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Comparison of Total Protein Concentration in Skeletal Muscle as Measured by the Bradford and Lowry Assays

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The Lowry and Bradford assays are the most commonly used methods of total protein quantification, yet vary in several aspects. To date, no comparisons have been made in skeletal muscle. We compared total protein concentrations of mouse red and white *gastrocnemius*, reagent stability, protein stability and range of linearity using both assays. The Lowry averaged protein concentrations 15% higher than the Bradford with a moderate correlation ($r = 0.36$, $P = 0.01$). However, Bland–Altman analysis revealed considerable bias (15.8 ± 29.7%). Both Lowry reagents and its protein–reagent interactions were less stable over time than the Bradford. The linear range of concentration was smaller for the Lowry (0.05–0.50 mg/ml) than the Bradford (0–2.0 mg/ml). We conclude that the Bradford and Lowry measures of total protein concentration in skeletal muscle are not interchangeable. The Bradford and Lowry assays have various strengths and weaknesses in terms of substance interference and protein size. However, the Bradford provides greater reagent stability, protein–reagent stability and range of linearity, and requires less time to analyse compared to the Lowry assay.

Key words: Lowry assay, Bradford assay, total protein concentration, skeletal muscle, Spectrophotometry.

Abbreviations: BCA, bicinchoninic acid; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenedinitrilotetraacetic acid; KCl, potassium chloride; wt/vol, weight per volume.

The central dogma of biology culminates with protein synthesis, thus it is expected that most biochemical and molecular laboratory research will involve protein analysis, particularly for the comparison of protein expression and activity. Therefore, determination of total protein concentration is an essential first step prior to further analyses. Over the years, several spectrophotometric assays have been developed to accomplish this task, including the biuret *(1)*, Lowry *(2)*, BCA *(3)* and Bradford *(4)* protocols, each with varying degrees of accuracy, sensitivity, repeatability and ease. Differences in tissue types, amino acid content, protein conformation and background principles employed in each method may account for the discrepancies among these colorimetric assays *(5)*.

The biuret method of protein quantification involves the reaction of copper (II) ions to protein peptide bonds under alkaline conditions, changing the colour of the solution from blue to purple *(1)*. However, this assay lacks sensitivity and consumes a large volume of sample not often available in laboratory research *(6)*. Alternatively, the Lowry assay established in 1951 incorporates the reaction of copper (II) ions, but requires considerably less sample volume. The principle behind this method involves two reactions: (i) the reduction of copper (II) ions by protein amides in alkaline solution, and (ii) the reduction of oxygen atoms via the Folin-phenol reagent used to turn the solution blue *(2)*. The bicinchoninic acid (BCA) method of protein quantification is similar to both the biuret and Lowry assays *(3)*. It involves one step: the reaction of the BCA sodium salt with copper (II) ions in an alkaline environment to form an intense purple complex, however, samples must be heated and then cooled before analysis *(3)*. Lastly, the Bradford assay developed in 1976 follows a different reaction principle, which involves the binding of proteins to Coomassie brilliant blue, to form a protein–dye complex that shifts the absorption maximum of the dye from 465 to 595 nm, turning the color of the solution from red-brown to blue *(4)*. Despite the existence of several protocols to establish total protein concentration, the two most commonly used methods for protein quantification are the Lowry and Bradford assays, and are examined in this paper.

Irrespective of the protocol used, the colour produced from the above mentioned assays is assumed to increase proportionally to increases in total protein content. In theory, the total protein concentration of a select sample is constant, and thus either assay should provide concentrations that are equally comparable or at the least interchangeable. However, previous investigations comparing the two methods have revealed significant differences in terms of interfering substances, reaction times, ability to detect small proteins and accuracy in a variety of samples including biological fluids, algal

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proteins, bean seeds, human tears and wine (5, 7–15). To date, no such comparisons have been made in skeletal muscle. Therefore, the objective of this study was to compare total protein concentration determined by the Lowry and Bradford assays within skeletal muscle tissue. We hypothesize that both methods would be comparable and/or interchangeable. It is important to note the novelty of this study: it is the first published work that illustrates and discusses the differences between both the Lowry and Bradford methods of protein quantification specifically in skeletal muscle tissue. Exercise physiologists among other researchers routinely use skeletal muscle for analysis of several molecular mechanisms, and therefore it is important to establish whether total protein concentration determined by the Lowry and Bradford assays are comparable and/or interchangeable.

MATERIALS AND METHODS

Sample Preparation—White and red *gastrocnemius* ($n = 52$) samples were used for analysis. Tissues were minced and homogenized using a glass-Teflon Porter–Elvenhejm homogenizer (4% wt/vol) in buffer containing 50 mmol/l potassium phosphate (dibasic), 5 mmol/l EDTA, 0.5 mmol/l DTT, 1.15% KCl and a protease inhibitor cocktail (Sigma P8215; St. Louis, MI, USA) at pH 7.4. The soluble protein fractions of the homogenates were separated by centrifugation at 600 *g* for 10 min at 4°C.

Determination of Protein Concentration—To determine differences in total protein concentration measured by the Lowry (2) and Bradford (4) spectrophotometric techniques, we measured protein concentrations of white ($n = 26$) and red ($n = 26$) *gastrocnemius*. Lowry: total protein concentration was determined at 750 nm using bovine serum albumin (BSA) as a standard, as previously described (2). Bradford: Total protein concentration was determined at 595 nm according to manufacturer's protocol for a standard 1-ml cuvette assay (Bio-Rad, 500-0203, Mississauga, ON). Measured concentrations were corrected to account for dilutions in both assays.

Reagent Stability—To determine changes in absorbance attributed to reagent composition and not protein content, the stability of the reagent solutions were measured over time. Briefly, the reference blank contained de-ionized water, while sample cuvettes contained either Lowry/Folin solutions or 1× Bradford reagent after acclimatization to room temperature. No protein was added to the cuvettes. Absorbance was measured continuously at either 750 nm or 595 nm at the following time points: 0, 2, 5, 10, 20, 30, 40, 50 and 60 min.

Protein Stability—To determine the stability of the protein–reagent interaction and to determine the time point at which measurements should be obtained *via* spectrophotometry, the stability of the copper/Folin/protein reaction (Lowry), and the interaction of the protein–dye complex (Bradford) were analysed over time. Standard curves and protein samples were prepared following the respective protocols for the Lowry and Bradford methods as described above. Lowry samples ($n = 3$) and Bradford samples ($n = 5$) were measured continuously at the following time points: 0, 3, 5, 7, 10, 12, 15, 17, 20, 25, 30, 35, 40, 50 and 60 min. Time 0 min corresponds to the first reading obtained immediately after combination of the Folin–phenol reagent (Lowry) and protein to Coomassie brilliant blue (Bradford). Note: the normal 15–20-min incubation post combination of the phenol mix (Lowry) and the 10-min incubation period required for the Bradford assay prior to reading protein concentrations were not complete at time 0 min.

Range of Linearity—To determine the linear range of absorbance *vs*. concentration of samples (the range at which Beer's law is upheld), we measured the range of detection of increasing concentrations of BSA. Briefly, absorbance was measured using the following BSA concentrations: Lowry—0, 0.05, 0.1, 0.25, 0.4, 0.5, 0.6, 0.8 and 1.0 mg/ml; Bradford—0, 0.125, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 mg/ml. Standard curve protocols were followed to generate linearity curves for both Lowry and Bradford assays, as mentioned above.

Statistical Analysis—Total protein concentration was analysed *via* two methods: (i) Pearson product-moment correlation coefficient (r), and (ii) Bland–Altman test (16). The linear range of concentrations for both methods was calculated *via* linear regression analysis. All calculations and analyses were done using GraphPad Prism v. 4.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Determination of Total Protein Concentration—Mean protein concentrations determined by the Lowry were on average 15% ($P < 0.001$) higher than those of the Bradford (Table 1). Values measured by the Bradford technique fell within a smaller range and had a standard deviation about 10% lower than that of the Lowry (Table 1). Calculation of the correlation coefficient revealed a moderate correlation ($r = 0.36$, $P = 0.01$) with a slope of 0.39 ± 0.15 between the two methods, yet displayed a distinct deviation of the linear regression line (bold) from the line of identity (broken) (Fig. 1A). Comparisons made *via* the Bland–Altman test revealed a considerable overestimation bias by the Lowry technique ($15.8 \pm 29.7\%$), and a substantial lack of agreement (range: –42% to +74%) between the two methods (Fig. 1B). These results suggest the Lowry and Bradford methods of protein quantification in skeletal muscle are not interchangeable.

Reagent Stability—Changes in absorbance attributed to reagent composition and not protein content, revealed Bradford reagents were more stable over an hour than those of the Lowry (Fig. 2). Analysis of Bradford reagents (Fig. 2B) displayed a small linear increase in absorbance over time (3.0% difference from time 0–60 min), while Lowry reagents (Fig. 2A) showed a greater linear decrease in absorbance over time (5.6% difference from time 0–60 min). This suggests that measurements of total protein concentration determined by the Bradford more accurately represent protein concentrations within a sample and not due to reagent variability.

Table 1. **Statistical characteristics of skeletal muscle protein concentration for 52 samples determined by the Lowry and Bradford assays.**

Assay	Minimum concentration (mg/ml)	Maximum concentration (mg/ml)	Mean concentration (mg/ml)	Standard deviation (mg/ml)	Standard error (mg/ml)
Lowry	2.88	8.76	5.95	1.45	0.20
Bradford	2.84	8.21	5.08	1.31	0.18

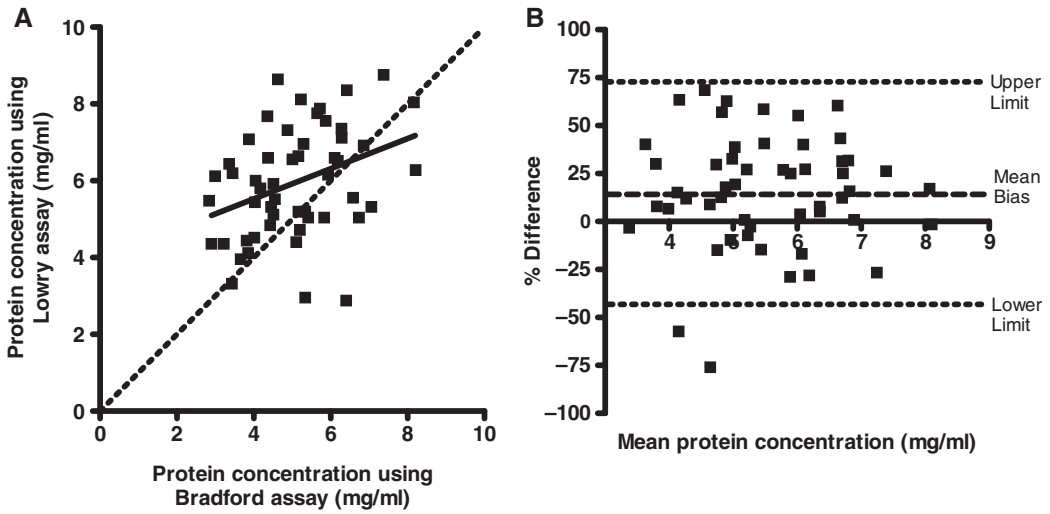

Fig. 1. **Comparison of total protein concentration determined by the Lowry and Bradford assays.** (A) Correlation between total protein concentration (mg/ml) of mouse red and white *gastrocnemius* ($r = 0.36$; slope = 0.39 ± 0.15; $P = 0.01$); solid line = linear regression line; dashed line = line of identity. (B) Bland–Altman comparison of total protein concentration, lack of agreement (mean bias) = $15.8 \pm 29.7\%$; range, –42% to +74%.

Protein Stability—The stability of the protein–reagent interaction displayed distinct differences depending on the spectrophotometric assay used (Fig. 3). The copper/Folin/protein reaction of the Lowry assay did not reach peak protein concentrations or stability, but instead continued to increase with time for an hour (protein concentrations were 2.2-fold higher at 60 min *vs*. 0 min; Fig. 3A). The interaction of the protein–dye complex of the Bradford assay did however, reach peak concentrations between 5 and 10 min following the addition of protein to reagent mixtures (Fig. 3B), after which protein concentrations remained relatively stable (5.8% difference from protein concentrations measured between 5 and 60 min). This suggests that the Bradford assay provides more accurate readings of protein concentration compared to the Lowry assay.

Range of Linearity—The linear range of concentration determined by increases in protein concentration proportional to increases in absorbance revealed a smaller range of detection for the Lowry than the Bradford assay (Fig. 4). The Lowry assay displayed a curvilinear increase in absorbance with increasing BSA concentration (Fig. 4A), while only concentrations between 0.05 and 0.50 mg/ml revealed a linear regression. The Bradford assay, on the other hand, displayed linear regression for the full range (0–2.0 mg/ml) of BSA concentrations (Fig. 4B), suggesting its greater adherence to the Beer–Lambert Law.

DISCUSSION

The novelty of this study demonstrates the differences between both the Lowry and Bradford methods of protein quantification in skeletal muscle tissue. Comparisons of Lowry and Bradford techniques for protein quantification revealed significant differences between concentrations in a variety of samples (*5*, *7–15*), however, no previous comparisons between the two methods have been made in skeletal muscle. Our calculation of the correlation coefficient between the two methods found a significant yet moderate correlation, however, this may be attributed to a mathematical artefact due to our large sample size ($n = 52$). Linear regression analysis also revealed a distinct deviation of the regression line from the line of identity (Fig. 1A). More importantly, the correlation coefficient does not describe the interchangeability between methods but only reflects the degree to which two measures are related (*16*). When one aims to assess two methods that perform the same function, such as the Lowry and the Bradford, it is not surprising that a relationship would appear. Therefore, the Bland–Altman test represents a true measure of comparison between two techniques that perform the same function. Our results indicate that although both the Lowry and Bradford assays measure total protein concentration in skeletal muscle, the large bias ($15.8 \pm 29.7\%$) and considerable range of disagreement (–42% to +74%) between the two methods reflect a significant lack of interchangeability (Fig. 1B).

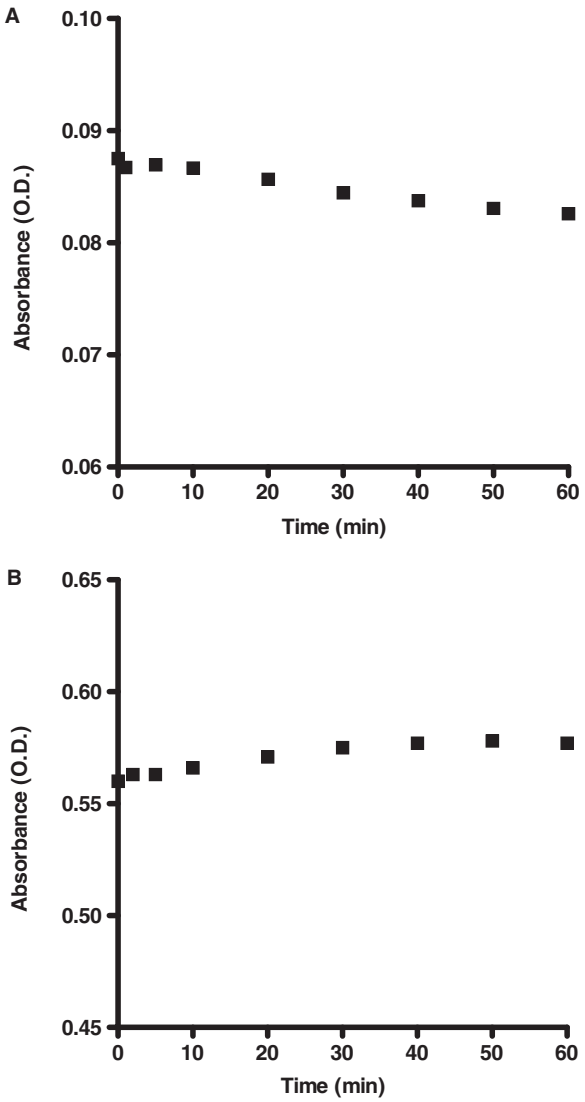

Fig. 2. **Stability of Lowry and Bradford reagents measured over time.** Absorbance (O.D.) *vs*. time (min) analysis of Lowry reagents read at 750 nm (A) and Bradford reagents read at 595 nm (B). Percent difference between absorbance values from time 0 to 60 min was lower for the Bradford (3.0%) than the Lowry (5.6%).

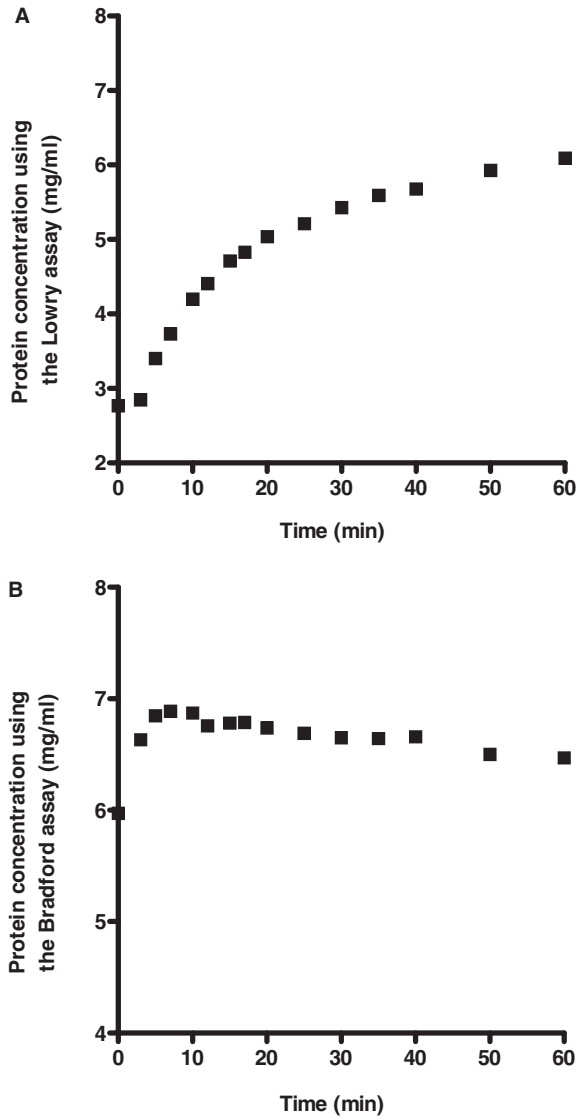

Fig. 3. **Reagent–protein interaction profiles for the Lowry ($n = 3$) and Bradford ($n = 5$) assays measured over time (min).** Protein concentrations (mg/ml) increased continuously over an hour with the Lowry assay (A), while peak values were obtained between 5 and 10 min with the Bradford assay (B).

The differences in total protein concentration obtained by the Lowry and Bradford assays may be attributed to several factors including: reagent stability, protein stability and interference of substances. The Lowry assay requires its reagents (both Lowry and Folin mixtures) to be prepared immediately before analysis due to several reported decreases in accuracy of protein concentrations when previously combined reagents are used (*2*, *17*). Our results indicate that Lowry reagents combined prior to analysis are stable over an hour with a small linear decrease in absorbance of 5.6% (Fig. 2A). However, the Bradford reagent proved to be slightly more stable over an hour compared to the Lowry reagents with a linear increase in absorbance of 3.0% (Fig. 2B).

Total protein concentration was also more stable with the Bradford assay compared to the Lowry assay (Fig. 3). According to the Lowry protocol, protein concentration is to be measured after a 15–20-min incubation of the Folin–phenol reagent to the protein/Lowry mixture. However, protein concentrations measured at 20 min (5.04 mg/ml) were 7% higher than those measured at 15 min (4.71 mg/ml). In fact, the protein concentrations for the Lowry assay increased continuously over time without reaching peak concentrations or stability (Fig 3A), and there was a 29% difference in concentrations measured between 15 and 60 min, clearly indicating that total protein concentrations determined by the Lowry assay are not stable. The Bradford protein–dye complex, however, reached peak protein concentrations between

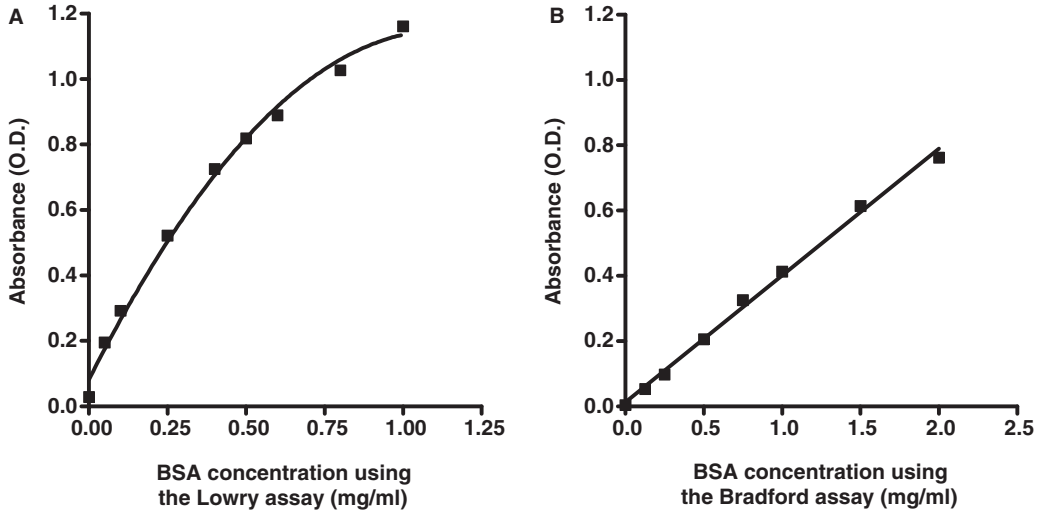

Fig. 4. **Linear range of protein quantification for the Lowry and Bradford assays.** Absorbance (O.D.) *vs*. BSA concentration (mg/ml) profile for the Lowry assay illustrates a second-order polynomial with increasing BSA concentration, $y = 0.082 + 1.896x - 0.843x^2$, $r^2 = 0.994$, while concentrations between 0.05 and 0.50 mg/ml reveal a linear regression of $y = 1.396x + 0.148$, $r^2 = 0.992$ (A). The Bradford assay demonstrates a linear regression with increasing BSA concentrations, $y = 0.389x + 0.012$, $r^2 = 0.996$ (B).

5 and 10 min post combination of protein to reagent and remained relatively stable thereafter. Our analysis revealed a 0.3% difference between protein concentrations determined between 5 and 10 min, and only a 5.5% difference in protein concentrations measured between 5 and 60 min. Therefore, we recommend measuring total protein concentration in skeletal muscle between 5 and 10 min post combination of protein to reagent using the Bradford and not the Lowry assay. The importance of measuring total protein concentration at peak values with either method reflects the maximum interaction. Reading protein concentrations at time points aside from peak values suggests a possible underestimation of protein content due to decreases in binding interactions with select compounds. However, an alternative interpretation may be suggested where reading protein concentrations at peak time points could erroneously overestimate protein content due to false interactions with non-protein species and reagents. Nevertheless, it is customary for researchers to follow the former rather than the later.

Another factor that favours the use of the Bradford assay over the Lowry is the relative ease at which it is performed. Aside from the initial set up of disposable cuvettes and acclimatization of the Bradford reagent to room temperature, the Bradford assay requires only one step: the addition of protein to reagent, which requires an incubation time of 5–10 min before absorbance can be measured. The set up for the Lowry assay, on the other hand, requires: set up of tests tubes, acclimatization of the Lowry and Phenol reagents to room temperature, sample preparation and two long incubation times before spectrophotometric measurements can be taken (10 min after addition of Lowry reagent to protein and 20 min after addition of phenol). The overall procedure for the Lowry assay usually takes somewhere between 60 and 90 min to analyse a set of 10 samples, whereas the Bradford assay takes somewhere between 15 and 20 min. Despite the shorter time frame, the Bradford assay requires a slightly greater relative cost of about $2.00 to analyse a set of 10 samples (including standard curve), while the Lowry costs $1.66, but this does not take into account the cost attributed to labour and time, which is much greater for the Lowry assay.

Protein quantification is also largely affected by substance interference, and although we did not measure this directly, it cannot be overlooked. Peterson *et al*. (*17*) performed a detailed analysis of substances that interfere with the Lowry assay and found interference issues with several dozen compounds including: specific amino acids, buffers, chelating agents, detergents, salts, reducing agents, ions and lipids, to name a few. Peterson also determined the maximum tolerable limits of these substances, above which interference would occur yielding inaccurate values obtained by the Lowry. The muscle homogenization buffer used in this experiment consisted of 50 mM potassium phosphate, 5 mM EDTA, 0.5 mM DTT and 1.15% KCl, with both potassium phosphate and potassium chloride amounts being well above the maximum tolerable limit established by Peterson (*17*), and may account for the lack of inter-changeability in our data, in spite of being a common homogenization buffer for skeletal muscle. The Bradford assay, however, has less interference of substances, with only strong alkaline buffers and large amounts of detergents yielding erroneous values (*10*). More importantly, the homogenization buffer used in this experiment was more tolerable and did not exceed any of the maximum tolerable limits for the Bradford assay as set by the manufacturer. However, homogenization buffer alone does not explain the substantial lack of agreement (range: –42% to +74%) between the two methods.

Previous comparative studies conducted on the Lowry and Bradford techniques have also shown differences between the two assays due to substance interference. The Lowry assay provided 20% higher values due to ethanol and Tris, but underestimations due to sodium citrate, while the Bradford suffered no interference (*10*). Comparisons in marine research found that the Lowry consistently provided 20% higher values for protein content of marine phytoplankton as compared with the Bradford, even after trichloroacetic acid precipitation to remove unwanted interfering substances (*7*). Furthermore, comparisons on pegylated proteins showed that the Lowry significantly overestimated activated pegylated protein concentration due to interference with *N*-hydroxysuccinimide, a main component introduced during pegylation, while the Bradford assay did not suffer interference (*8*). However, in a study conducted on plasma proteins, researchers determined that the Lowry was more sensitive in biological fluids because the Bradford is unable to detect proteins of smaller sizes with a lower limit of detection between 3 and 5 kDa (*5*, *17*). In cell-culture experiments, researchers found that the Bradford consistently provided lower protein concentrations than the Lowry (*18*), and protein content measurements of membrane-containing fractions were also significantly underestimated by the Bradford assay due to loss in dye-binding activity after storage in –20°C (*9*). In contrast, comparisons in purified wine fractions determined that the Bradford underestimated protein content by 50–80% due to interference by alcohol and phenols, and that the Lowry overestimated protein content due to interfering substances but could be precipitated out by ethanol and acetone (*15*). Yet, another study conducted to compare both methods on total protein concentrations in human tears, determined that protein content obtained by either method were not only strongly correlated, but were also interchangeable upon appropriate usage of linear equations using the Bland–Altman test.(*13*). Therefore, the issue of substance interference must be thoroughly explored and not overlooked before selection of either the Lowry or Bradford assay.

Differences in linear range of concentration may also account for the lack of inter-changeability between the Lowry and Bradford assays. Our analyses indicate that the Bradford assay has a greater linear range of detection (0–2.0 mg/ml), while concentrations >0.50 mg/ml in the Lowry assay deviated towards a curvilinear direction (Fig. 4). A linear standard curve is required for extrapolating sample concentrations and adhering to the Beer–Lambert law, the principle behind spectrophotometric techniques. Therefore, the Bradford assay upholds the Beer–Lambert law through a greater range of linearity compared to the Lowry assay, and as such provides a more accurate relative measurement of total protein concentration.

Our analysis of both the Lowry and Bradford spectrophotometric methods of protein quantification in skeletal muscle revealed that the two methods were not interchangeable. The inability to compare skeletal muscle protein concentrations between both assays is a significant and novel finding for laboratory research since total protein concentration is relevant for all protein analysis. The Lowry and the Bradford techniques both have various strengths and weaknesses concerning stability, linearity, interfering substances, protein size and time required to perform analyses, all of which should be considered before selection of the appropriate assay. The Bradford assay is unable to detect low molecular weight proteins with a lower limit of detection of 3–5 kDA (*10*), however the Lowry assay is subject to greater substance interference. From our experiments in skeletal muscle, we have determined that the Bradford assay provides greater reagent stability, better protein stability, larger range of linearity and requires less time to perform analyses compared to the Lowry assay. Therefore, the Bradford assay is superior to the Lowry assay for protein quantification in skeletal muscle.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Gornall, A.G., Bardawill, C.J., and David, M.M. (1949) Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* **177**, 751–766
2. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
3. Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., and Klenk, D.C. (1985) Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76–85
4. Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
5. Okutucu, B., Dincer, A., Habib, O., and Zihnioglu, F. (2007) Comparison of five methods for determination of total plasma protein concentration. *J. Biochem. Biophys. Methods* **70**, 709–711
6. Ohnishi, S.T. and Barr, J.K. (1978) A simplified method of quantitating protein using the biuret and phenol reagents. *Anal. Biochem.* **86**, 193–200
7. Berges, J., Fisher, A., and Harrison, P. (1993) A comparison of Lowry, Bradford and Smith protein assays using different protein standards and protein isolated from the marine diatom *Thalassiosira pseudonana*. *Mar. Biol.* **115**, 187–193
8. Gong, X.W., Wei, D.Z., He, M.L., and Xiong, Y.C. (2006) Lowry method for the determination of pegylated proteins: the error, its reason, and a method for eliminating it. *Anal. Biochem.* **354**, 157–158
9. Kirazov, L.P., Venkov, L.G., and Kirazov, E.P. (1993) Comparison of the Lowry and the Bradford protein assays as applied for protein estimation of membrane-containing fractions. *Anal. Biochem.* **208**, 44–48

10. Lucarini, A.C. and Kilikian, B.V. (1999) Comparative study of Lowry and Bradford methods: interfering substances. *Biotechnol. Tech.* **13**, 149–154
11. Magnani, M., Stocchi, V., Serafini, G., and Chiarantini, L. (1988) Quantitation of electrophoretic eluted proteins. *Ital. J. Biochem.* **37**, 96–103
12. Marks, D.L., Buchsbaum, R., and Swain, T. (1985) Measurement of total protein in plant samples in the presence of tannins. *Anal. Biochem.* **147**, 136–143
13. Ng, V. and Cho, P. (2000) The relationship between total tear protein concentrations determined by different methods and standards. *Graefe's Arch. Clin. Exp. Ophthalmol. (Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol.)* **238**, 571–576
14. Paredes-Lopez, O., Guevara-Lara, F., Schevenin-Pinedo, M.L., and Montes-Rivera, R. (1989) Comparison of procedures to determine protein content of developing bean seeds (*Phaseolus vulgaris*). *Plant Foods Hum. Nutr.* **39**, 137–148
15. Vincenzi, S., Mosconi, S., Zoccatelli, G., Pellegrina, C.D., Veneri, G., Chignola, R., Peruffo, A., Curioni, A., and Rizzi, C. (2005) Development of a new procedure for protein recovery and quantification in wine. *Am. J. Enol. Vitic.* **56**, 182–187
16. Bland, J.M. and Altman, D.G. (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1**, 307–310
17. Peterson, G.L. (1979) Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. *Anal. Biochem.* **100**, 201–220
18. Guerlava, P., Izac, V., and Tholozan, J.L. (1998) Comparison of different methods of cell lysis and protein measurements in *Clostridium perfringens*: application to the cell volume determination. *Curr. Microbiol.* **36**, 131–135