients^[10,19,22] and lend further support to the theory that thyroid hormones have a profound effect on mitochondrial energy expenditure.^[25]

We also found areas containing pyknotic nuclei and central nuclear migration, consistent with severe hypothyroid myopathy,^[26] as well as regions demonstrating a loss in sarcolemmal integrity and blebbing,^[18,27,28] along with inflammatory cell infiltration into necrotic fiber areas. This contrasts with other studies where necrotic muscle fibers were found in the absence of interstitial inflammatory cells.^[13]

In patients with severe hypothyroidism, selective type II fiber atrophy was reported to be due to impaired glycogen utilization and accompanied by an increased type I fiber area which gives the muscle a “bulky” appearance.^[27,29] A link between the severity of myopathic symptoms, the degree of type II fiber atrophy and loss, and increased central nuclear displacement has been recognized.^[22] A correlation between clinical severity of hypothyroidism and the degree of myofibrillar loss and abnormal glycogen accumulation has been established.^[18] These observations are consistent with our report.

This report identifies specific skeletal muscle morphological changes that are characteristic of subclinical hypothyroidism. Establishing consistent morphological markers of subclinical hypothyroidism prior to disease progression could justify an earlier, more efficacious treatment with thyroid hormone. This treatment strategy may diminish morbidity by preventing disease progression from subclinical to overt hypothyroidism. Earlier initiation of

Figure 3. Subclinical hypothyroid patient 4.



Skeletal muscle taken from patient 4. Several mitochondria (M) have moved from their normal position between myofibrils (see Fig. 2) to within a sarcomere. They appear large, elongated and irregular in shape with a lower density of inner membranes. The curved mitochondria has lost some internal membrane structure (↑), suggesting that apoptosis may be taking place.

disarray in type I fibers, and this was observed to correlate with the severity and duration of hypo-

this therapy may ultimately translate into an improvement in patients' lifestyle. Further research to establish reproducibility and clinical effectiveness appears to be warranted from these case studies.

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References

- Legrys, V.A., Hartman, K., and Walsh, J.F.: The clinical consequences and diagnosis of hypothyroidism. *Clin. Lab. Sci.*, 4:203-208, 2004.
- Surks, M.I., Ortiz, E., and Daniels, G.H., et al.: Subclinical thyroid disease. *JAMA*, 291:228-238, 2004.
- Monzani, F., Caraccio, N., Del Guerra, P., et al.: Neuromuscular symptoms and dysfunction in subclinical hypothyroid patients: beneficial effect of L-T₄ replacement therapy. *Clin. Endo.*, 51:237-242, 1999.
- Col, N.F., Surks, M.I., and Daniels, G.H.: Subclinical thyroid disease. *JAMA*, 291:239-243, 2005.
- Wilson, G.R., and Whit Curry, R.: Subclinical thyroid disease. *Amer. Fam. Phys.*, 72:1517-1524, 2005.
- Cooper, D.S.: Thyroid disease in the oldest old. *JAMA*, 292:2651-2654, 2004.
- Hennessey, J.V., Chromiak, J.A., Della Ventura, S., et al.: Increase in percutaneous muscle biopsy yield with a suction-enhancement technique. *J. Appl. Physiol.*, 82:1739-1742, 1997.
- Cosmas, A.C., Monteiro, M., Levesque, S., et al.: Ultrastructural changes in skeletal muscle of postmenopausal women following twelve months of aerobic exercise and progressive resistance training. Proceedings of the 31st. annual American Aging Association/16th. Annual meeting of American College of Clinical Gerontology, 18, 2002.
- Weibel, E.R.: Stereological principles for morphometry in electron microscopic cytology. *Int. Rev. Cytol.*, 26:235-302, 1969.
- Jiskra, J.: Myopathy syndrome in undiagnosed hypothyroidism. *Vnitr. Lek.*, 47:642-646, 2001.
- Lochmuller, H., Reimers, C.D., Fischer, P., et al.: Exercise-induced myalgia in hypothyroidism. *Clin. Invest.*, 12:999-1001, 1993.
- Rodolico, R.A., Toscano, S., Benvenega, A., et al.: Myopathy as the persistently isolated symptomatology of primary autoimmune hypothyroidism. *Thyroid*, 8:1033-1038, 1998.
- Norris, F.H., and Panner, J.: Hypothyroid Myopathy. *Arch. Neurol.*, 11:574-589, 1961.
- Jerusalem, F., Engel, A.G., and Peterson, H.A.: Human muscle fiber fine structure: Morphometric data on controls. *Neurol.*, 25:127-134, 1975.
- Jerusalem, F., Engel, A.G., and Peterson, H.A.: Human muscle fiber fine structure: Morphometric data on controls. *Neurol.*, 25:127-134, 1975.
- Santoro, C., Cosmas, A., Forman, D., et al.: Exercise training alters skeletal muscle mitochondrial morphometry in heart failure patients. *J. Cardiovas. Risk*, 9:377-381, 2002.
- Kelley, D.E., Jing, H., Menshikova, E.V., et al.: Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*, 51:2944-2950, 2002.
- Lin, R.T., Liu, C., Tai, C., et al.: Hypothyroid myopathological and ultrastructural study. *Kaohsiung J. Med. Sci.*, 16:68-75, 2000.
- Modi, G.: Cores in hypothyroid myopathy: a clinical, histological and immunofluorescence study. *J. Neuro. Sci.*, 175:28-32, 2000.
- Ono, S., Inouye, K., and Mannen, T.: Myopathology of hypothyroid myopathy: Some new observations. *J. Neurol. Sci.*, 77:237-248, 1987.
- Rodolico, C., Toscano, A., Benvenega, S., et al.: Myopathy as the persistently isolated symptomatology of primary autoimmune hypothyroidism. *Thyroid*, 8:1033-1038, 1998.
- Agostinucci, K., Manfredi, T.G., Cosmas, A., et al.: Vitamin E and liver mitochondrial morphometry. *Age*, 36:2393, 1999.
- Caraccio, N., Natali, A., Sironi, A., et al.: Muscle metabolism and exercise tolerance in subclinical hypothyroidism: A controlled trial of L-thyroxine. *J. Clin. Endocrinol. Metab.*, 90:4057-4062, 2005.
- Bertoni-Freddari, C., Fattoretti, P., Casoli, T., et al.: Morphological plasticity of synaptic mitochondria during aging. *Brain Research*, 628:193-200, 1993.
- Evans, R.M., Watanabe, I., and Singer, P.A.: Central changes in hypothyroid myopathy: A case report. *Muscle & Nerve*, 13:952-956, 1999.
- Harper, M., and Seifert, E.L.: Thyroid hormone effects on mitochondrial energetics. *Thyroid*, 18:145-156, 2008.
- Khaleeli, A.A., Gohil, K., McPhail, G., et al.: Muscle morphology and metabolism in hypothyroid myopathy: effect of treatment. *J. Clin. Pathol.*, 36:519-526, 1983.
- Staub, J.J., Althaus, B.U., Engler, H., et al.: Spectrum of subclinical and overt hypothyroidism: effect on

thyrotropin, prolactin and thyroid reserve, and metabolic impact on peripheral target tissues. *Am. J. Med.*, 92:631-642, 1992.

29. Khaleeli, A.A., Griffith, D.G., and Edwards, R.H.T.:

The clinical presentation of hypothyroid myopathy and its relationship to abnormalities in structure and function of skeletal muscle. *Clin. Endocrin.*, 19:365-376, 1983.