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ORIGINAL ARTICLE

Myoglobin plasma level related to muscle mass and fiber composition – a clinical marker of muscle wasting?

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Abstract Progressive muscle wasting is a central feature of cancer-related cachexia and has been recognized as a determinant of poor prognosis and quality of life. However, until now, no easily assessable clinical marker exists that allows to predict or to track muscle wasting. The present study evaluated the potential of myoglobin (MG) plasma levels to indicate wasting of large locomotor muscles and, moreover, to reflect the loss of MG-rich fiber types, which are most relevant for daily performance. In 17 cancer-cachectic patients (weight loss 22%) and 27 age- and

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gender-matched healthy controls, we determined plasma levels of MG and creatine kinase (CK), maximal quadriceps muscle cross-sectional area (CSA) by magnetic resonance imaging, muscle morphology and fiber composition in biopsies from the vastus lateralis muscle, body cell mass (BCM) by impedance technique as well as maximal oxygen uptake (VO_2max). In cachectic patients, plasma MG, muscle CSA, BCM, and VO_2max were 30–35% below control levels. MG showed a significant positive correlation to total muscle CSA (r=0.65, p<0.001) and to the CSA fraction formed by type 1 and 2a fibers (r=0.80, p<0.001). However, when adjusted for body height and age by multiple regression, MG yielded a largely improved prediction of total CSA (multiple r=0.83, p<0.001) and of fiber type 1 and 2a CSA (multiple r=0.89, p<0.001). The correlations between CK and these muscle parameters were weaker, and elevated CK values were observed in 20% of control subjects despite a prior abstinence from exercise for 5 days. In conclusion, plasma MG, when adjusted for anthropometric parameters unaffected by weight, may be considered as a novel marker of muscle mass (CSA) indicating best the mass of MG-rich type 1 and 2a fibers as well as VO_2max as an important functional readout. CK plasma levels appear to be less reliable because prolonged increases are observed in even subclinical myopathies or after exercise. Notably, cancer-related muscle wasting was not associated with increases in plasma MG or CK in this study.

Keywords Cancer cachexia · Muscle wasting · Muscle biopsy · Muscle morphology · Fiber composition

Introduction

Cachexia has been recognized as a life-threatening syndrome of progressive weight loss associated with cancer, cardiorespiratory, or inflammatory diseases. It is experienced by up to 50% of cancer patients and may account for more than 20% of cancer-related deaths [1–5]. The major manifestation of cachexia, besides lipolysis, is muscle wasting, which in many cancer patients is only partly attributable to malnutrition, anemia, inactivity, or therapeutic interventions. Muscle wasting is considered to be critical for impaired mobility, quality of life, and prognosis. The underlying massive netto-proteolysis, which appears to target especially myosin as the functional contractile protein, is caused by a metabolic dysregulation that is just about to be unraveled in animal and in vitro models [6–9] or humans [10, 11] Progressive protein loss leads to muscle fiber shrinkage and reduction in cross-sectional area (CSA), and a wasting-related reduction in muscle CSA of most relevant locomotor muscles like the quadriceps femoris muscle together with associated changes in fiber composition and capillarization explain a major portion of the loss of aerobic capacity (VO_2max) in healthy subjects as well as in cancer patients [12–14] (Hildebrandt et al., unpublished data). Accordingly, the reduction in VO_2max is most prominent in gastrointestinal cancer types with the highest incidence of cachexia as opposed to non-cachectic breast cancer patients [15–17]. As daily endurance performance required for mobility is best assessed by VO_2max as a functional readout of muscle mass and fiber composition together with cardiorespiratory functions, it may be of great clinical value to identify an easily assessable and reliable marker of reduced muscle mass, which is more precisely related to muscle mass and fiber composition than the widely used body weight or body impedance analysis. At present, no such parameter exists, and muscle mass determination by dual energy X-ray absorptiometry (DEXA), magnetic resonance imaging (MRI), or computed tomography (CT) is limited to studies and can hardly be routinely established given the high costs and low availability of these methods.

We therefore addressed the question, to what extent plasma MG, as an easily measurable blood parameter, could be a surrogate parameter for relevant muscle mass and fiber composition. The idea behind this approach was that the functionally important aerobic slow-twitch type 1 (and 2a) fibers are the primary source of plasma MG, a rather small, solely cytoplasmatic protein (17 kDa), which has a very short half-time (5.5 h) for renal elimination after severe muscle damage. In comparison, CK, which is less fiber specific, is present in different cellular compartments, and elevations of CK plasma levels are often associated with even subclinical myopathies and are known to be much more prolonged upon severe exercise or muscle damage than that of plasma MG levels [18, 19]. Therefore, CK plasma levels were hypothesized to be less indicative of a functionally relevant muscle mass.

The present study therefore examined the relation of MG and of CK to the total quadriceps muscle CSA determined by MRI in severely cachectic patients suffering from gastrointestinal cancer as well as in healthy controls similar in age, gender, and body weight at health. Additionally, in surgically obtained biopsies, we determined each fiber type's fractional CSA of the vastus lateralis muscle as the most important large locomotor muscle and studied their relation to MG or CK. We found a close positive and highly significant correlation between MG and total muscle CSA and the CSA formed by fiber type 1 (or 1 and 2a), which was considerably strengthened when MG was adjusted for body height and age by multiple regression (r=0.83 and r=0.89, respectively). All subjects had abstained from exercise for at least 5 days to exclude exercise-induced muscle damage that is a well-known cause of increases in plasma MG [18–20]. As all MG values were found to be in the normal range—especially in the cachectic cancer patients—severe muscle wasting appears not to involve myocellular membrane

damage or myolysis. Our explorative, invasive study in a limited sample size generates the hypothesis that muscle mass may be predicted from MG and easily assessable anthropometric parameters unaffected by weight loss. Less invasive studies on larger cohorts are warranted to evaluate whether MG may be considered as a novel clinically feasible marker to track muscle wasting and fiber composition.

Materials and methods

Cancer-cachectic patients and control subjects

Seventeen mobile patients with the diagnosis of a malignoma and a cancer-related cachexia defined as a progressive weight loss of >10% within 6 months (inclusion criteria) were recruited as outpatients of the Medical University Clinic of Heidelberg (Department of General and Visceral Surgery or Department of Internal Medicine I or II) as well as from regional oncological practices. Five types of cancer associated with a high risk of progressive cachexia were admitted to the study resulting in the following distribution among the randomly selected patients: three gastric cancer (Union internationale contre le cancer (UICC) staging: one with IIIa and two with IV), seven pancreatic cancer (UICC staging: one with II and six with III), three colon cancer (UICC staging: one with II, one with IIIa, and one with IIIc), one bronchogenic carcinoma (UICC staging IV), and three chronic lymphatic leukemia (CLL) (Binet-staging: C). The patients were included into the study irrespective of prior or simultaneous chemo- or radiotherapy. Nine patients had completed chemotherapy before inclusion, while six patients were under chemotherapy at the time of the study. Additional radiotherapy had been completed by four patients: two with pancreatic cancer, one with gastric cancer, one with bronchial cancer. Care was taken that any sampling or measurement was dated not before at least 8 days after any radio- or chemotherapeutic intervention.

Twenty-seven healthy controls were recruited by public announcement such that they were comparable to the cancer patients with respect to age, gender, and the body weight reported for health.

Patients and controls, by instruction, had abstained from any severe exercise at least 5 days before the examinations. All subjects and patients underwent an initial clinical examination for exclusion criteria that included routine laboratory parameters in venous blood samples, blood pressure measurement in sitting position, and electrocardiogram at rest and during an incremental exercise test for determination of VO_2max. The following main study parameters were obtained within 10 days: maximal quadriceps muscle CSA by MRI, muscle fiber composition by biopsy, VO_2max, body composition analysis by bioimpedance, and venous blood sampling for MG, CK, and other parameters. Exclusion criteria were any known or newly diagnosed severe neuromuscular, renal, inflammatory, cardiovascular, respiratory, hepatic, or psychiatric disease. Subjects or patients were without orthopedic problems of the lower extremity or spine that would have limited daily mobility and activity. Informed written and oral consent was obtained from all patients and control subjects. The study was approved by the Ethical Committee of the University of Heidelberg and performed according to the Declaration of Helsinki (1996) and to good clinical and laboratory practices.

Measurements and equipment

Venous blood analysis After an overnight, fast blood samples were drawn from an antecubital vein for clinical routine laboratory diagnostic in the central laboratory of the Medical University Clinic of Heidelberg. Additionally, whole blood samples were drawn and immediately centrifuged at 2,000 rpm for 10 min at 4°C to obtain serum or ethylenediaminetetraacetic acid (EDTA) plasma, which were stored at −75°C for further analysis. Interleukin-6 (IL-6) plasma levels in EDTA plasma were measured in duplicate using a commercial enzyme-linked immunosorbent assay (IBL, Hamburg, Germany). Plasma MG levels were determined in serum samples by a two-step sandwich enzyme immunoassay using the chemoilluminiscence detector device Centaur (Bayer, Leverkusen, Germany). Other blood parameters, i.e., cholinesterase, hemoglobin, albumin, and creatinine were obtained by routine laboratory methods.

Magnetic resonance imaging MRI of the right thigh was performed in the supine position on a 1.5-T clinical MR system (MAGNETOM Symphony, Siemens AG Medical Solutions, Erlangen, Germany) using the manufacturer's standard phased array coil for signal reception. The imaging protocol comprised an axial and coronal T1-weighted spin-echo sequence (repetition time/echo time in milliseconds, 500/13), an axial (4,000/50) and coronal (5,130/63) T2-weighted fat-suppressed short tau inversion recovery sequence, and a fat-suppressed T1-weighted turbo spin-echo (TSE) sequence (500/13). Muscle CSA of the right quadriceps femoris was determined including the maximal thigh circumference at 12-, 20-, and 28-cm distances from the trochanter major. All MR images were displayed as soft-copies in a fully electronic, monitored fashion using our picture archiving and communication system (PACS) with large-screen, high-resolution cathode-ray tube displays, which enabled the review of eight images simultaneously and the individual selection of the different MR sequences, e.g., to clarify the fascial boundaries of the quadriceps muscle. CSA was assessed on T1-weighted images using a computerized digitizer as part of the PACS standard tool. All

MR examinations were jointly randomized and shown to the readers in order of randomization. The reader was blinded to identifying parameters such as the subject's name and clinical data. This analysis of quadriceps muscle CSA renders precise and reproducible measurement of muscle CSA and a very high positive correlation between the values obtained by two independent blinded readers ($r>0.98$). Muscle CSA of the whole quadriceps femoris muscles was determined because, especially in the proximal and distal axial MRI scans, the fascial boundaries between the lateral and deep vastus muscles could often not be identified, as it has been described before [21].

Sampling and analysis of muscle biopsy Eleven out of 17 cachectic patients and 15 out of 27 controls gave their informed consent to muscle biopsy. The biopsies were taken from the vastus lateralis muscle at about mid-thigh level by means of the technique of Bergström [22] applied after careful local anesthesia and disinfection. The muscle samples were immediately shock-frozen in liquid nitrogen-cooled isopentane and stored at −80°C. For histochemistry and morphometric analysis, serial transverse sections (6 μm) were cut in a cryotome at −20°C. For fiber population analysis, transverse sections were stained for myofibrillar ATPase after preincubation at pH 4.35 (5 min, room temperature), pH 4.6 (5 min, room temperature), and pH 10.5 (15 min, 37°C) according to [23] as previously described [21]. The fiber types 1, 2a, 2ax, and 2x were identified according to their acid-sensitive ATPase-staining intensity after preincubation at pH 4.6, as well as the fiber types 1, 2c, and 2 after incubation at pH 10.5. Biopsies with less than 100 fibers were excluded from the analysis. The mean fiber number that could be analyzed per sample was 220±142. Furthermore, type 2ax fibers were subsumed in 2a fibers, as their fraction was below 1%. Microscopic video recordings of the ATPase-stained cross-sections (pH 4.6) were digitized by a PC-based image analysis system (VIBAM 0.0-VFG1 frame grabber). Fiber CSAs were determined at a 200-fold magnification. Statistical analysis was performed including the three main fiber types 1, 2a, and 2x.

Body composition Body composition was analyzed by measurement of electrical impedance and reactance under standardized conditions regarding body position, electrode localization, and overnight fast using the TVI-10 body composition analyzer purchased from FM Service GmbH (Leverkusen, Germany) in combination with the BIA-Star program by RECAL Biomed (Heidelberg, Germany) for calculation of absolute and percentage values of body fat, fat-free mass, total body water (TBW), and body cell mass (BCM) based on body height and actual measured body weight, as previously described [24]. The theoretical basis of this method has been described elsewhere [25].

Maximal oxygen uptake (VO_2max) VO_2max was determined breath-by-breath by the spirometric system ZAN680 (ZAN Ferraris Cardiorespiratory, Oberthulba, Germany) during an incremental exercise test on a cycle ergometer type Ergoline 100 (Ergoline, Windhagen, Germany) that increased work load from a starting level of 50 W in steps of 25 W every 2 min until exhaustion. VO_2max was calculated as the maximal mean value covering 10 s. VO_2max could be obtained from 19 controls and 12 cachectic patients only.

Statistics

Parameters measured on a continuous quantitative scale were described statistically using means and their standard error. Comparisons between cachectic patients and controls were performed by the Student's t test for unpaired observations. Bivariate correlations of MG, CK, or other parameters to CSA and each fractional fiber type's CSA, BCM, or VO_2max were assessed using linear regression (Pearson correlation coefficient r) and graphically presented as scatter plots including the linear regression line; r^2 was used as a measure of explained variability in linear regression.

In addition to univariate regression, a multiple stepwise regression analysis was performed between muscle CSA or fiber type 1 and 2a CSA as dependent variables and MG, body height, gender, and age as independent variables. Three of them, i.e., MG, body height, and age, remained in the multiple regression as independent predictors of muscle CSA or fiber type 1 and 2a CSA. The respective multiple regression equation was used to calculate individual *predictions* for muscle CSA and fiber type 1 and 2a CSA. These predictions were plotted as x values (based on MG, body height, and age) against individually corresponding *measured values* (y values) of both muscle CSA and fiber type 1 and 2a CSA (see Fig. 3a and b). Regression lines together with r and p values were determined for the total group of subjects as well as for the two subgroups of cachectic patients and controls. Moreover, 95% prediction intervals were calculated according to [26] using the formula

$$ax + b \pm 1.96\sqrt{s^2\left(1 + \frac{1}{n} + \frac{(x-\bar{x})^2}{\sum (x_i - \bar{x})^2}\right)}$$

where a is the regression coefficient, x is the multivariate predictor (independent variable), b is the y-axis intercept, s^2 is the variance, n is the number of observations, and $\bar{x}$ is the mean of all individual x. This approach to compare the two methods of assessing muscle mass was used because both methods involve different units, which prohibits the application of the Bland–Altman analysis assuming identical units between the methods to be compared.

A significance level of $p \leq 0.05$ was chosen for all statistical tests. The program SPSS (version 11.5, SPSS, Chicago, IL, USA) was used for all statistical tests.

Results

The 17 cachectic cancer patients and the 27 controls were not significantly different with regard to proportion of gender, to age, body height, and body weight at health (Table 1). Cachectic patients had a significantly lower actual body weight and body mass index due to a cancer-related mean weight loss of 22.3% and a mean weight loss rate of 3.45±0.79% per month (Table 1). The bioimpedance analysis of body composition revealed that the 19-kg difference in mean body weight was caused by a 10-kg lower BCM representing mainly muscle mass, a 5-kg lower fat mass, and lower TBW. Muscle CSA was found to be by 32% lower in cachectic patients compared to controls (Table 1). These data document the well-known fact that, besides fat mass, skeletal muscle mass is the primary target tissue of cancer-related wasting. Muscle CSA and BCM were significantly positively correlated ($r=0.93$, $p<0.001$), and MRI of the most important and largest locomotor muscle could thus be considered a reliable measure of overall muscle wasting.

Table 1 Anthropometric data, body composition, muscle CSA, as well as venous blood parameters

	Control	Cachexia	*p* value
n (female/male)	27 (13/14)	17 (8/9)	
Age (years)	57.9±2.4	52.5±1.6	0.07
Body weight (kg)	78.4±3.0	59.3±3.0	0.000***
Body height (cm)	173.1±1.6	171.1±1.6	0.482
Body mass index (kg m^{-2})	26.0±0.8	20.1±0.7	0.000***
Body weight at health (kg)	78.4±3.0	75.6±3.8	0.628
Weight loss (%)	–	22.3 ±28	
BCM (kg)	33.8±1.3	23.4±1.7	0.000***
Body fat (%)	22.7±1.2	17.9±1.5	0.017*
TBW (%)	62.5±1.5	56.1±1.1	0.001**
Muscle CSA (cm^2)	74.4±2.7	50.8±3.8	0.000***
CK (U l^{-1})	131.7±12.9	52.1±9.9	0.000***
MG (μg l^{-1})	42.0±6.0	27.0±2.8	0.000***
Cholinesterase (kU l^{-1})	8.91±0.44	6.24±0.65	0.001**
Hemoglobin (g dl^{-1})	15.0±0.4	12.5±0.4	0.000***
IL-6 (pg l^{-1})	2.86±0.38	8.15±3.0	0.092
Albumin (g l^{-1})	44.8±0.4	39.4±2.7	0.071
Creatinine (mg dl^{-1})	0.85±0.03	0.58±0.08	0.008**
VO_{2max} (l min^{-1})	2.60±0.16[a]	1.29±0.20[b]	0.000***
VO_{2max}/body weight (ml min^{-1} kg^{-1})	32.8±1.7[a]	21.3±2.4[b]	0.001**

Data show the mean±SEM.
Muscle CSA Cross-sectional area (maximum) of the quadriceps femoris muscle, *VO_2max* maximal oxygen uptake (aerobic capacity)
*$p<0.05$ by Student's *t* test cachexia vs control
**$p<0.01$ by Student's *t* test cachexia vs control
***$p<0.001$ by Student's *t* test cachexia vs control
[a] $n=19$
[b] $n=12$

MG plasma levels were found to be in the normal range (see Fig. 1a), and mean values in cancer-cachectic patients were 48% below that of controls (Table 1). CK plasma levels were about 60% lower in cancer-cachectic patients compared to the control group (Table 1), which included supranormal values in at least five control subjects despite abstinence from exercise 5 days before the study (Fig. 1c). Plasma creatinine levels were found within the normal range excluding a relevant renal insufficiency; however, within this normal range, the significant creatinine reduction in cachectic patients compared to controls should be noted (Table 1). Moreover, cholinesterase, a plasmatic indicator of hepatic function, i.e., protein synthesis, revealed a significant reduction in cachectic patients occurring within the normal range as well (Table 1). The same was true for albumin (Table 1), which is known to be reduced in cancer patients due to vascular escape [27]. Cachectic patients had a slight but significant anemia, with subnormal values found in the total group (Table 1) as well as in the female and male subgroup (not shown). Plasma levels of IL-6, a cytokine predominantly discussed as an etiologic factor of muscle wasting, were found to be increased in cachectic patients compared to controls without reaching the level of statistical significance (Table 1). There was no significant correlation between IL-6 and MG plasma levels or muscle CSA. VO_2max, in absolute terms, was about 51% lower in cachectic patients compared to controls, and after VO_2max normalization for body weight, a significant reduction remained amounting up to 35% (Table 1).

We found a significant positive correlation between individual MG plasma levels and BCM or muscle CSA in the total group of subjects (Fig. 1a and b). This correlation of MG to BCM or muscle CSA was also significant when analyzing men and women separately (women $r=0.45$, $p=0.05$ or $r=0.53$, $p=0.03$; men $r=0.60$, $p=0.003$ or $r=0.68$, $p<0.001$, respectively). Moreover, MG was found to be significantly correlated to BCM or CSA within the group of healthy controls ($r=0.61$, $p=0.001$ or $r=0.56$, $p=0.005$, respectively), while the respective correlations within the smaller group of cachectic patients were $r=0.38$, $p=0.15$ or $r=0.40$, $p=0.1$. Plasma levels of CK showed a significant but weaker positive correlation to BCM or muscle CSA (Fig. 1c and d). There was a significant positive correlation between MG and CK plasma levels ($r=0.62$, $p<0.001$).

Cholinesterase plasma levels were also positively correlated with BCM ($r=0.42$, $p=0.006$) or muscle CSA ($r=0.45$, $p=0.003$). Weaker but still significant correlations were found between plasma creatinine and BCM ($r=0.33$, $p=0.044$) or muscle CSA ($r=0.36$, $p=0.033$).

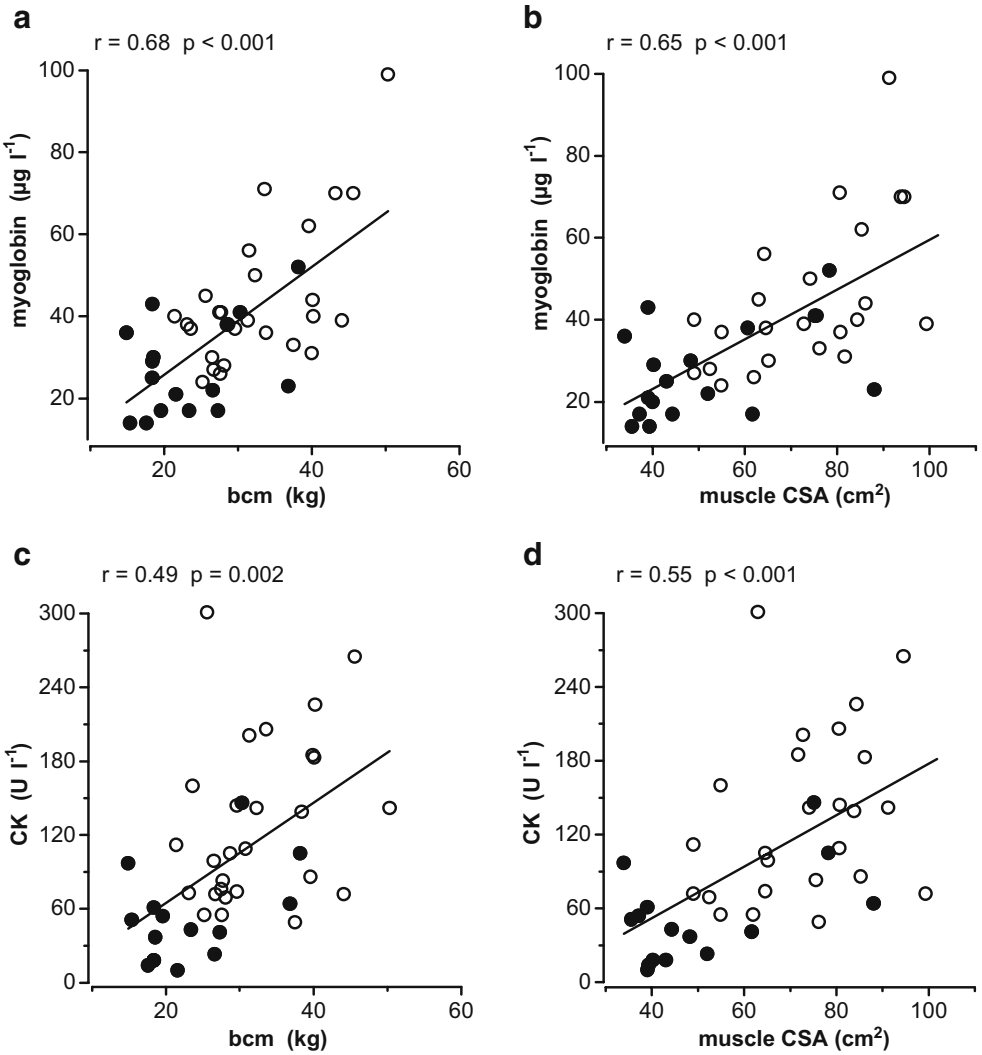

Fig. 1 Correlation between plasma MG (**a**, **b**) or CK (**c**, **d**) and BCM (**a**, **c**) or quadriceps muscle CSA (**b**, **d**). The regression line, the correlation coefficient *r*, and the *p* value are given for the total group of subjects. *Open circles* controls, *closed circles* patients

Table 2 presents fiber size and composition as well as total fiber type CSA for the three fiber types 1, 2a, and 2x in 26 biopsies obtained from 11 cachectic patients and 15 controls. We found a markedly reduced size of type 1 (−20%), type 2a (−55%), and type 2x (−45%) fibers in cachectic patients as compared to control subjects (Table 2). However, the fractional area of any of the three fiber types within the biopsies was similar between the two groups under test (Table 2). Within the given quadriceps CSA as determined by MRI, the total area contributed by type 1, type 2a, or type 2x fibers was found to be reduced by 22, 25, and 30% in cachectic patients compared to controls (Table 2). The overall reduction in fiber size of about 40% could well account for the 32% decrease in muscle CSA in cachectic patients below the control level.

Within the 11 patients and 15 controls from whom the muscle biopsies were obtained, we found significant positive correlations between plasma levels of MG and the total CSA formed by type 1 and by type 2a fibers (Fig. 2a and b). The closest correlation (r=0.80) was observed between MG and

Table 2 Skeletal muscle fiber type composition (vastus lateralis muscle)

	Controls	Cachexia	*p* value
Samples analyzed	15	11	
Fiber size			
Type 1 (µm^2)	4,675±381	3,723±364	0.099
Type 2a (µm^2)	6,942±225	3,207±374	0.195
Type 2x (µm^2)	3,765±446	2,105±401	0.016*
Fractional fiber area within the biopsy			
Type 1 (%)	44.2±3.2	50.2±3.2	0.316
Type 2a (%)	40.3±3.6	35.6±3.4	0.385
Type 2x (%)	15.5±3.3	14.2±4.2	0.802
Total fiber area within quadriceps CSA			
Type 1 (cm^2)	34.7±2.0	27.1±3.4	0.068
Type 2a (cm^2)	26.8±2.1	19.9±2.7	0.058
Type 2x (cm^2)	11.5±2.7	8.2±2.9	0.412

Data show the mean ± SEM
*p<0.05 by Student's t test cachexia vs control

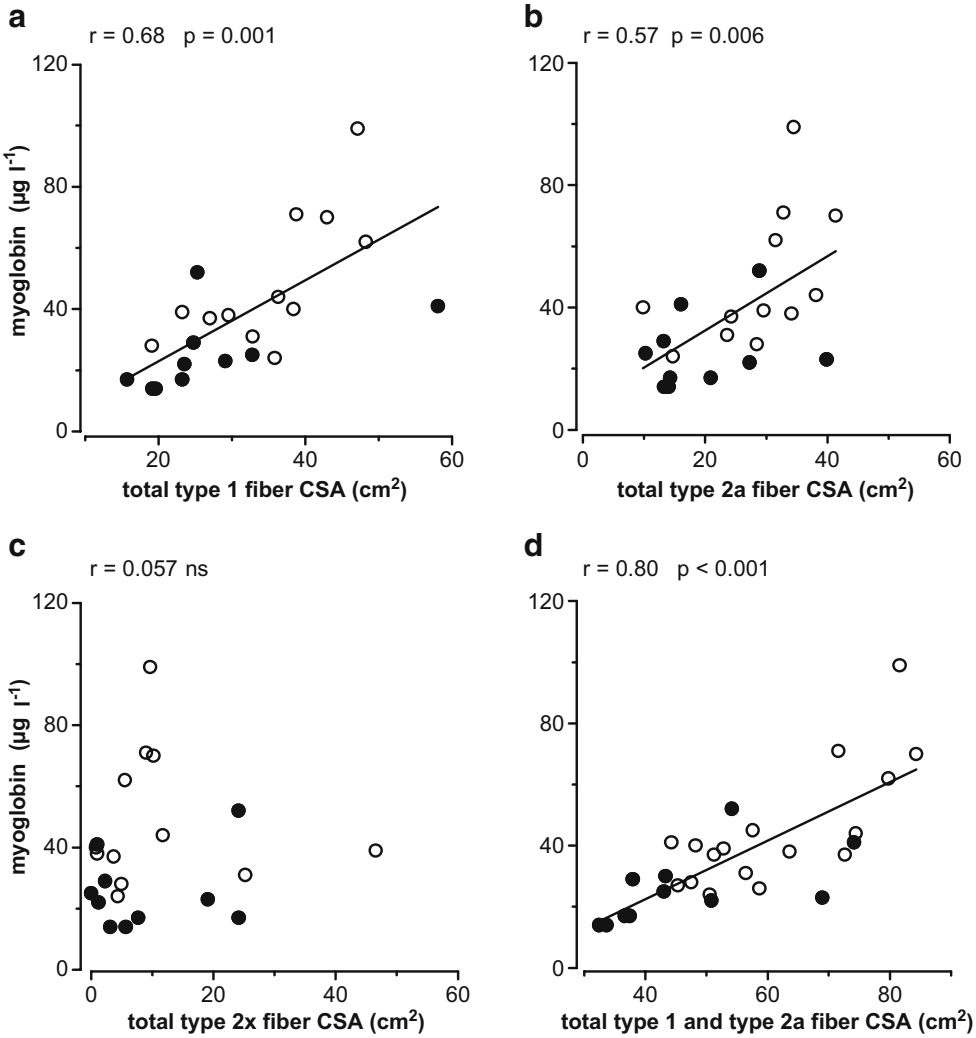

Fig. 2 Correlation between plasma MG and the total muscle CSA portion rendered by type 1 fiber (**a**), type 2a fiber (**b**), type 2x fiber (**c**), as well as by the sum of type 1 and 2a fibers (**d**). The regression line, the correlation coefficient *r*, and the *p* value are given for the total group of subjects. *Open circles* controls, *closed circles* patients

the CSA of the sum of type 1 and 2a fibers (Fig. 2d), and this correlation was also found within each of the two groups under test (healthy controls: $r=0.82$, $p=0.002$; cachectic cancer patients: $r=0.61$, $p=0.06$) and, moreover, within the subgroup of women ($r=0.77$, $p=0.26$) as well as within the subgroup of men ($r=0.76$, $p=0.002$). In contrast, no significant correlation was found between MG and the total CSA of type 2x fibers. Moreover, MG was significantly related to VO_2max ($r=0.62$, $p<0.001$), which may be considered to reflect the impact of the MG-containing, i.e., the mainly aerobically working, muscle mass on VO_2max.

CK plasma levels showed a weaker relation to the total CSA of type 1 fibers, however, a close relation to the CSA of type 2a fibers ($r=0.59$, $p=0.002$). Moreover, significant correlations were found between CK and the fiber size of type 1 and type 2x (not shown).

By multiple stepwise regression analysis, we evaluated which anthropometric parameters that are easily assessable and unaffected by weight loss would contribute besides MG to the variability of muscle CSA and fiber type 1 and 2a CSA. We identified body height and age as independent factors. The bivariate correlation of body height to muscle CSA was found to be significant for all subjects ($r=0.59$, $p<0.001$) as well as separately for cachectic patients ($r=0.64$, $p<0.01$) and for controls ($r=0.64$, $p<0.01$). Age revealed its small but significant contribution to muscle CSA only upon multiple regression.

When MG was adjusted for body height and age in the multiple regression (x=MG×0.539+body height×0.892−age×0.65−75.409), a larger portion of the measured muscle CSA variability could be explained (predicted) than by MG alone (69% vs 42%, multiple $r=0.83$, $p<0.001$ vs $r=0.64$, $p<0.001$). Regarding the fiber type 1 and 2a CSA fraction, the analogue MG adjustment according to the multiple regression (x=MG×0.558+body height×0.489−age×0.518−21.59) explained a very large portion (80%, $r=0.89$, $p<0.001$) of MG-containing muscle fraction as compared to a non-

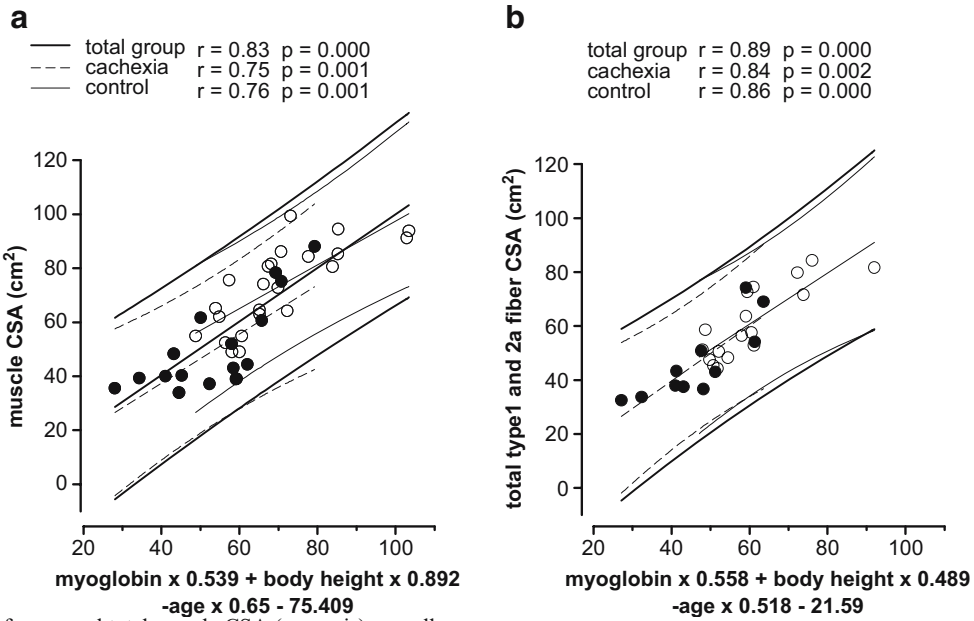

Fig. 3 Prediction of measured total muscle CSA (**a**, *y*-axis) as well as of the CSA fraction of type 1 and 2a fibers (**b**, *y*-axis) by plasma MG adjusted for body height and age (*x*-axis). The adjustment of MG for body height and age was based on the multiple regression (see *x*-axis title), which was obtained for individual values of the total group of subjects with regard to both the measured total CSA (**a**) and the measured CSA fraction formed by type 1 and 2a fibers (**b**). Both the prediction plots **a** and **b** include the upper and lower 95% prediction limits, i.e., the 95% prediction intervals, the regression lines, the correlation coefficients (multiple *r*), and the *p* value for the total group of subjects as well as of the subgroups of both the cachectic patients (*closed circles*) and the healthy controls (*open circles*). Each group's regression line and 95% prediction interval is indicated by the *legend*. For details, see "Materials and methods"/"Statistics"

adjusted MG (42%, r=0.65, p<0.001). In Fig. 3, the *measured* individual values of total muscle CSA or fiber type 1 and 2a CSA (*y*-axis Fig. 3a or *y*-axis Fig. 3b, respectively) are plotted against the such adjusted MG (*x*-axis), i.e., the individual *predictions* as the independent variable. Moreover, Fig. 3a and b includes the regression lines, r and *p* values as well as the upper and lower 95% prediction limits (intervals; for calculation, see "Materials and methods") of the total group as well as both the groups of cachectic patients and of controls. There was good agreement in regression lines and the 95% prediction intervals between all groups.

Highly significant correlations of adjusted MG to total muscle CSA and fiber type 1 and 2a CSA fraction were also obtained in separately analyzed subgroups of women (r=0.68, p=0.002 and r=0.90, p=0.002, respectively) and men (r=0.70, p<0.001 and r=0.83, p<0.001, respectively).

Adjustment of MG for body height and age also improved its correlation to VO_2max (r=0.756, p<0.001) compared to unadjusted MG alone (r=0.62, p<0.001).

Discussion

The present cross-sectional study investigated the relation of plasma MG and CK to BCM and to the CSA as well as fiber composition of the quadriceps muscle as the largest and most relevant locomotor muscle within the total group of severely cachectic cancer patients as well as healthy controls of varying physical activity.

As a first important finding, MG plasma levels were not only related to total muscle CSA but also revealed a closer correlation to the type 1 and type 2a fiber CSA, which was calculated from total CSA by MRI and fractional fiber type areas in biopsies. The correlation was closest (r=0.80, p<0.001) when taking the sum of type 1 and type 2a areas, i.e., the fiber types predominantly containing MG, and was found to be also significant when analyzing the group of men, women, cachectic patients, or healthy controls separately. Through adjustment of MG for body height and age using multiple regression, the prediction of total muscle CSA as well as fiber type 1 and 2a CSA fraction by MG could be considerably improved and rendered multiple r values of 0.83 and 0.89, i.e., explanation of 69 and 80% of the CSA variability, respectively. Moreover, highly significant and similarly strong correlations were obtained within the two subgroups of cachectic patients and controls, who in addition revealed similar regression lines and 95% prediction intervals (Fig. 3a and b), suggesting that prediction of muscle mass by the adjusted MG may cover the range of health as well as of severe wasting as associated with several types of cancer. Moreover, highly significant correlations between adjusted MG and muscle CSA were found within the subgroups of female and male subjects.

Our invasive study in a limited number of subjects thus gives rise to a strong hypothesis toward the usefulness of MG in non-invasive and clinically feasible assessment of muscle mass, to be studied in large cohorts including different wasting conditions and longitudinal designs.

As a second important finding, plasma MG as well as CK levels were significantly lower in cachectic patients, whose muscle CSA and BCM were 32% lower than that of controls. As both, patients and controls, had abstained from severe exercise 5 days before examination, it is suggested that cancer-related muscle wasting may not include muscle damage or even myolysis that would be easily indicated by rises in MG or CK plasma levels. In fact, our histomorphological screening of more than five transverse sections of all biopsies revealed no indication for myolysis or necrosis associated with cachexia. Moreover, upon individual analyses of muscle biopsies using the TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay to measure DNA fragmentation (apoptosis) on transverse sections, we found no TUNEL-positive nuclei. However, the result concerning absence of apoptotic nuclei in skeletal muscle should not be over-interpreted because the time-window to detect apoptosis may be very small, and one muscle fiber contains a huge amount of nuclei.

Our findings may be of significance for clinical management of wasting conditions especially cancer. Muscle wasting as a major feature of the severe and complex cachexia syndrome is frequently observed in cancer as well as in chronic cardiopulmonary or other diseases and crucially determines performance, quality of life, and even therapy outcome [1, 3–5]. The performance state and mass of skeletal muscle are now being recognized as a new therapeutic target especially because regular exercise, a stimulus to maintain muscle mass and function at all ages, appears to have rather large beneficial effects on survival of cancer patients besides its well-established cancer-preventing effect [28–30].

However, quantification of muscle mass during progressive wasting, i.e., a loss of up to 50% or more, is still far from being clinically implemented, and no easily assessable routine surrogate parameters exist except for the largely unreliable parameters of body weight and reported performance status. Methods to precisely assess muscle mass like DEXA, MRI, and CT remain somewhat limited to studies. Thus, an adequate routine parameter that is clearly related to muscle mass for longitudinal follow-up is needed, which can be included into clinical routine at low costs and is less critically dependent on intraindividual standardization as, e.g., required for bioimpedance analysis.

The presently found close correlation between MG, especially when adjusted for the easily assessable anthropometric parameters, and the quadriceps muscle CSA (i.e., the mass determined by MRI) or the fiber-type specific muscle mass (determined by MRI and biopsy) may potentially allow for a clinical use to track muscle mass, provided the suggested non-invasive prediction model is confirmed in larger cohorts and longitudinal studies demonstrate an intraindividual correlation as well. For clinical routine purposes, of course, another important well-studied source of plasma MG, i.e., the myocardial damage, e.g., via infarction, has to be excluded.

The measurement of plasma MG levels may have important advantages over that of CK, which presently showed similar but weaker correlations to muscle CSA (Fig. 1d) or the three fiber type-specific CSA fractions (not shown): (a) After acutely induced muscle damage, e.g., due to severe exercise, intramuscular injections, or bagatelle trauma, normalization of acutely increased plasma MG levels occurs within 24 h, i.e., quicker than that of CK levels [18, 31], which may to some extent also explain our findings of a weaker correlation between CK and CSA; (b) many, if not most, myopathies are associated with variable CK elevations at no or marginal MG increases in the plasma; (c) according to the present analyses, MG is closely correlated to the fiber 1 and 2a fraction of total CSA or mass, which represent the main source of skeletal muscle MG. Thus, MG may be suggested as a novel marker to render information on muscle fiber composition besides muscle mass itself. Muscle fiber composition is an important information about muscle wasting and muscle performance because a most recent study from our laboratory has shown that there may be substantial fiber transition at the cost of type 1 fiber, which along with overall muscle atrophy and reduced capillarization contributes to the low aerobic capacity (VO_2max) in cancer-cachectic patients compared to age-, gender-, and weight-matched controls (Hildebrandt et al., unpublished data). In the present study, we found a significant correlation between plasma MG and VO_2max, which may reflect the impact of fiber 1 and 2a on both MG release and VO_2max.

An obvious limitation of our study is the rather inhomogeneous and small group of cancer-cachectic patients. The difficult recruitment of cachectic cancer patients with poor prognosis may at least in part be due to the fact that these patients, which have to cope with an invasive and mainly non-curative treatment, may not easily agree to an additional invasive biopsy or hospital-based and time-consuming MRI. The time for recruitment of the group of cancer patients and the larger group of controls was 2.5 years and 1 year, respectively.

In summary, our cross-sectional study shows that plasma MG is closely related to a quadriceps muscle CSA as a robust index of locomotor muscle mass, especially to the mass of MG-containing fiber types that are crucial for daily performance. Adjustment for body height and age as independent factors of muscle mass considerably improves the prediction

of muscle mass, with significant and very close positive correlations in the total as well as the subgroups of cachectic cancer patients or healthy controls. Our explorative invasive study thus suggests adjusted MG as a clinical surrogate parameter for the tracking of functionally important muscle mass undergoing wasting. Further studies in larger cohorts are warranted to evaluate the usefulness of this non-invasive predictor including intraindividual longitudinal follow-ups in cancer and in the numerous other important clinical conditions associated with severe muscle wasting.

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