

Research Article

Limitations of Fat-Free Mass for the Assessment of Muscle Mass in Obesity

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Keywords

Bioelectrical impedance analysis · Fat-free mass · Four-compartment model · Sarcopenia · Skeletal muscle mass

Abstract

Background: A high amount of adipose tissue limits the accuracy of methods for body composition analysis in obesity. **Objectives:** The aim was to quantify and explain differences in fat-free mass (FFM) (as an index of skeletal muscle mass, SMM) measured with bioelectrical impedance analysis (BIA), dual energy X-ray absorptiometry (DXA), air displacement plethysmography (ADP), and deuterium dilution in comparison to multicompartiment models, and to improve the results of BIA for obese subjects. **Methods:** In 175 healthy subjects (87 men and 88 women, BMI 20–43.3 kg/m², 18–65 years), FFM measured by these methods was compared with results from a 3- (3C) and a 4-compartment (4C) model. FFM_{4C} was compared with SMM measured by magnetic resonance imaging. **Results:** BIA and DXA overestimated and ADP underestimated FFM in comparison to 3C and 4C models with increasing BMI (all $p < 0.001$). Differences were largest for DXA. In obesity, BIA results were improved: $\text{value}_{\text{corrected}} = \text{value}_{\text{uncorrected}} - a(\text{BMI} - 30 \text{ kg/m}^2)$, $a = 0.256$ for FFM and $a = 0.298$ for SMM. SMM accounts for 45% of FFM in women and 49% in men. **Conclusions:** In obesity, the use of FFM is limited by a systematic error of reference methods. In addition, SMM accounts for about 50% of FFM only. Corrected measurement of SMM by BIA can overcome these drawbacks.

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Introduction

Measurement of body composition is especially important in obesity because a low muscle mass can be obscured by a high amount of adipose tissue. “Hidden cachexia” and “hidden sarcopenia” have been increasingly recognized as high-risk phenotypes associated with adverse health outcomes like asthma and high cholesterol levels [1], and they also limit therapeutic success and affect patient prognosis [2, 3]. Identification of muscle loss at an early stage as well as monitoring of body composition during therapeutic interventions are therefore critical and require repeat measurements with noninvasive and clinically accessible technology. In this regard, bioelectrical impedance analysis (BIA) has gained importance because of technological advances and improved validation of outcome measures [4]. This is supported by the growing number of publications that provide impedance-based reference data for fat-free mass (FFM) or skeletal muscle mass (SMM) [5–10].

The validity of BIA for body composition analysis in obesity is challenged by the assumption of a constant hydration of FFM that is violated by the higher hydration of FFM in adipose tissue and the higher ratio of extracellular (ECW) to intracellular water (ICW) in the adipose tissue part of connective tissue [11]. This drawback however applies to all body composition techniques that are based on a 2-compartment model that divides the body into fat and fat-free mass, and can only be ruled out by a 3-compartment (3C) model that avoids the assumption of a constant hydration by measuring the water content of FFM [12]. Additional limitations come from the assumption of a constant mineral content of FFM because a higher percentage of fat mass (FM) was associated with a lower bone mineral density and could thus decrease the mineral content of FFM [13]. A 4-compartment (4C) model independently measures the water and mineral content of FFM, and thus provides the most accurate tool for FM and FFM measurements in obesity [14]. A 4C model that requires the combination of densitometry by air displacement plethysmography (ADP) or underwater weighing, deuterium (D₂O) dilution for measurement of total body water (TBW), and dual energy X-ray absorptiometry (DXA) for measurement of bone mineral content (BMC) is however cumbersome and not suitable for clinical practice. Therefore, the aim of the present study was (i) to quantify the impact of adiposity on the systematic error of BIA and other 2-compartment methods for the assessment of FFM by comparison versus a 3C and 4C model and (ii) to mathematically correct BIA equations for the systematic error with increasing adiposity. To evaluate the necessity of the additional DXA measurement required by the 4C model, we evaluated both, the 3C and the 4C model, as a reference.

Finally, the increase in connective tissue (i.e., adipose tissue) with obesity impairs the “metabolic quality” of FFM [4]. As a secondary aim of the study, the relationship between FFM and SMM was analyzed in order to evaluate the use of FFM as a proxy for SMM in obesity.

Subjects and Methods

In a first phase of this study, 153 Caucasian men and women with a BMI <35 kg/m² were recruited from the area of Kiel, Germany, and FFM and SMM prediction equations for the seca medical body composition analyzer (mBCA) devices were developed [4, 15]. A seca mBCA 515 device (seca gmbh & co. kg., Hamburg, Germany) was used for BIA measurements, and a 4C model based on D₂O dilution, DXA, and ADP was used as reference for FFM, whereas whole-body magnetic resonance imaging (MRI) and sodium bromide (NaBr) dilution were used as references for SMM and ECW, respectively. Details of the study protocol were described previously [4, 15]. In a second phase, 35 obese Caucasian men and women with a BMI ≥30 kg/m² were examined using the same study protocol. Ten subjects of the first phase

and 3 of the second phase had to be excluded from the study due to missing or implausible reference measurements. The results of the remaining 175 men and women aged 18–65 years were analyzed.

The subjects were asked to fast overnight and come to the study center between 07:00 and 07:30 in the morning. Whole-body MRI measurements took place at a separate appointment not more than 4 days apart. Subjects were excluded from the study if they fulfilled one of the following criteria: acute and/or chronic diseases (e.g., hypertension, renal and cardiac insufficiency), regular intake of medications (except for contraceptives), amputation of limbs, electrical implants as cardiac pacemaker, metallic implants (except for tooth implants), pregnancy or breastfeeding period, current alcohol abuse, and extensive tattoos at the arms or legs. Edema of the ankles were excluded by inspection and manual compression if appropriate.

Anthropometrics

Body height and weight were obtained on a measuring station (seca 285) with an accuracy of ± 50 g up to 100 kg and ± 75 g up to 150 kg for the scale and ± 2 mm for the stadiometer. BMI was classified as normal weight (BMI ≥ 18.5 , < 25 kg/m²), overweight (BMI ≥ 25 , < 30 kg/m²) and obesity (BMI ≥ 30 kg/m²) with obesity class I (BMI ≥ 30 , < 35 kg/m²), obesity class II (BMI ≥ 35 , < 40 kg/m²), and obesity class III (BMI ≥ 40 kg/m²).

Bioelectrical Impedance Analysis

The seca mBCA 515 device consists of a platform with an integrated scale and a handrail system. Each side of the ascending handrail carries 6 electrodes, of which 2 were chosen depending on the person's height. To get the right choice of grip position, the subject has to stand upright with outstretched arms. Another 2 pairs of electrodes contact the feet. This 8-electrode technique enables segmental impedance measurements. Details of the device were previously described [15]. The accuracy for measurements of the right and left body side at frequencies of 5 and 50 kHz is 5 Ω for the impedance and 0.5° for the phase angle. Prediction equations use BIA values obtained at 5 and 50 kHz [15].

Participants were asked not to exercise within 12 h and drink alcohol within 24 h before the impedance measurement. The duration of each BIA measurement was 75 s.

Reference Methods

FM was calculated using a 3C model and a 4C model that include body volume (by ADP), TBW (by D₂O dilution), BMC (by DXA), and weight using the following equations [14]:

$$FM_{3C} (kg) = 2.220 \times \text{body volume (L)} - 0.764 \times TBW (L) - 1.465 \times \text{weight (kg)}$$

$$FM_{4C} (kg) = 2.7474 \times \text{body volume (L)} - 0.7100 \times TBW (L) + 1.4599 \times BMC (kg) - 2.0503 \text{ weight (kg)}.$$

FFM_{3C} and FFM_{4C} were calculated as the difference between body weight and FM.

Body volume was measured with ADP using the BOD POD™ device (Cosmed, Italy). FM_{ADP} was calculated from body density using Siri's [16] equation, and FFM_{ADP} was calculated as the difference to body weight. A whole-body DXA scan was performed to measure BMC, FFM_{DXA}, and lean soft tissue using a Hologic Discovery A densitometer and the whole-body software 12.6.1:3 (Hologic, Inc., Bedford, MA, USA). D₂O dilution was used to measure TBW and NaBr dilution to assess ECW. FFM_{D₂O} was calculated by $FFM_{D_2O} (kg) = TBW (L) / 0.732$. ICW was calculated as the difference between TBW and ECW. Details of these reference methods were described previously [15].

Total SMM (excluding head and neck) and visceral adipose tissue were measured by MRI using a Magnetom Avanto 1.5-T scanner (Siemens Medical Systems, Erlangen, Germany). Details were described previously [4].

Table 1. Characteristics of the study population

	Women (n = 88)	Men (n = 87)	All (n = 175)
Age, years	38.5±13.0	38.6±11.6	38.5±12.3
Weight, kg	75.8±20.9	88.8±16.7***	82.3±20.0
Height, cm	168.4±6.6	179.8±5.8***	174.1±8.4
BMI, kg/m ²	26.6±6.5	27.5±5.2	27.0±5.9
FMI _{4C} , kg/m ²	9.8±4.9	7.1±4.1***	8.4±4.7
FFM _{4C} , kg	47.8±7.3	66.0±7.2***	56.9±11.7
SMM _{MRI} , kg	21.7±3.8	32.5±4.0***	27.1±6.7

FMI, fat mass index; FFM, fat-free mass; SMM, skeletal muscle mass; 4C, 4-compartment model; MRI, magnetic resonance imaging. *** $p < 0.001$ vs. women (t test).

Statistics

Data analyses were performed with R software, version 3.0.1 (R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics and errors are presented as means ± SD. Differences between women and men as well as significant errors were analyzed by t test. The FM index (FMI) was calculated using the following formula: $FMI = FM/height^2$. Significance of the Pearson correlation coefficients is based on Fisher's z -transformation. For correction equations, a linear regression was calculated using the uncorrected value from subjects with $BMI \geq 30$ kg/m². $BMI - 30$ kg/m² was used as independent variable, and the intercept was fixed to zero at $BMI = 30$ kg/m² to enforce continuity between uncorrected and corrected values at the threshold of $BMI = 30$ kg/m². Regressions were calculated for FFM, SMM (total and segmental), TBW, ECW, and visceral adipose tissue. Significance of coefficients was assessed by t test, and equations were corrected for $BMI \geq 30$ kg/m² using these coefficients. The pure error was calculated as:

$$\text{Pure error} = \sqrt{\frac{\sum (\text{predicted value} - \text{reference value})^2}{\text{number of observations}}}$$

A p value <0.05 was considered significant, values <0.01 and <0.001 are indicated.

Results

Characteristics of the study population stratified by gender are given in Table 1. Men were heavier and taller than women and also had higher amounts of SMM and FFM, whereas FMI was higher in women. The BMI ranged from 20 to 43.3 kg/m², and the prevalence of normal weight, overweight, and obesity class I–III was 49, 24, 13, 10, and 4%, respectively.

Table 2 shows the error of FFM for normal-weight, overweight, and obese subjects measured using BIA, DXA, ADP, or D₂O dilution, and the 3C and the 4C model as a reference. BIA overestimated FFM in obese subjects for both models (3C and 4C). DXA overestimated FFM in all BMI groups and for both models; the systematic error increased with BMI (Tables 2, 3) and was largest in obese subjects. By contrast, ADP underestimates FFM in all BMI groups and for both models with the highest negative systematic error in obese subjects. No significant systematic error was found for D₂O dilution. The highest systematic error was found for both models when FFM was measured with DXA in obese subjects. Significant differences between the 3C and the 4C model were only found in normal-weight subjects.

Table 2. Error of fat-free mass (FFM) measured by bioelectrical impedance analysis (BIA), dual-energy X-ray absorption (DXA), air displacement plethysmography (ADP), and deuterium (D2O) dilution in comparison with a 3-compartment (3C) and a 4-compartment (4C) model, stratified by normal weight, overweight, and obesity

	Normal weight (n = 86)	Overweight (n = 42)	Obesity (n = 47)
FFM _{BIA} – FFM _{3C} , kg	-0.25±1.83	-0.26±1.92	1.39±2.41***
FFM _{DXA} – FFM _{3C} , kg	1.09±1.34***	2.21±1.95***	5.25±2.10***
FFM _{ADP} – FFM _{3C} , kg	-0.90±1.17***	-1.07±1.38***	-1.53±1.33***
FFM _{D2O} – FFM _{3C} , kg	-0.11±0.97	-0.14±1.15	0.26±1.04
FFM _{BIA} – FFM _{4C} , kg	-0.02±1.83	-0.25±1.82	1.34±2.40***
FFM _{DXA} – FFM _{4C} , kg	1.32±1.33***	2.22±1.97***	5.19±2.05***
FFM _{ADP} – FFM _{4C} , kg	-0.67±1.10***	-1.06±1.38***	-1.58±1.48***
FFM _{D2O} – FFM _{4C} , kg	0.12±1.15	-0.13±1.28	0.20±1.03
FFM _{3C} – FFM _{4C} , kg	0.23±0.33***	0.01±0.37	-0.06±0.37

*** $p < 0.001$: error significantly different from zero by t test.

Table 3. Correlations between the error of fat-free mass (FFM) assessed by different methods and potential determinants

	FFM _{BIA} – FFM _{4C}	FFM _{DXA} – FFM _{4C}	FFM _{ADP} – FFM _{4C}	FFM _{D2O} – FFM _{4C}
BMI	0.36***	0.73***	-0.31***	0.03
FMI _{4C}	0.46***	0.77***	-0.23**	0.18*
BMC _{DXA} /FFM _{4C}	-0.16*	-0.29***	0.60***	0.06
TBW _{D2O} /FFM _{4C}	0.20**	0.19*	-0.70***	n.c.
ECW _{NaBr} /ICW _{D2O, NaBr}	0.21**	0.28***	0.23**	0.01

BIA, bioelectrical impedance analysis; 4C, 4-compartment model; DXA, dual energy X-ray absorptiometry; ADP, air displacement plethysmography; D2O, deuterium dilution; FMI, fat mass index; BMC, bone mineral content; TBW, total body water; ECW, extracellular water; ICW, intracellular water; NaBr, sodium bromide dilution. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$: correlation coefficients significantly different from zero.

The error of all methods increased with increasing FMI (the negative error in case of ADP; Table 3). In addition, the error of FFM_{ADP}, FFM_{DXA}, and FFM_{BIA} also correlated with water and mineral content of FFM as well as the ECW/ICW ratio.

Figure 1 explains how BIA results for FFM were corrected for obese subjects. Without correction, BIA overestimates FFM at high FMI, and 22% of the variance of the difference between FFM_{BIA} and FFM_{4C} are explained by FMI (Fig. 1A). Instead of FMI, BMI was used for correcting FFM because it is independent of impedance measurements and can be used as a proxy for adiposity. Figure 1B shows that a systematic overestimation of FFM_{BIA} only occurs when BMI exceeds 30 kg/m². A linear regression including subjects with BMI ≥ 30 kg/m² was therefore used for the correction of FFM_{BIA} in obese subjects. To enforce continuity, the correction is zero at BMI = 30 kg/m² (Fig. 1B). FFM was corrected by the following formula:

$$FFM_{BIA, \text{ corrected}} = FFM_{BIA, \text{ uncorrected}} - 0.256 \times (\text{BMI} - 30 \text{ kg/m}^2).$$

No correction was applied for subjects with a BMI < 30 kg/m². This correction reduces the overestimation of FFM by BIA in obese subjects from 1.34 ± 2.40 to -0.06 ± 2.15 kg (online

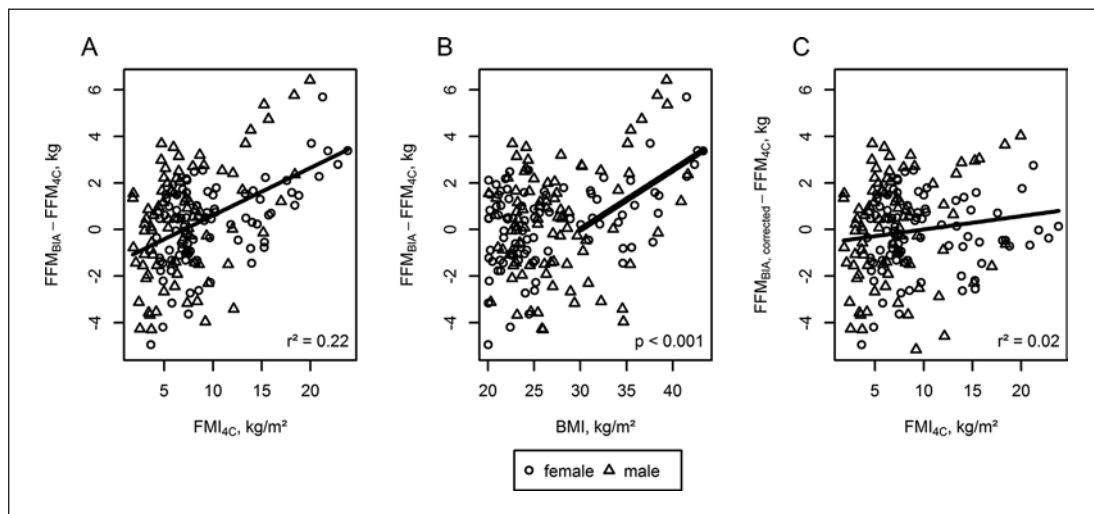


Fig. 1. Development of correction of fat-free mass (FFM) measured with bioelectrical impedance analysis (BIA). **A** Fat mass index (FMI) dependency of the error of uncorrected FFM in comparison to a 4-compartment (4C) model. **B** BMI dependency of the error for BMI ≥ 30 kg/m² which is used as correction, $FFM_{BIA} - FFM_{4C} = 0.256 (BMI - 30 \text{ kg/m}^2)$. **C** Error of corrected FFM vs. FMI.

suppl. Table S1; for all online suppl. material, see www.karger.com/doi/10.1159/000499607), and the variance in the difference between FFM_{BIA} and FFM_{4C} explained by FMI is reduced from 22 to 2% (Fig. 1C). A similar correction can be applied to SMM, which reduces the error by BIA in obese subjects from 1.63 ± 2.40 to 0.01 ± 2.11 kg:

$$SMM_{BIA, \text{corrected}} = SMM_{BIA, \text{uncorrected}} - 0.298 \times (BMI - 30 \text{ kg/m}^2).$$

Further corrections can be applied to other BIA equations using the correction factors listed in online supplemental Table S1.

A linear relationship was found between FFM according to the 4C model and SMM measured by MRI (online suppl. Fig. 1). SMM accounts for 45% of FFM in women and for 49% of FFM in men. A significant correlation between the corresponding residuals and BMI was found in men ($r = 0.22$, $p < 0.05$) but not in women. As a parameter describing the “quality” of FFM, the ECW/ICW ratio correlates with the residuals with $r = -0.26$ ($p < 0.05$) in men and $r = -0.54$ ($p < 0.001$) in women. In contrast to BIA measurements of appendicular SMM (based on MRI as reference), SMM will be overestimated when appendicular lean soft tissue measured by DXA is used as a proxy (online suppl. Fig. S2).

Measuring the FFM with BIA using the FFM correction formula and calculating the SMM from FFM using the equations presented in online supplemental Figure 1 leads to a pure error for SMM of 1.50 kg for all subjects and 2.14 kg for obese subjects when compared to SMM measured by MRI, whereas measuring the SMM with BIA using the SMM correction formula leads to a pure error of 1.41 kg for all subjects and 2.09 kg for obese subjects.

Discussion

The primary aim of the present study was to quantify the impact of obesity on the systematic error of FFM measurements by BIA, DXA, ADP, and D₂O dilution in comparison to a 3C and a 4C model and to correct BIA equations. We found that the error of all 2-compartment

methods correlated with FMI (Table 3). BIA and DXA overestimated and ADP underestimated FFM with increasing obesity (Table 2). The overestimation of FFM in obesity by BIA equations was partly explained by a higher hydration of FFM (Table 3). In obesity, a higher hydration of FFM is due to an increased water fraction of FFM in adipose tissue [17]. For obesity, these effects were not adequately accounted for in the original BIA equations [4, 15], since inclusion criteria for the reference population used to generate the BIA algorithm were limited to BMI <35 kg/m² [15]. On the other hand, overestimation of FFM by DXA can be explained by an underestimation of trunk FM by fan beam DXA devices [18]. Since mineral content of the TBW is largely responsible for X-ray attenuation and differentiation between FM and FFM [19, 20], a higher hydration of the FFM in adipose tissue also adds to the overestimation of FFM by DXA in obesity. However, the error of FFM measured by BIA or DXA was more dependent on BMI and FMI than on differences in hydration (Table 3). The accuracy of FFM measured by ADP depends on the assumption of a constant density of FFM [21]. A higher hydration or a lower BMC of the FFM in obesity therefore leads to a lower density and hence an underestimation of FFM, which is in accordance with the correlations given in Table 3.

The systematic error of FFM measured by D₂O dilution was not significant in any BMI group, neither for the 3C nor for the 4C model. Therefore, D₂O dilution is a valid method for measuring FFM in obesity, but is too time consuming for clinical practice. Differences between the 3C and the 4C model were only found in normal-weight subjects, suggesting that a 3C model might be sufficient in overweight and obese subjects if DXA measurements are not suitable.

The present study shows that the BIA equations can be improved for measurements in obese persons when a correction term for subjects with BMI ≥30 kg/m² is used (Fig. 1; online suppl. Table S1). This correction leaves results for nonobese subjects unchanged and avoids abrupt changes of results with increasing BMI. Correction factors were statistically significant ($p < 0.001$) for FFM, total SMM, SMM of the legs, TBW, and visceral adipose tissue, but not for SMM of the arms and ECW (online suppl. Table S1). In our study population (which was partly also used for the generation of BIA equations), the systematic error of the corrected BIA equation for FFM is lower than the systematic error of uncorrected DXA and ADP results. However, the standard deviation of the error in obesity is smaller in DXA and ADP (Table 2; online suppl. Table S1), which indicates a higher precision of those methods. As an alternative approach for the correction of BIA equations, we used a nonlinear (quadratic) correction over the complete BMI range. This approach resulted in a similar pure error compared to the linear correction beginning at BMI = 30 kg/m².

A linear relationship was found between FFM according to the 4C model and SMM assessed by MRI (online suppl. Fig. S1). FFM can therefore be used for the prediction of SMM. However, SMM accounts for only 45–49% of FFM in women and men. This relationship also explains why appendicular muscle mass is overestimated when measured by DXA (online suppl. Fig. S2). While muscle tissue volume is measured with MRI, the results of DXA represent lean soft tissue mass which is FFM without bone. Therefore, lean soft tissue of the extremities as a proxy for SMM leads to an overestimation of muscularity [22]. Definitions of a low muscle mass that are based on different methods therefore differ between guidelines from the European Group on Sarcopenia in Older People and the International Consensus for Cancer Cachexia [23]. The prevalence of low muscle mass in patients with cancer may depend on the method of muscle measurement [24]. Using the seca mBCA 515 device, the prediction of SMM has advantages over the prediction of FFM because the pure error in comparison to MRI is smaller for the corrected SMM equation than for SMM calculated from corrected FFM (1.41 vs. 1.50 kg for all and 2.09 vs. 2.14 kg for obese subjects).

As a limitation to our study, participants were healthy, apart from being obese, and results may not be valid in subjects with diseases leading to disturbed hydration. Also, corrections

were calculated for BIA equations implemented in the seca mBCA 515 device and cannot be applied to other BIA instruments. Furthermore, the 3C and 4C models as a reference were not independent because they included information derived from ADP, D₂O dilution, and DXA.

In conclusion, FFM measured by the reference methods DXA and ADP has a systematic error in obesity. These methods are therefore no appropriate standards for body composition in obesity. In clinical practice, the use of corrected BIA measurements for FFM or SMM can be a suitable alternative as long as BIA equations are validated versus a 4C or 3C model or MRI, like the equations implemented in the seca mBCA 515 device.

Statement of Ethics

This study was approved by the Medical Ethics Committee of the Christian Albrechts University Kiel, Germany, and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All subjects provided their fully informed and written consent before participation.

Disclosure Statement

Björn Jensen and Kristin Klückmann are employed by seca. Anja Bosy-Westphal serves and Manfred J. Müller served as consultant for seca. Wiebke Braun, Corinna Geisler, and Markus Both declare to have no conflict of interest.

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Author Contributions

B. Jensen and A. Bosy-Westphal designed research, analyzed data, and wrote the paper. W. Braun, C. Geisler, and M. Both conducted research. K. Klückmann designed and coordinated the research. M.J. Müller designed the research and contributed to the critical review and intellectual content of the manuscript. All authors read and approved the final manuscript.

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