Skeletal muscle weakness in patients with sarcoidosis and its relationship with exercise intolerance and reduced health status

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Web-only Appendix

Methods

All measurements took place at the outpatient department of the respiratory rehabilitation division of the University Hospital Gasthuisberg in Leuven, Belgium. Technicians and laboratory workers performing blood analyses and functional tests were unaware of the group-classification of the participants (e.g. sarcoidosis patient or healthy control subject).

Measurements

Venous blood analyses

To minimize possible 'noise' of circadian rhythm (1) and/or of exercise (2) on circulating levels of various cytokines, venous blood was drawn from supine subjects between 08.30 and 09.00 ante meridian, before functional testing. Interleukin (IL)-6 and interleukin-8 (CXCL8) were determined using Human Inflammation Cytometric Bead Array (Becton Dickinson Biosciences, San Diego, CA; range: 1.0 to 625 pg/ml) (3). Tumor necrosis factor-alpha (TNF-α) and its soluble receptors (sTNF-R55 and sTNF-R75) were measured with enzyme-linked immuno assays (EASIA KAC 1752 (range: 5.0 to 130.0 pg/ml), 1762 (range: 0.5 to 47.0 ng/ml) and 1772 (range: 1.7 to 120 ng/ml), respectively, Biosource Europe S.A., Nivelles, Belgium). Insulin-like growth factor-I (IGF-I) was determined using a radio-immuno assay as described previously (4) (range: 45 to 3500 ng/ml). IGF binding protein 3 (IGFBP-3) was assessed using a solid phase immunoradiometric assay (IRMA C.T. BC 1014, Biocade S.A., Liège, Belgium, range: 390 to 7800 ng/ml)).

Functional tests

Pulmonary function

Forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) were measured according to the European Respiratory Society guidelines for pulmonary function testing (5). In addition, the diffusing capacity for carbon monoxide ($T_{L,CO}$) was measured by the single breath method (Sensor Medics 6200, Bilthoven, the Netherlands). Results were expressed as a percentage of the predicted normal values of Quanjer and co-workers (5).

Skeletal muscle force tests

Handgrip force was evaluated using a hydraulic dynamometer (Yamar Preston, Jackson MI). It was assessed at the dominant side with the elbow joint in 90 degrees flexion and the lower arm and wrist in neutral position. Isometric quadriceps peak torque was measured using a Cybex Norm (Enraf-Nonius, Delft, the Netherlands). Peak extension torque was evaluated in sitting position at 60 degrees of knee flexion and 90 degrees of hip flexion. Both tests were performed at least three times, and the best of two reproducible tests was used for analysis. Values were related to reference values of Mathiowetz and co-workers (6) and Decramer and co-workers (7), respectively.

Respiratory muscle force tests

Inspiratory and expiratory muscle strength was measured using a modification of the technique of Black and Hyatt (8). An electronic pressure transducer was used to measure the generated pressure. The signal was recorded for further evaluation. At least five attempts were made to measure expiratory muscle strength from total lung capacity and to measure inspiratory muscle strength from residual volume. Both were determined as the pressure that could be sustained for at least 1 second. Tests were repeated until the variability among the three best attempts was less then 5%. The highest value was expressed as a percent of the predicted value (9).

Functional exercise capacity

Functional exercise performance was measured by a 6-minute walking test.This test was performed in a 53-meter hospital corridor. Encouragement was standardized (10). Transcuteneous oxygen saturation and heart rate were measured during this test. At the end of the test symptom Borg-scores for dyspnea and fatigue were obtained from all participants.The best of two tests was used and expressed as percent of the predicted value (11).

Symptom-limited peak exercise test

Peak exercise capacity was assessed by a maximal incremental cycle exercise test (Partn'air 5400; Medisoft, Dinant, Belgium). After a 2-minute resting period and 3 minutes of unloaded cycling, participants started at 20 watts and cycled until symptom limitation at an incremental workload (+30 watt·2 minutes^{-1}). Oxygen consumption, carbon dioxide output, and ventilation were measured breath by breath. Heart rate was monitored constantly. At the end of the test symptom Borg-scores for dyspnea and fatigue were obtained from all participants. Peak external work rate and peak oxygen uptake were normalized for height, age and gender (12).

Questionnaires

Health status was measured with the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) (13). It includes eight multiple-item scales that assess physical functioning (the extent to which health limits physical acitivities such as self-care, walking climbing stairs, bending, lifting, and moderate and vigorous exercise), physical role (the extent to which physical health interferes with work or other daily activities, including accomplishing less than wanted, limitations in the kind of activities, or difficulty in performing activities), pain (the intensity of pain and effect of pain on normal work, both inside and outside home), general health (the personal evaluation of health, including current health, health outlook, and resistance to illness), vitality (feeling energetic and full of pep versus feeling tired and worn out), social functioning (the extent to which physical health or emotional problems interfere with normal social activities), emotional role (the extent to which emotional problems interfere with work or other daily activities, including decreased time spent on activities, accomplishing less, and not working as carefully as usual), and mental health (general mental health, including depression, anxiety, behavioral-emotional control, general positive effect). Scores can range from 0 (worst) to 100 (optimal).

Participants completed the EQ-5D (formerly the EuroQoL), which provides a simple descriptive profile and a single index value for health status (14). It comprised of 5 dimensions of health: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Moreover, all participants self-rated their health status on a graduated (0-100) visual analogue scale. The Hospital Anxiety and Depression Scale (HADS) was used to assess the presence of clinical symptoms of anxiety and depression (15). Seven questions are related to anxiety and 7 questions are related to depression. Each item is rated on 4-point scale (0 to 3), giving maximum scores of 21 for anxiety and depression, respectively. A score > 10 points is suggestive for the presence of clinical symptoms of anxiety or depression. The domain fatigue of the Chronic Respiratory Disease Questionnaire (CRDQ) was used to assess fatigue (16). It comprised of 4 questions related to fatigue. Each question is rated on a 7-point scale (1 to 7). Scores can range from 4 (worst) to 28 (no fatigue complaints).

Statistical analyses

All statistical procedures were performed using SAS package 6.08 and V8 (SAS Institute, Cary, North Carolina, USA). Data have been tested for normality with the Kolmogorov-Smirnov test. Results are presented as mean (standard deviation) or as median (interquartile range, IQR). A two-tailed unpaired t-test (continuous), a Mann-Whitney U test or a chi square test (binomial) was used to determine differences between sarcoidosis patients and healthy control subjects. Differences between means and their 95% confidence intervals (95% CI) are reported. Moreover, differences between medians were calculated with bootstrapping technique. Sampling was performed with repeated replacement (5000 replications). The resulting sample of effects then was used to calculate its 95% CI (17). Due to the large number of comparisons a two-sided level of significance was set at $p \leq 0.01$ (18). Pearson product moment correlation (continuous, r) or Spearman rank correlation (r_s) were used to determine the relationship between skeletal muscle force and inflammatory and anabolic markers, and to determine the relationships between physiological factors, exercise capacity and health status in sarcoidosis. *A priori*, a two-sided level of significance was set at $p \leq 0.05$ (18).

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Reconstruction of original figure of Delobbe and co-workers

Figure S2 of the *web-only appendix* is a reconstruction of the original Figure 2 of Delobbe and co-workers (19). For both groups peak values of peak oxygen uptake were taken (for patients with sarcoidosis: 2.128 liters per minute; for healthy control subjects: 2.909). Afterwards, we calculated the absolute values for oxygen uptake at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% of the achieved peak oxygen uptake. Moreover the heart rate values for the respective mean oxygen values were estimated from an enlarged copy of the original Figure 2 (19).

References *web-only appendix*

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Legend Figure S1. Heart Rate over Oxygen Uptake in Sarcoidosis and Healthy Subjects.

Mean (standard deviation) heart rate (H_R) over mean oxygen uptake (VO_2) in patients with sarcoidosis (●, heart rate = 66 + 54·oxygen uptake) and in healthy subjects (□, heart rate = 64 + 47·oxygen uptake). Heart rate is expressed in beats per minute (BPM), and oxygen uptake in liters per minute (l/min).

Legend Figure S2. Reconstruction of the Relationship between Heart Rate and Oxygen Uptake.

Mean (standard deviation) heart rate (H_R) over mean oxygen uptake (VO_2) in patients with sarcoidosis (●) and in healthy control subjects (□), reconstructed from values reported by Delobbe and co-workers (19). Heart rate is expressed in beats per minute (BPM), and oxygen uptake in liters per minute (l/min).

Table S1. Circulating Levels of Inflammatory and Anabolic Markers in Sarcoidosis Patients and in Healthy Subjects

	Sarcoidosis		Healthy		Differences between medians (95%CI)	p-value
IL-6, pg·ml^{-1}	2.40	(1.50-3.70)	2.10	(1.00-4.90)	-0.3 (-1.75 to 1.00)	0.56
CXCL8, pg·ml^{-1}	3.05	(2.30-4.20)	2.00	(1.70-3.80)	-1.05 (-2.15 to 0.85)	0.29
TNF-α, pg·ml^{-1}	24.1	(20.2-30.5)	15.3	(12.9-22.5)	-8.8 (-12.6 to -0.91)	0.01
STNF-R55, ng·ml^{-1}	1.77	(1.57-2.06)	1.67	(1.59-1.85)	-0.10 (-0.36 to 0.12)	0.31
STNF-R75, ng·ml^{-1}	4.34	(3.83-4.95)	3.54	(2.72-4.42)	-0.80 (-1.50 to 0.11)	0.04
IGF-I, ng·ml^{-1}	138	(125-160)	143	(119-184)	5 (-25 to 40)	0.40
IGFBP-3, ng·ml^{-1}	2576	(2320-2880)	2644	(2502-2776)	68 (-198 to 284)	0.62

Values are expressed as median (interquartile range) and as differences between medians (95% confindence interval, 95%CI). IL-6=interleukin-6; CXCL8=interleukin-8; TNF-α=tumor necrosis factor-alpha; sTNF-R55=soluble TNF receptor p55; sTNF-R75=soluble TNF receptor p75; IGF-I=insulin-like growth factor-I; IGFBP-3=IGF binding protein 3 (IGFBP-3).

Table S2. Circulating Levels of Inflammatory and Anabolic Markers in Sarcoidosis Patients Taking Steroids and Those Not Taking Steroids

	Taking Steroids		**No Steroids**		Differences between medians (95%CI)	p-value
IL-6, pg·ml^{-1}	3.20	(2.40-6.60)	1.80	(1.00-2.40)	-1.4 (-4.8 to -0.4)	0.005
CXCL8, pg·ml^{-1}	3.20	(2.60-4.40)	2.70	(2.10-4.20)	-0.5 (-2.0 to 1.1)	0.25
TNF-α, pg·ml^{-1}	22.6	(12.5-24.5)	27.9	(20.6-44.4)	5.3 (-2.8 to 22.8)	0.10
STNF-R55, ng·ml^{-1}	1.75	(1.64-2.06)	1.78	(1.50-2.18)	0.03 (-0.39 to 0.43)	0.90
STNF-R75, ng·ml^{-1}	4.38	(3.83-4.90)	4.31	(3.62-4.96)	-0.07 (-0.85 to 0.91)	0.74
IGF-I, ng·ml^{-1}	144	(125-164)	134	(95-157)	-10 (-49 to 29)	0.67
IGFBP-3, ng·ml^{-1}	2689	(2364-2838)	2446	(2125-2922)	-244 (-592 to 309)	0.57

Values are expressed as median (interquartile range) and as differences between medians (95% confindence interval, 95%CI). IL-6=interleukin-6; CXCL8=interleukin-8; TNF-α=tumor necrosis factor-alpha; sTNF-R55=soluble TNF receptor p55; sTNF-R75=soluble TNF receptor p75; IGF-I=insulin-like growth factor-I; IGFBP-3=IGF binding protein 3 (IGFBP-3).

Table S3. Scores on Questionnaires in Sarcoidosis Patients Taking Steroids and Those Not Taking Steroids

	Taking Steroids		No Steroids		Differences between medians (95% CI)	p-value
SF-36 (0 to 100)						
Physical functioning	35	(30-60)	67.5	(45-75)	32.5 (-10 to 42.5)	0.07
Role functioning physical	0	(0-50)	12.5	(0-50)	12.5 (-37.5 to 50)	0.51
Bodily pain	40	(40-50)	55	(50-70)	15 (-5 to 30)	0.16
General health	30	(20-60)	45	(20-60)	15 (-15 to 40)	0.55
Vitality	45	(20-65)	40	(30-60)	-5 (-30 to 25)	0.81
Social functioning	63	(38-88)	69	(50-88)	6 (-37.5 to 37.5)	0.95
Role functioning emotional	100	(0-100)	100	(67-100)	0 (-33.3 to 100)	0.72
Mental health	72	(44-80)	60	(44-80)	-12 (-36 to 22)	0.50
HADS (0 to 21)						
Anxiety	6	(2-9)	9	(6-11)	3 (-1 to 7)	0.14
Depression	8	(3-13)	7	(3-11)	-1 (-8 to 6)	0.50

Table S3. Scores on Questionnaires in Sarcoidosis Patients Taking Steroids and Those Not Taking Steroids, continued

Values are expressed as median (interquartile range) and as differences between medians (95% confindence interval). Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) included 8 multiple-item scales. Hospital Anxiety and Depression Scale (HADS) included 7 questions related to anxiety and 7 questions are related to depression.

Table S4. Correlations between Questionnaires and Muscle Force in Sarcoidosis Patients

	QPT	Depression	Fatigue
SF-36			
Physical functioning	0.70 [‖]	-0.67 [‖]	0.57 [§]
Role functioning physical	0.59 [§]	-0.54 [§]	0.67 [‖]
Bodily pain	0.62 [§]	-0.68 [‖]	0.62 [§]
General health	0.31	-0.42 *	0.78 [‖]
Vitality	0.44 [†]	-0.77 [‖]	0.78 [‖]
Social functioning	0.50 [‡]	-0.82 [‖]	0.57 [§]
Role functioning emotional	0.30	-0.61 [§]	0.74 [‖]
Mental health	0.56 [§]	-0.76 [‖]	0.89 [‖]

Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36, expressed in arbitrary units, points), quadriceps peak torque (QPT, expressed as a percent of the predicted value), depression measured with Hospital Anxiety and Depression Scale, and CRDQ-domain fatigue (both expressed in arbitrary units, points). * p=0.06, [†] p=0.05, [‡] p=0.02, [§] p=0.01, [‖] $p \leq 0.001$