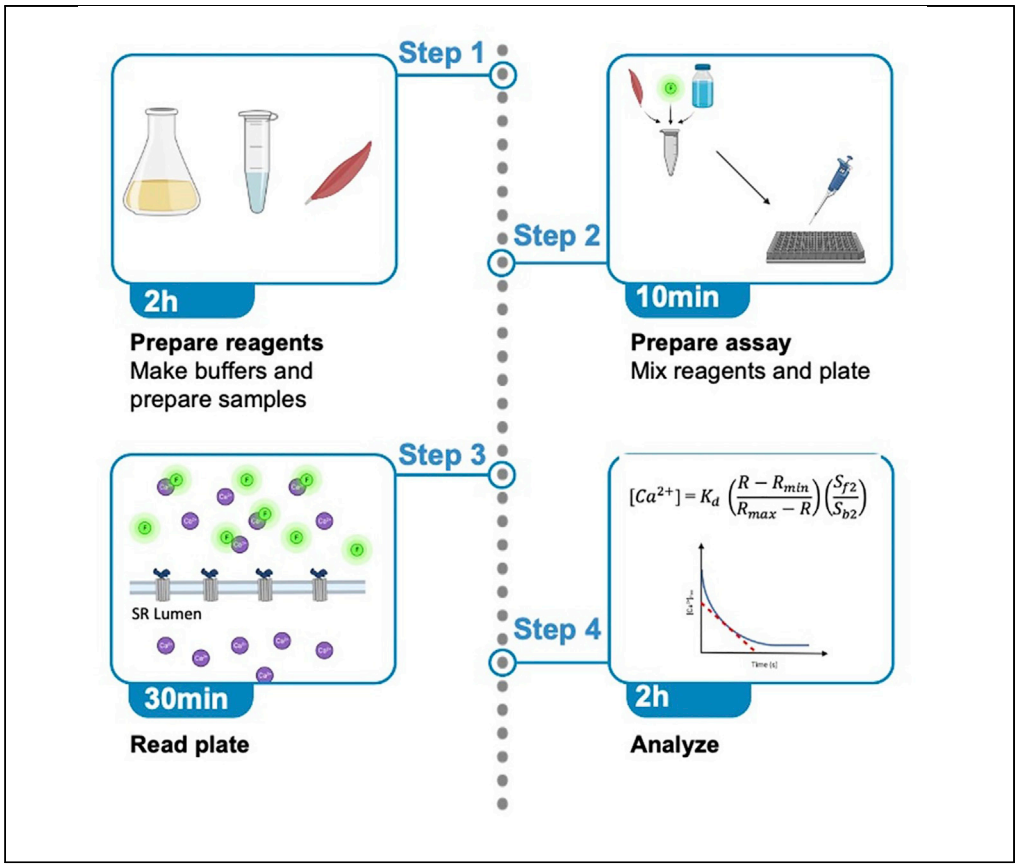
STAR Protocols

CellPress
OPEN ACCESS

Protocol

Measuring SERCA-mediated calcium uptake in mouse muscle homogenates



This protocol employs the indo-1 Ca^{2+} fluorophore to quantify Ca^{2+} uptake by the sarco(endo) plasmic reticulum Ca^{2+} ATPase pump in murine muscle homogenates and allows for real-time kinetic measurement of Ca^{2+} mobilization within the muscle homogenate. This protocol can be easily adapted for other tissue types and can be modified to single-emission/single-excitation Ca^{2+} dyes. Fitted to a 96-well plate, this assay can be readily performed in most laboratories with minimal sample requirement and the option of multiple replicates.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Mia S. Geromella, Jessica L. Braun, Val A. Fajardo

vfajardo@brocku.ca

Highlights

Measure real-time ATP-dependent Ca^{2+} uptake in muscle homogenates

Process multiple samples in duplicate or triplicate by fitting onto a plate reader

Applicable for non-muscle tissue such as brain homogenates

Steps for assessing rates of Ca^{2+} uptake and area-under-the-curve analysis

Geromella et al., STAR Protocols 4, 101987
March 17, 2023
https://doi.org/10.1016/j.xpro.2022.101987

STAR Protocols

CellPress
OPEN ACCESS

Protocol

Measuring SERCA-mediated calcium uptake in mouse muscle homogenates

Mia S. Geromella,[1,2,3] Jessica L. Braun,[1,2,3] and Val A. Fajardo[1,2,4,5,*]

[1]Department of Kinesiology, Brock University, St. Catharines, ON L2S 3A1, Canada

[2]Centre for Bone and Muscle Health, Brock University, St. Catharines, ON L2S 3A1, Canada

[3]These authors contributed equally

[4]Technical contact

[5]Lead contact

*Correspondence: vfajardo@brocku.ca
https://doi.org/10.1016/j.xpro.2022.101987

SUMMARY

This protocol employs the indo-1 Ca^{2+} fluorophore to quantify Ca^{2+} uptake by the sarco(endo)plasmic reticulum Ca^{2+} ATPase pump in murine muscle homogenates and allows for real-time kinetic measurement of Ca^{2+} mobilization within the muscle homogenate. This protocol can be easily adapted for other tissue types and can be modified to single-emission/single-excitation Ca^{2+} dyes. Fitted to a 96-well plate, this assay can be readily performed in most laboratories with minimal sample requirement and the option of multiple replicates.

For complete details on the use and execution of this protocol, please refer to Braun et al. (2022),[1] Braun et al. (2021a),[2] Braun et al. (2021b),[3] Cleverdon et al. (2022),[4] and Geromella et al. (2022).[5]

BEFORE YOU BEGIN

The protocol below describes the specific steps for measuring SERCA-mediated calcium uptake in murine skeletal and cardiac muscle homogenate. This assay has been modified from the originally described cuvette-based assay[6–10] to fit a 96-well plate. Notably, we have also successfully used this protocol in HEK293 cells, C2C12 cells, and murine brain tissue.

Institutional permissions

All animal experiments were in compliance with the Brock University Animal Care Committee and the Canadian Council on Animal Care. Researchers will need to check and/or obtain permissions from the relevant institutions before conducting this protocol.

Prepare reagents

Timing: 2 h

1. Prepare sample homogenizing buffer.
   a. Dissolve 250 mM sucrose, 5 mM HEPES, 0.2 mM phenylmethylsulfonyl fluoroide, and 0.2% [w/v] NaN_3 in distilled H_2O (dH_2O), and bring the pH to 7.5.

   Note: protease and phosphatase inhibitor cocktails are not recommended in the buffer as they can interfere with the assay.

2. Prepare Ca^{2+} uptake reaction buffer.

a. Dissolve 200 mM KCl, 20 mM HEPES, 10 mM NaN_3, 5 μM TPEN, and 15 mM $MgCl_2$ in dH_2O and bring the pH to 7.0. Store at –20°C for up to 1 year.

Note: 5 mM oxalate can be optionally added to the reaction buffer. This will buffer Ca^{2+} levels within the sarco(endo)plasmic reticulum (SR/ER), thereby preventing back-inhibition by lowering the amount of free Ca^{2+} in the SR/ER.[11] Therefore, the addition of oxalate can provide a measure of SERCA's maximal capacity to transport Ca^{2+} into the SR. However, the addition of oxalate may not be truly be physiologically representative and thus, it is important to consider the desired outcome measure. In conditions where severe Ca^{2+} dyshomeostasis is expected, (e.g., diseased or damaged states[4]) it is suggested that oxalate be added to allow for a read of Ca^{2+} uptake. For more information, see troubleshooting 1.

3. Prepare other reagents.
   a. Dissolve 250 mM ATP in dH_2O (pH = 7.0).

   Note: It is recommended to aliquot 300–500 μL, store at –20°C and avoid >5 freeze-thaw cycles (can be stored up to 1 year).

   b. Dissolve 50 mM EGTA in dH_2O (pH = 7.0).

   Note: Can be stored at 2°C–8°C for up to 1 year.

   c. Dissolve 100 mM Ca^{2+} or $CaCl_2$ in dH_2O.

   Note: Can be stored at 2°C–8°C for up to 1 year.

   d. Dissolve 2 mM Indo-1 in 50 mM glycine (pH = 11.0).

   Note: It is recommended to aliquot 15 μL and store away from light at –20°C (can be stored up to 1 year).

Tissue preparation

Timing: 1–3 h

4. Following dissection of the desired muscles (or other tissues), snap-freeze samplesin liquid nitrogen and store at –80°C.
5. Prior to the assay, homogenize skeletal muscle samples 1:10 and cardiac muscle samples 1:5 with homogenizing buffer.
   a. E.g., an 8 mg muscle sample will be homogenized in 80 μL of buffer.

   Note: if you are anticipating slower SERCA mediated Ca^{2+} uptake rates, this ratio can be altered accordingly (e.g., cardiac muscle and brain tissues display slower rates of SERCA Ca^{2+} uptake (Figure 1, *data not shown for brain*) and are thus homogenized 1:5).

   Note: the assay works best with freshly homogenized samples and is the way we process our samples; however, the assay has worked with samples that were previously freeze-thawed 2–3 times.

   b. Homogenize by hand using glass conical homogenizers and keep the whole homogenate for use in the assay (i.e., no centrifugation).

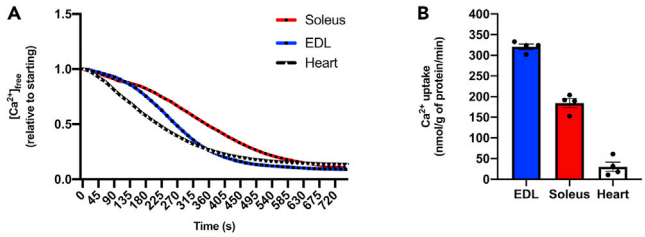


Figure 1. Ca^{2+} uptake curves and rates of Ca^{2+} uptake obtained in soleus, extensor digitorum longus (EDL) and cardiac muscle homogenates

(A) Calcium uptake, displayed as $[Ca^{2+}]_{free}$ made relative to each individual starting $[Ca^{2+}]$ over time for soleus, EDL, and cardiac muscle.

(B) Rates of Ca^{2+} uptake for each of the three muscle types measured using tangent analysis at 1,000 nM $[Ca^{2+}]_{free}$. (n = 4 per group, run in duplicate).Note: heart homogenate was more concentrated than that of skeletal muscle, however when making Ca^{2+} uptake rates relative to protein content, the rates are notably slower in the heart compared to skeletal muscle, as expected. Values are means ± SEM.

Note: spinning the homogenate to pellet membranes will compromise the assay as intact SR/ER membranes are required for proper SERCA function and the use of the membrane- impermeant Indo-1.

Note: we have also had success using skeletal muscle tissue homogenized using beads (both metal and silica) via FAST Prep as well as a Qiagen TissueLyser, again with whole homogenate being used for the assay.

Note: for use of cells, resuspend pellets in 150–200 µL (depending on the size of the pellet) and vortex in the same homogenizing buffer. For further information regarding the use of cells with this assay, please see Braun et al. and Geromella et al.[2,5]

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Sucrose	Sigma-Aldrich, Oakville, ON	S0389
N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid), HEPES	Sigma-Aldrich, Oakville, ON	H3375
Phenylmethylsulfonyl fluoroide (PMSF)	Sigma-Aldrich, Oakville, ON	PMSF-RO
Sodium azide (NaN_3)	Sigma-Aldrich, Oakville, ON	S2002
Potassium chloride (KCl)	Sigma-Aldrich, Oakville, ON	P9541
N,N,N′,N′-Tetrakis(2-pyridylmethyl) ethylenediamine (TPEN)	Sigma-Aldrich, Oakville, ON	P4413
Magnesium chloride ($MgCl_2$)	Sigma-Aldrich, Oakville, ON	M8266
Adenosine triphosphate (ATP)	Sigma-Aldrich, Oakville, ON	A2383
Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA)	Sigma-Aldrich, Oakville, ON	E3889
Calcium chloride ($CaCl_2$)	Sigma-Aldrich, Oakville, ON	C4901
Indo-1 (membrane-impermeant)	Biotum	50041
Fluo-4 (cell-impermeant)	Thermo Fisher, Waltham, MA	F14200
Cyclopiazonic acid (CPA)	Sigma-Aldrich, Oakville, ON	239805
Glycine	Sigma-Aldrich, Oakville, ON	G7126
Potassium oxalate	Sigma-Aldrich, Oakville, ON	P0963

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Softmax Pro 7 Software	Molecular Devices, San Jose, CA	N/A
Biological samples		
Murine (WT male, C57BL/6, 3–4 months) soleus homogenate	The Jackson Laboratory	N/A
Murine (WT male, C57BL/6, 3–4 months) extensor digitorum longus homogenate	The Jackson Laboratory	N/A
Murine (WT male, C57BL/6, 3–4 months) cardiac muscle homogenate	The Jackson Laboratory	N/A
Murine (WT male, C57BL/6, 3–4 months) red gastrocnemius homogenate	The Jackson Laboratory	N/A
Other		
All black 96-well plates	Thermo Fisher, Waltham, MA	7805
All black 1.5 mL microcentrifuge tubes	Fisher Scientific, Waltham, MA	15386548
Eppendorf repeater pipette	Sigma-Aldrich, Oakville, ON	EP4982000322
SpectraMax M2 multi-mode microplate reader	Molecular Devices, San Jose, CA	https://www.moleculardevices.com/products/microplate-readers/acquisition-and-analysis-software/softmax-pro-software?cmp=7010g000000nNEh&utm_source=AdWords&utm_medium=cpc&utm_campaign=MPR-Brand_Regional&utm_adgroup=%7Badgroup%7D&utm_location=1005010&utm_keyword=%2Bsoftmax%20pro%207&utm_device=c&utm_devicemodel=&utm_placement=&utm_adpostion=&utm_target=&utm_network=g&utm_creative=434045065811&gclid=Cj0KCQiA4aacBhCUARIsAI55maGPMqs81u7yfZRxBOkgfpBg02J_r_j6rSaEPog2VffBFZxerHvgmswaAoBKEALw_wcB

MATERIALS AND EQUIPMENT

Homogenizing buffer (1 L)		
Reagent	Final concentration	Amount
Sucrose (MW = 342.3)	250 mM	85.575 g
HEPES (MW = 238.3)	5 mM	1.192 g
PMSF (MW = 174.19)	0.2 mM	0.035 g
NaN_3 (MW = 65.01)	0.2% (w/v)	2.000 g
dH_2O	N/A	1 L
Total	**N/A**	**1 L**
Bring buffers to a pH of 7.5 and store at +4°C, can be stored up to 1 year.		

Ca^{2+} uptake reaction buffer (200 mL)		
Reagent	Final concentration	Amount
KCl (MW = 74.55)	200 mM	2.982 g
HEPES (MW = 238.3)	20 mM	0.953 g
NaN_3 (MW = 65.01)	10 mM	0.130 g
TPEN (MW = 424.54)	5 μM	0.425 mg
$MgCl_2$ (MW = 95.21)	15 mM	0.285 g
dH_2O	N/A	1 L
Total	**N/A**	**1 L**
Bring buffers to a pH of 7.0 and store at –20°C, can be stored up to 1 year.		

- M2 Molecular Devices Multimode plate reader with shaking, single excitation and dual-emission capabilities.
- Black 96 well plates.
- All-black 1.5 mL Eppendorf tubes.

Alternatives: A plate reader with only single emission capabilities can be used with alternative cell-impermeant Ca^{2+} fluorophores such as Fluo-4 (troubleshooting 2).

STEP-BY-STEP METHOD DETAILS

Preparing the reaction mixture

Timing: 5 min

This step is for combining the necessary materials to create the reaction mixture that is plated and used to obtain a measure of starting free Ca^{2+}.

1. Set plate reader to 37°C.
2. In black 1.5 mL Eppendorf tubes add 200 μL of Ca^{2+} uptake buffer, 20 μL of muscle homogenate, and 1 μL of Indo-1 (2 mM).

   Note: ensure to shake the Ca^{2+} uptake buffer and vortex the homogenate prior to addition to the tube.

   Note: the amount of tissue sample added can vary and may need to be optimized. For example, cardiac and brain tissue or cells may require 60 μL of homogenate. This can be altered freely as uptake rates are made relative to the amount of protein in each well.

3. Vortex the reaction mixture and plate 100 μL in duplicate in an all-black 96-well plate.
4. Using an excitation wavelength of 355 nm and dual emission at 405 nm and 485 nm, read the plate to gain values of the starting free Ca^{2+} concentration in each well.

   ⚠ CRITICAL: Once Indo-1 is added, the amount of light exposure should be kept to a minimum.

Running and calibrating the kinetic plate

Timing: 15–45 min

This step will guide through the process of running the kinetic plate as well as the calibration steps.

5. Remove the plate from the plate reader and add 4 μL of 250 mM ATP to each well.

   Note: It is recommended to use a repeater pipette for this step so that the ATP is added to all wells as quickly as possible.

6. Using the same excitation and emission settings as stated in step 4, read the plate kinetically, with continuous or interval shaking, for 30 min at 20 s intervals.

   Note: It is likely that the interval separation depends on the plate reader being used and the amount of samples on the plate. However, we recommend running intervals that are no more than 30 s apart, and therefore, the user may have to minimize the amount of samples run on a single plate in order to accommodate this (troubleshooting 3).

7. Once the plate is finished reading, add 10 μL of 50 mM EGTA to each well in order to obtain a "minimum" value for calibration.

   Note: To ensure an accurate "min" value, EGTA can be added in increments of 10 μL, with a read after each 10 μL addition, until the values become stable between reads (i.e., the values do not continue to decrease with each addition of EGTA).

8. Following EGTA, add 80 μL of 100 mM Ca^{2+} solution to each well and read the plate, in order to obtain a "maximum" value for calibration.

   Note: This value must be higher than the starting Ca^{2+} read at the very least, but Ca^{2+} can continue to be added, with the plate being read after each addition, being mindful of the maximal volume of the wells (troubleshooting 4).

   Optional: To gain a measure of Ca^{2+}-leak, add a SERCA inhibitor, such as cycolpiazonic acid (1 μL of 40 mM stock, dissolved in DMSO), to each well and continue the kinetic plate read for an additional 10–15 min (or until a plateau is reached). The calibration steps can then be performed following leak measurements. This step also ensures that the amount of Ca^{2+} uptake during this protocol was mediated by the SERCA pump.

   Note: The timing of the kinetic read will vary from experiment to experiment, and the timing listed in the steps is just a recommended starting point. Generally, the plate can be run until samples have reached a plateau or until a pre-determined endpoint.

   Pause point: The plate can be paused throughout the kinetic read to add a reagent/treatment to the samples and then resumed once ready. However, it is important that any breaks in the assay be as brief as possible.

EXPECTED OUTCOMES

With this protocol, one can anticipate obtaining free [Ca^{2+}] levels over time, corresponding to the amount of Ca^{2+} taken up by SERCA (Figure 1A). With this data, differences in SERCA-mediated Ca^{2+} uptake rates between muscle (or tissue) types can be clearly observed (Figure 1B) and is valuable for investigating the effects of different pathologies, conditions, or treatments on SERCA function. From the data, a variety of measures can be obtained, discussed further in the following section.

QUANTIFICATION AND STATISTICAL ANALYSIS

1. Export raw fluorescence data and the ratio (R) of Ca^{2+}-bound fluorescence (405 nm) to Ca^{2+}-free fluorescence (485 nm) can be calculated for each time point.
2. The free Ca^{2+} concentration ($[Ca^{2+}]_{free}$) for each time point is then calculated using the following equation:

$$\left[Ca^{2+}\right] = K_d\left(\frac{R - R_{min}}{R_{max} - R}\right)\left(\frac{S_{f2}}{S_{b2}}\right)$$

Where K_d is the dissociation constant of Indo-1 (250 nM). R_{min} is the ratio of bound:unbound Indo-1 after adding EGTA. R_{max} is the ratio of bound:unbound Indo-1 after adding $CaCl_2$. S_{f2} is the fluorescence emission at 485 nm (Ca^{2+}-free Indo-1) in the EGTA portion and S_{b2} is the fluorescence emission at 485 nm in the 5 M $CaCl_2$ portion. In otherwise healthy muscle, starting [Ca^{2+}] levels typically range between 1,000–3,000 nM and following uptake can be decreased to approximately 500 nM or below. In muscle from disease models, the starting [Ca^{2+}] levels may be higher, which may be

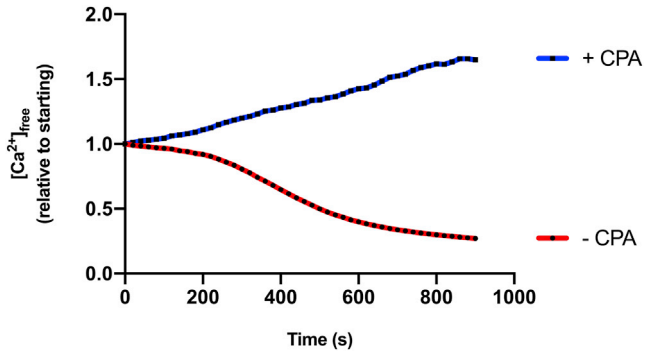


Figure 2. Ca^{2+} uptake is virtually absent in muscle homogenates when SERCA is inhibited with cyclopiazonic acid (CPA)

Representative Ca^{2+} uptake trace in red gastrocnemius muscle homogenates in the presence and absence of CPA.

indicative of impaired SERCA function, but some recommendations for running the assay and data analysis in this case are discussed in troubleshooting 5.

3. With this data, the following measures can be performed:
   a. Starting $[Ca^{2+}]$.
   b. Plot of Ca^{2+} uptake over time (either relative to starting or raw data, Figure 1).
   c. Time to 50% decay.
   d. Tangent analyses at desired $[Ca^{2+}]$ (e.g., 500 nM, 1,000 nM, etc.).
      i. We typically take instantaneous tangents at different concentrations using values 200 nM above and below the desired $[Ca^{2+}]$. This can be done with plotting software (i.e., Logger Pro™, Vernier).
   e. Area under the curve.
   f. Amount of Ca^{2+} taken up over desired time period.

Note: any measures should be made relative to protein content in each well to account for differences in protein concentrations of each sample.

LIMITATIONS

There are various limitations to this protocol that users must be aware of. First, starting $[Ca^{2+}]_{free}$ is not normalized. This can be circumvented by adding low amounts of $CaCl_2$ (0.5 μL of 10 mM $CaCl_2$ to each well) prior to beginning the assay, or in the case of high starting $[Ca^{2+}]_{free}$ low amounts of EGTA (0.2–0.5 μL of 50 mM EGTA) can be added to each well; however, Indo-1 is a high affinity Ca^{2+} dye and thus too much Ca^{2+} in the reaction mixture will lead to unreliable results. Further, this assay utilizes skeletal muscle homogenates and does not discount the contribution from other Ca^{2+} transporters and channels present. We observe that pre-treatment of homogenates with CPA completely abolishes Ca^{2+} uptake (Figure 2) and thus provides an indication that most, if not all, of the Ca^{2+} uptake is mediated by the SERCA pump. Users should perform this control test with CPA to ensure that Ca^{2+} uptake is mediated by SERCA. If CPA treatment does not abolish the Ca^{2+} uptake entirely, then subtracting Ca^{2+} uptake rates in the presence of CPA from those in the absence of CPA will provide a SERCA-specific rate. High levels of Ca^{2+} leak out of the SR/ER, as can occur in certain pathologies/treatments, may confound the amount and rates of Ca^{2+} uptake measured with this protocol. For example, high levels of Ca^{2+} leak into the cytosol will result in higher emission of bound Indo-1, resulting in the appearance of less Ca^{2+} uptake and thus slower rates. Results should always be interpreted in this context and followed up with alternative methods based on relevancy and feasibility, such as Western blotting, SR/ER phospholipid analysis, ionophore ratios, etc. Finally, our rates of Ca^{2+} uptake appear to be slower than those previously published using a similar protocol with a

spectrofluorometer,[6,8,12] though we attribute this to a lack of consistent stirring with the 96-well plate adaptation.

TROUBLESHOOTING

Problem 1

Severe SERCA dysfunction is anticipated (prepare reagents step 2 in before you begin).

Potential solution

In situations where severe Ca^{2+} dyshomeostasis is expected at the level of the triad, one can add 5 mM oxalate to the Ca^{2+} uptake reaction buffer to chelate SR/ER Ca^{2+} levels and prevent back-inhibition on the SERCA pump. This will allow for more, and uninterrupted, Ca^{2+} uptake, though it should be noted that this is not physiological and in control conditions, the rates of SERCA Ca^{2+} uptake will be supraphysiological.

Problem 2

The plate reader only has single-emission read-out (step 4).

Potential solution

If a plate reader does not have dual emission capabilities, alternative Ca^{2+} indicators can be used. The indicator should be cell-impermeant and the calculation and analysis will differ slightly than the present protocol but can be found in other publications using the selected indicator. In our hands, we have had success with Fluo-4 (Thermofisher, F14200) with the following equation:

$$[Ca^{2+}] = K_d\left(\frac{F - F_{min}}{F_{max} - F}\right)$$

Where K_d is the dissociation constant of Fluo-4 (335 nM), F is fluorescence, F_{min} is fluorescence in the presence of EGTA, and F_{max} is fluorescence in the high Ca^{2+} portion (5 M $CaCl_2$). As an example, in our hands, this assay also works with a 2 μM concentration of Fluo-4 (Thermofisher, F14200) in the reaction mixture and the K_d of 335 nM. Otherwise, the protocol remains the same and similar troubleshooting steps can be taken for optimization.

Problem 3

No Ca^{2+} uptake is observed (step 6).

Potential solution

Should the assay be run and no Ca^{2+} uptake is observed, a few things can be adjusted. First, ensure that the plate reader protocol is set up to have continuous, or frequent interval shaking throughout the read. Second, more sample can be added to the reaction mixture, especially if slower rates/lower protein concentrations are expected (e.g., soleus, cardiac muscle, brain, etc.). This will increase the protein content in each well, which will be accounted for in the analysis, and in our hands typically gives a signal. Third, we have seen, on occasion, samples requiring several minutes before uptake begins. Thus, allowing the plate to run longer may be a solution.

Problem 4

The high Ca^{2+} values are not getting higher than the starting Ca^{2+} values (step 8).

Potential solution

In this case, if the maximum values are not higher than starting and no more Ca^{2+} can be added due to volume limitations, the calibration can be performed separately. A new reaction mixture can be made with the Ca^{2+} uptake buffer, sample, and Indo-1, and plated in duplicate. Following plating, EGTA and then Ca^{2+} can be added immediately and the ATP addition and kinetic read can be skipped. This will provide the high and low calibration points necessary to calculate free Ca^{2+} concentrations.

Problem 5

The starting Ca^{2+} levels appear very high (quantification and statistical analysis, step 2).

Potential solution

In cases where the starting Ca^{2+} levels appear high, or are high in a specific experimental group (e.g., Cleverdon et al.[4]), a few things can be done. First, it is possible to add small amounts of 50 mM EGTA (0.2–0.5 μL) to the individual wells in order to bring all wells to a similar starting point prior to adding ATP and initiating uptake. In this case, all samples will be run with a similar starting [Ca^{2+}] and may make obtaining the R_{max} easier if problems are encountered there (also see troubleshooting 3). Alternatively, the Ca^{2+} uptake curves can be made relative to their individual starting [Ca^{2+}] levels for analysis in which case area under the curve or 50% decay rate can be obtained (see quantification and statistical analysis).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Val A. Fajardo (vfajardo@brocku.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

This study did not generate/analyze [datasets/code].

ACKNOWLEDGMENTS

This work was supported by an NSERC Discovery Grant to V.A.F. J.L.B. is supported by a Canadian Institutes of Health Research (CIHR) doctoral award. V.A.F. is supported by a Canada Research Chair (Tier 2) Award.

AUTHOR CONTRIBUTIONS

M.S.G. and J.L.B. helped conceive the protocol, optimized the protocol, performed data collection and analysis, and wrote, reviewed, and edited the manuscript. V.A.F. conceived the protocol, helped optimize the protocol, and reviewed and edited the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Braun, J.L., Ryoo, J., Goodwin, K., Copeland, E.N., Geromella, M.S., Baranowski, R.W., MacPherson, R.E.K., and Fajardo, V.A. (2022). The effects of neurogranin knockdown on SERCA pump efficiency in soleus muscles of female mice fed a high fat diet. Front. Endocrinol. *13*, 957182. https://doi.org/10.3389/fendo.2022.957182.

2. Braun, J.L., Teng, A.C.T., Geromella, M.S., Ryan, C.R., Fenech, R.K., MacPherson, R.E.K., Gramolini, A.O., and Fajardo, V.A. (2021a). Neuronatin promotes SERCA uncoupling and its expression is altered in skeletal muscles of high-fat diet-fed mice. FEBS Lett. *595*, 2756–2767. https://doi.org/10.1002/1873-3468.14213.

3. Braun, J.L., Geromella, M.S., Hamstra, S.I., Messner, H.N., and Fajardo, V.A. (2021b). Characterizing SERCA function in murine skeletal muscles after 35-37 Days of spaceflight. Int. J. Mol. Sci. *22*, 11764. https://doi.org/10.3390/ijms222111764.

4. Cleverdon, R.E.G., Braun, J.L., Geromella, M.S., Whitley, K.C., Marko, D.M., Hamstra, S.I., Roy, B.D., MacPherson, R.E.K., and Fajardo, V.A. (2022). Sarco(endo)plasmic reticulum Ca(2+)-ATPase function is impaired in skeletal and cardiac muscles from young DBA/2J mdx mice. iScience *25*, 104972. https://doi.org/10.1016/j.isci.2022.104972.

5. Geromella, M.S., Ryan, C.R., Braun, J.L., Finch, M.S., Maddalena, L.A., Bagshaw, O., Hockey, B.L., Moradi, F., Fenech, R.K., Ryoo, J., et al. (2022). Low dose lithium supplementation promotes adipose tissue browning and sarco(endo)plasmic reticulum Ca(2+) ATPase uncoupling in muscle. J. Biol. Chem. *298*, 102568. https://doi.org/10.1016/j.jbc.2022.102568.

6. Tupling, R., and Green, H. (2002). Silver ions induce Ca2+ release from the SR in vitro by acting on the Ca2+ release channel and the Ca2+ pump. J. Appl. Physiol. *92*, 1603–1610. https://doi.org/10.1152/japplphysiol.00756.2001.

7. Tupling, A.R., Vigna, C., Ford, R.J., Tsuchiya, S.C., Graham, D.A., Denniss, S.G., and Rush, J.W.E. (2007). Effects of buthionine sulfoximine treatment on diaphragm contractility and SR Ca2+ pump function in rats. J. Appl. Physiol. *103*, 1921–1928. https://doi.org/10.1152/japplphysiol.00529.2007.

8. Bombardier, E., Smith, I.C., Vigna, C., Fajardo, V.A., and Tupling, A.R. (2013).

Ablation of sarcolipin decreases the energy requirements for Ca2+ transport by sarco(endo)plasmic reticulum Ca2+-ATPases in resting skeletal muscle. FEBS Lett. *587*, 1687–1692. https://doi.org/10.1016/j.febslet.2013.04.019.

9. Gamu, D., Juracic, E.S., Fajardo, V.A., Rietze, B.A., Tran, K., Bombardier, E., and Tupling, A.R. (2019). Phospholamban deficiency does not alter skeletal muscle SERCA pumping efficiency or predispose mice to diet-induced obesity. Am. J. Physiol. Endocrinol. Metab. *316*, E432–E442. https://doi.org/10.1152/ajpendo.00288.2018.

10. Fajardo, V.A., Bombardier, E., Irvine, T., Metherel, A.H., Stark, K.D., Duhamel, T., Rush, J.W.E., Green, H.J., and Tupling, A.R. (2015). Dietary docosahexaenoic acid supplementation reduces SERCA Ca2+ transport efficiency in rat skeletal muscle. Chem. Phys. Lipids *187*, 56–61. https://doi.org/10.1016/j.chemphyslip.2015.03.001.

11. Inesi, G., and de Meis, L. (1989). Regulation of steady state filling in sarcoplasmic reticulum. Roles of back-inhibition, leakage, and slippage of the calcium pump. J. Biol. Chem. *264*, 5929–5936.

12. Fajardo, V.A., Bombardier, E., McMillan, E., Tran, K., Wadsworth, B.J., Gamu, D., Hopf, A., Vigna, C., Smith, I.C., Bellissimo, C., et al. (2015). Phospholamban overexpression in mice causes a centronuclear myopathy-like phenotype. Dis. Model. Mech. *8*, 999–1009. https://doi.org/10.1242/dmm.020859.