

Acute effects of taurine on sarcoplasmic reticulum Ca^{2+} accumulation and contractility in human type I and type II skeletal muscle fibers

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Dutka TL, Lamboley CR, Murphy RM, Lamb GD. Acute effects of taurine on sarcoplasmic reticulum Ca^{2+} accumulation and contractility in human type I and type II skeletal muscle fibers. *J Appl Physiol* 117: 797–805, 2014. First published August 14, 2014; doi:10.1152/jappphysiol.00494.2014.—Taurine occurs in high concentrations in muscle and is implicated in numerous physiological processes, yet its effects on many aspects of contractility remain unclear. Using mechanically skinned segments of human vastus lateralis muscle fibers, we characterized the effects of taurine on sarcoplasmic reticulum (SR) Ca^{2+} accumulation and contractile apparatus properties in type I and type II fibers. Prolonged myoplasmic exposure (>10 min) to taurine substantially increased the rate of accumulation of Ca^{2+} by the SR in both fiber types, with no change in the maximum amount accumulated; no such effect was found with carnosine. SR Ca^{2+} accumulation was similar with 10 or 20 mM taurine, but was significantly slower at 5 mM taurine. Cytoplasmic taurine (20 mM) had no detectable effects on the responsiveness of the Ca^{2+} release channels in either fiber type. Taurine caused a small increase in Ca^{2+} sensitivity of the contractile apparatus in type I fibers, but type II fibers were unaffected; maximum Ca^{2+} -activated force was unchanged in both cases. The effects of taurine on SR Ca^{2+} accumulation 1) only became apparent after prolonged cytoplasmic exposure, and 2) persisted for some minutes after complete removal of taurine from the cytoplasm, consistent with the hypothesis that the effects were due to an action of taurine from inside the SR. In summary, taurine potentiates the rate of SR Ca^{2+} uptake in both type I and type II human fibers, possibly via an action from within the SR lumen, with the degree of potentiation being significantly reduced at low physiological taurine levels.

contractile apparatus; taurine; sarcoplasmic reticulum; single fiber

TAURINE IS A SMALL ORGANIC acid found in particularly high concentrations in cardiac and skeletal muscle and is thought to be involved in numerous important processes such as modulating osmotic pressure, cation homeostasis, ion channel activity, proinflammatory mediation, protein stabilization, and acting as an antioxidant, to name a few (6, 19, 21, 40, 42). Given its many roles, it is not surprising that taurine deficiency can lead to pathogenesis in both cardiac and skeletal muscle (6, 21, 40). Furthermore, the pathogenesis that stems from a lack of taurine has been shown to be partially ameliorated with therapeutic oral taurine supplementation (4, 35). Additionally, there is considerable interest in the health consequences of a strict vegan diet, which is devoid of taurine (24, 38), and also in possible ergogenic effects of increasing muscle taurine content (1, 14, 35, 39, 42, 50, 51).

Taurine content in skeletal muscle has been shown to be highly fiber-type dependent, with type I fibers containing approximately twice as much taurine as type II fibers in both rat muscles (22) and in camel gluteal muscles (7). In vastus lateralis muscle of untrained healthy young adults, the taurine level is ~12 to 14 mM in type I fibers and between ~3 and 11 mM in type II fibers (15, 17, 46) (amounts given here in units of mmoles per liter cytoplasmic water). Oral taurine supplementation has been found to increase muscle taurine content in rats (14), but in healthy humans such supplementation did not raise muscle taurine content above the normal level (13). However, muscle taurine content can be significantly lower in chronic renal failure patients (3). Furthermore, cardiomyopathies develop in cats with taurine-deficient diets (36), and severe skeletal muscle impairment is observed in taurine transporter knockout mice (48). In both cases, taurine supplementation partially reversed these deleterious effects, suggesting taurine is important for normal muscle function.

In chemically skinned cardiac trabeculae of rats (43), taurine (up to 30 mM) was found to cause a minor increase in the Ca^{2+} sensitivity of the contractile apparatus without any change to maximum force. In mechanically skinned extensor digitorum longus (EDL) fibers of rats, which are almost exclusively type II fibers, 20 mM taurine seemed instead to cause a small decrease in contractile apparatus Ca^{2+} sensitivity (2). In contrast to such minor effects on the contractile apparatus, the presence of 5–30 mM taurine markedly potentiated the peak and area of caffeine-induced force responses in both the skinned EDL muscle fibers (2) and cardiac trabeculae (43). Steele et al. (43) attributed this effect to taurine increasing Ca^{2+} loading into the sarcoplasmic reticulum (SR), while Bakker and Berg (2) proposed the effect could have been due either to increased Ca^{2+} accumulation by the SR or to sensitization of Ca^{2+} release through Ca^{2+} release channel-ryanodine receptors (RyRs). It is presently unknown 1) whether physiological levels of taurine exert comparable effects on SR Ca^{2+} accumulation in human skeletal muscle fibers, 2) whether the effects of taurine differ between type I and type II muscle fibers, and 3) precisely how taurine exerts its apparent effects on net Ca^{2+} uptake.

Using both type I and type II fibers from human vastus lateralis muscle, we sought to determine whether the presence of taurine affects 1) the contractile apparatus properties, 2) SR Ca^{2+} uptake, as suggested by the studies on rat tissue (2, 43), and 3) the sensitivity of SR Ca^{2+} release and, furthermore, to ascertain if taurine exerts its effects on SR Ca^{2+} accumulation from an action on the myoplasmic side or from inside the SR lumen. This was achieved using mechanically skinned fibers in which excitation-contraction coupling remains functional (26) and where the myoplasmic taurine concentration could be

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rapidly and precisely manipulated, with each fiber able to act as its own control. We examined the effects of 0 to 20 mM taurine, more than spanning the physiological range in human skeletal muscle type I and type II fibers. We hypothesized that acutely altering taurine concentrations would have only minor effects on the contractile apparatus, and that increasing the myoplasmic taurine concentration across this range would appreciably affect force responses, primarily by increasing SR uptake in both fiber types.

MATERIALS AND METHODS

Muscle biopsy, subject details. Eleven healthy subjects, 10 men and 1 woman (age 23 ± 5 yr; height, 178 ± 6 cm; body mass, 76 ± 12 kg), gave written, informed consent and participated in this study, which conformed to the standards set by the Declaration of Helsinki. These subjects were all recreationally active [i.e., performed regular physical activities such as jogging and cycling (30–60 min, 2–3 times per week)], but were not specifically trained in any sport. All protocols and procedures performed were approved by the Human Research Ethics Committees at Victoria University and La Trobe University. After injection of a local anesthetic into the skin and fascia (1% lidocaine), a small incision was made in the middle third of the vastus lateralis of each subject, and a muscle sample was taken using a Bergstrom biopsy needle (30). An experienced medical practitioner took all biopsies at approximately constant depth. The excised muscle sample was rapidly blotted on filter paper to remove excess blood and placed in paraffin oil (Ajax Chemicals, Sydney, NSW, Australia) at $\sim 10^\circ\text{C}$ for 45 min before individual muscle fibers were dissected.

Fiber mounting. The muscle biopsy was pinned at resting length in a petri dish lined with Sylgard 184 (Dow Corning, Midland, MI) and immersed in paraffin oil and kept cool ($\sim 10^\circ\text{C}$) on an icepack. Using jeweler's forceps, segments of individual fibers were mechanically skinned (25) and individually mounted at 120% of resting length on a force transducer (AME801, Horten) with a resonance frequency of >2 kHz. The fiber segment was then transferred to a 2-ml Perspex bath containing standard K⁺-based solution that broadly mimics the myoplasmic environment (see below). All force measurements were made at room temperature ($23 \pm 2^\circ\text{C}$).

Solutions. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO), unless specified otherwise. The standard potassium-hexamethylene-diamine-tetraacetate (K-HDTA) solution used for examining SR Ca²⁺ release experiments contained the following (in mM): 50 HDTA²⁻ (Fluka, Buchs, Switzerland); 8 total ATP; 36 Na⁺; 126 K⁺; 8.5 total Mg²⁺ (giving 1 mM free [Mg²⁺], where brackets denote concentration); 10 phosphocreatine; 0.05 total EGTA; 90 HEPES; pH 7.1 and pCa ($= -\log_{10} [\text{Ca}^{2+}]$) ~ 7.0 , except where stated. Where required, the SR of the skinned fiber was totally depleted of releasable Ca²⁺ by exposing the fiber to a K-HDTA solution with 30 mM caffeine and low (0.05 mM) free Mg²⁺ (2.15 mM total Mg²⁺) and with 0.5 mM EGTA (pCa > 8.0) present to chelate much of the released Ca²⁺ (12), referred to hereafter as the "full release solution." This caffeine-low [Mg²⁺] solution is a very potent stimulus, which is insensitive to subtle changes in Ca²⁺ release, and provides a reliable indication of the total amount of releasable Ca²⁺ in the SR (12, 33). In contrast, the experiments examining the sensitivity of the RyRs to Ca²⁺-induced Ca²⁺ release (CICR) used a submaximal stimulus [8 mM caffeine in the presence of normal (i.e., 1 mM) free Mg²⁺] with little Ca²⁺ buffering, made by adding caffeine to the standard K-HDTA solution containing only 0.05 mM EGTA (pCa ~ 7.0). Examination of the contractile apparatus properties required the use of heavily Ca²⁺-buffered solutions in which all HDTA was replaced with EGTA (i.e., relaxing solution) or Ca-EGTA (i.e., maximum Ca²⁺-activating solution), as described previously (9). The pCa of solutions (for pCa < 7.2) was measured with a Ca²⁺-sensitive electrode (Orion Research, Boston, MA). In addition, a strontium-

based solution (at pSr 5.3) was made by mixing relaxing solution (50 mM EGTA) with a Sr-EGTA solution similar to the maximum Ca²⁺-activating solution; this solution was used in the contraction experiments to indicate the likely fiber type under examination (a pSr 5.3 solution typically elicits $<5\%$ of maximum force in type II fibers and $>65\%$ of maximum in type I fibers; see Refs. 5, 9, 28).

Taurine is a highly soluble compound (solubility of 838 mM at 25°C ; see Ref. 18), and a 500 mM stock was made simply by dissolving it in standard K-HDTA solution. A matching stock with 500 mM sucrose (Ajax, Sydney, NSW, Australia) was also made up in standard K-HDTA solution. The effects of taurine solutions were compared with "control" solutions, with equimolar sucrose replacing taurine, to verify that any observed effects were not due simply to a change in osmolality. The osmolality of experimental solutions containing 20 mM sucrose or taurine was 315 ± 5 mosmol/kgH₂O, and for those containing 5 mM sucrose or taurine it was 300 ± 5 mosmol/kgH₂O. Both taurine and sucrose negligibly bind either Mg²⁺ or Ca²⁺ at pH 7.1. The preequilibration solution with 10 mM carnosine (0.5 mM EGTA, pCa > 8) was made by mixing K-HDTA solution with a 80 mM carnosine stock solution containing similar constituents, made as described previously (9).

Contractile apparatus experiments. The acute effect of 5, 10, and 20 mM taurine on the contractile apparatus was examined by directly activating the contractile apparatus in a series of heavily Ca²⁺-buffered solutions of different known pCa, with either sucrose (i.e., control condition) or with taurine (i.e., test condition) present. After a 5-min treatment with Triton X-100 (2% vol/vol in relaxing solution) and two subsequent 1-min wash periods (again in relaxing solution), the skinned fiber segment was exposed to a range of solutions with progressively higher free [Ca²⁺] (0.1–20 μM , i.e., pCa 7.0 to 4.7). These solutions were made by mixing appropriate amounts of the 50 mM EGTA (relaxing) and the 50 mM Ca-EGTA (maximum activating) solutions. This solution sequence elicited force responses resembling a "staircase" (see Fig. 1). Fibers were preequilibrated in relaxing solution with either taurine or sucrose for >2 min before the effects on Ca²⁺ activation were examined. For each given condition, the isometric force response elicited at each pCa was expressed as a percentage of the corresponding maximum Ca²⁺-activated force ("max."), plotted against the pCa value and a Hill curve fitted using GraphPad Prism 4 (GraphPad Software, San Diego, CA).

Ca²⁺-release experiments. After we mounted the skinned fiber segment on the force transducer and equilibrating it for 2 min in standard K-HDTA intracellular solution (50 μM EGTA, pCa ~ 7.0), the SR was depleted of all of its releasable Ca²⁺ by exposing the fiber to the "full release" solution (see earlier) for 1 min, eliciting a force response that was used as an estimate of the endogenous amount of Ca²⁺ that had been initially present in the SR (27, 28). Before each exposure to the full release solution, the fiber was first equilibrated for 15 s in K-HDTA solution with 0.5 mM EGTA solution, so as to raise the [EGTA] within the fiber before applying the potent caffeine-low [Mg²⁺] stimulus. After the full release, the fiber was washed for >1 min in K-HDTA solution with 0.5 mM EGTA to remove the caffeine and to raise the free [Mg²⁺] back up to 1 mM. The SR was then reloaded with Ca²⁺ to a particular level by bathing the fiber in "load" solution (K-HDTA solution with 1 mM total Ca-EGTA-EGTA at pCa 6.7) for a set time (5 s to 5 min), and then again depleted of all Ca²⁺ with the full release solution, and the fiber subsequently washed again. These load-release-wash cycles were performed with either sucrose or taurine present in all solutions (as in Fig. 2) or a combination of both present (as in Fig. 5). When exposed to the Ca²⁺ loading solution, the depleted SR initially takes up Ca²⁺ approximately linearly with load time and then at a progressively slower rate as the SR Ca²⁺ content approaches its maximal level (28, 33, 47).

The sensitivity of the Ca²⁺ release channels (RyRs) was tested using a submaximal caffeine (8 mM) solution with only very weak Ca²⁺ buffering (i.e., standard K-HDTA solution, containing 50 μM total EGTA, pCa ~ 7.0), so as to allow possible self-reinforcement of

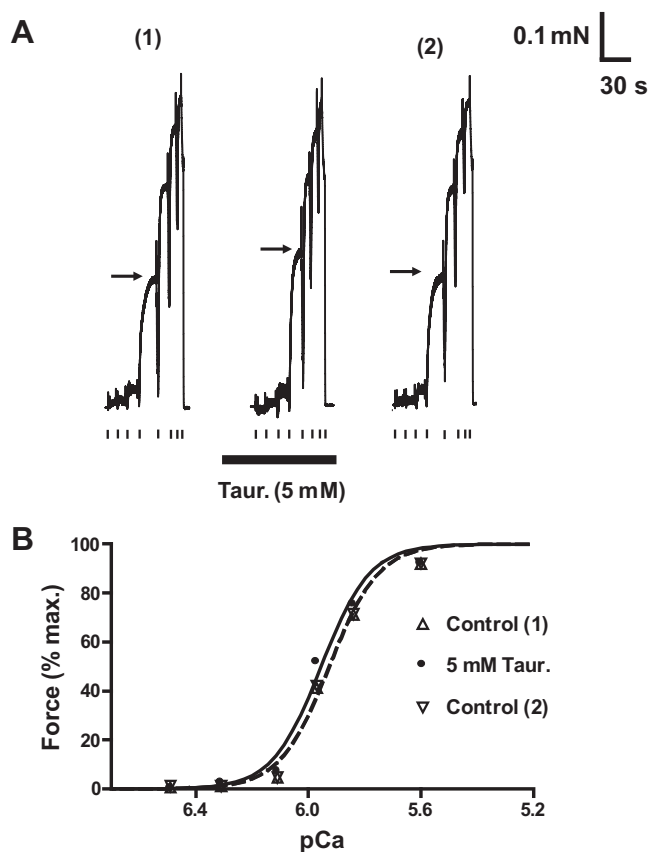


Fig. 1. Taurine has only minor effects on the contractile apparatus properties. *A*: force responses elicited in a human type I muscle fiber when directly activating the contractile apparatus with heavily Ca²⁺-buffered solutions with progressively higher free Ca²⁺ concentration ([Ca²⁺]) (pCa of successive solutions: >9, 6.49, 6.31, 6.11, 5.97, 5.84, 5.60, 4.7, then back to >9, marked by ticks under each force trace). Force-pCa staircases were elicited before and after taurine (Taur) (controls 1 and 2) and in presence of taurine. Horizontal arrow indicates force at pCa 5.97 in absence and presence of 5 mM taurine. *B*: Hill fits to force-pCa staircases in *A* (force at pCa 4.7 defined as maximum). There was a small increase in Ca²⁺ sensitivity with no change to maximum force in the presence of taurine [i.e., force-pCa curve shifted from control level (dashed line) to the left with 5 mM taurine (solid line)]. Note, this fiber displayed the largest shift observed in any fiber with 5 mM taurine ($\Delta pCa_{50} = +0.029$ pCa units from the control level), with the range in the 6 type I fibers examined being +0.003 to 0.029 pCa units (see Table 1 for mean data).

any CICR. By using such a submaximal stimulus (as in Fig. 4), it was possible to determine whether taurine modified the responsiveness of the RyRs to CICR. The procedure was similar to the cycles above but with additional intermediate steps: 1) the SR was first loaded for a set time in absence of taurine; 2) the fiber was preequilibrated in standard K-HDTA solution for 15 s and then exposed to the same solution with 8 mM caffeine for 15 s, with taurine or sucrose as appropriate present in both solutions; 3) all Ca²⁺ remaining in the SR was released by preequilibration in K-HDTA solution with 0.5 mM EGTA for 15 s followed by exposure to the full release solution for 1 min, with no taurine present; and, finally, 4) the fiber was washed in K-HDTA solution with 0.5 mM EGTA.

Western blot analysis. Following the physiological measurements, each fiber segment was placed in an Eppendorf tube containing 10 μ l of relaxing solution (50 mM EGTA, pCa > 9, described above) with 5 μ l of 3 \times solubilizing buffer, which contained 0.125 M Tris-HCl, 10% glycerol, 4% SDS, 4 M urea, 10% mercaptoethanol, and 0.001% bromophenol blue, pH 6.8. Fibers were stored at -80°C until analyzed. Western blots were performed to determine the myosin heavy

chain (MHC I or II) isoforms present, as described previously (9, 28, 32). Total denatured protein from individual fiber segments was separated on 4–15% Criterion TGX Stain Free gels (BioRad, Hercules, CA) and then transferred to nitrocellulose. Membranes were exposed to mouse primary antibodies diluted in 1% BSA in PBS-Tween 20 (anti-MHC I: 0.19 μ g/ml, mouse monoclonal IgM, clone A4.840; anti-MHC II: 0.15 μ g/ml, mouse monoclonal IgG, clone A4.74; Developmental Studies Hybridoma Bank, University of Iowa), following which the appropriate secondary antibody (i.e., either goat anti-mouse IgG or IgM) conjugated with horseradish peroxidase was added to the membranes. Bands were visualized using West Femto chemiluminescent substrate (ThermoScientific), and images captured using Quantity One software (BioRad).

Statistics. All values are presented as means \pm SE, with *n* denoting the number of individual fibers examined. Statistical significance ($P < 0.05$) was determined using paired Student's one- or two-tailed *t*-tests, as appropriate. Exponential curves were fitted to mean data of response area for various SR Ca²⁺ loading times (10 s to 5 min) in presence or absence of taurine (see Fig. 2*B*), as described previously (28).

RESULTS

Fiber typing. The fiber type of each fiber segment used in the physiology experiments was later ascertained by Western blot analysis of the MHC isoform(s) present (see MATERIALS AND METHODS). Of the 55 fibers from 11 subjects used in this study, 25 were classified as type I (containing only MHC I), 27 were classified as type II (containing only MHC II), and only 3 were considered of "mixed" type, containing appreciable levels of both MHC I and MHC II. The MHC II antibody used here is documented as detecting both MHC IIA and IIX, and so the type II fibers here were not subdivided into IIA, IIA/X, and IIX categories, but, based on other studies in similar untrained subjects, it is expected that the great majority of the fibers studied here were pure IIA fibers (20, 45). As our laboratory has reported previously for other vastus lateralis muscle fibers from young adults (9, 28), the fiber type (i.e., type I or type II) could also be gauged during the course of the physiology measurements by the responsiveness of the fiber to a Sr²⁺-containing solution at pSr 5.3 (see MATERIALS AND METHODS); the response to Sr²⁺ differs depending on troponin C isoform present (slow or fast), which was found to be consistently in accord with the MHC isoform(s) present.

Effect of taurine on contractile apparatus properties in human type I and type II fibers. The properties of the contractile apparatus in each skinned fiber segment were examined by directly activating the contractile apparatus in a sequence of solutions with the free [Ca²⁺] heavily buffered at progressively higher levels, with maximum Ca²⁺-activated force defined as that produced at pCa 4.7 (i.e., ~ 20 μ M free Ca²⁺) (see MATERIALS AND METHODS). As shown in Fig. 1, this was performed before, during, and after exposure to taurine, with equimolar sucrose replacing the taurine in the bracketing "control" cases. The presence of taurine caused a small concentration-dependent increase in the Ca²⁺ sensitivity in type I fibers (mean change in pCa₅₀ being +0.019 pCa units with 5 mM taurine and +0.035 pCa units with 20 mM taurine), but had no effect in type II fibers (see Table 1). The Hill coefficient and maximum Ca²⁺-activated force were not altered by the presence of taurine in either fiber type.

Effect of taurine on SR Ca²⁺ accumulation. Taurine has been shown to augment SR Ca²⁺ accumulation in mechani-

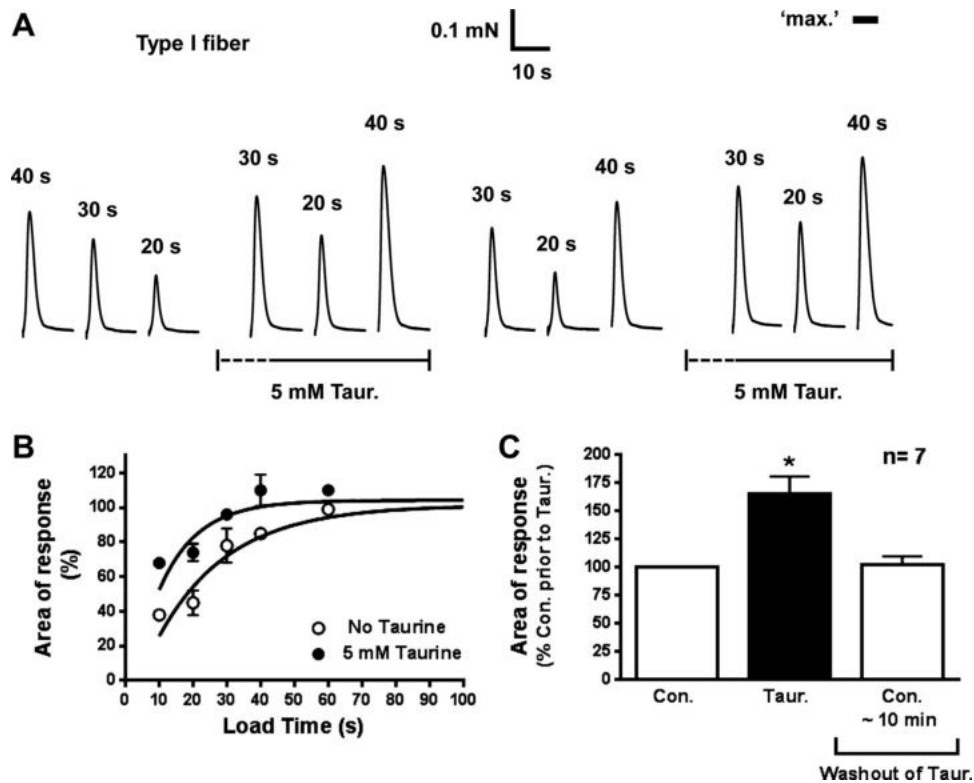


Fig. 2. Taurine increases the rate of sarcoplasmic reticulum (SR) Ca²⁺ accumulation in human vastus lateralis fibers. *A*: force responses elicited in a type I fiber upon emptying the SR of all releasable Ca²⁺ in full release solution (see MATERIALS AND METHODS) after prolonged equilibration with 5 mM taurine present or absent (replaced with equimolar sucrose). Fiber was loaded for indicated time (20–40 s) before each response. Taurine was present/absent for >10 min before SR was loaded and responses elicited. max, Maximum Ca²⁺-activated force produced by fiber at pCa 4.7. *B*: mean (±SE) of relative area (i.e., time integral) of full release force response in 4 type I fibers after loading for indicated time, with taurine present or absent as in *A*. All values are expressed relative to that found in same fiber with 60-s loading under control conditions (no taurine); data were obtained in 2–4 of the fibers at each point. Exponential curves were fitted to the two data sets constrained to same maximum level (see RESULTS). The degree of potentiation with 5 mM taurine was greater at lower load times. *C*: full reversibility of potentiation after taurine was washed out for >10 min. Values are means (±SE) of full release response in 4 type I and 3 type II fibers after SR was loaded for a given time (20–40 s) before, during, and after exposure to 5 mM taurine; values are expressed relative to control (Con) response in same fiber before exposure to taurine. *Significantly different from initial control ($P < 0.05$, Student's paired t -test).

cally skinned EDL muscle fibers (2) and in chemically skinned cardiac trabeculae (43) of rats. We sought first to determine whether this phenomenon also occurred in human vastus lateralis muscle fibers, and second, if there were any differences between type I and type II fibers, particularly given that they typically contain different amounts of taurine and different SERCA isoforms. Fiber segments were exposed to either 5

mM taurine or to zero taurine for ≥ 10 min, during which time the SR was loaded with Ca²⁺ (at pCa 6.7, with 1 mM total EGTA-Ca-EGTA) for a range of set times (i.e., 10–60 s) and then depleted of all releasable Ca²⁺ by exposing the fiber segment to the full release solution (see MATERIALS AND METHODS). This assay of the SR Ca²⁺ content produces highly reproducible force responses, which increase progressively in peak size and

Table 1. Summary of effect of taurine on contractile apparatus properties

| Treatment | Max (%Con) | pCa ₅₀ | | Δ pCa ₅₀ (Relative to Con) | Δ h (Relative to Con) | n |
|------------|-------------|-------------------|---------------|--|------------------------------|---|
| | | Con | in Taur | | | |
| Type I | | | | | | |
| 5 mM Taur | 99.6 ± 0.5 | 5.965 ± 0.030 | 5.984 ± 0.031 | +0.019 ± 0.005* | +0.0 ± 0.1 | 6 |
| 10 mM Taur | 98.5 ± 0.8 | 5.944 ± 0.024 | 5.969 ± 0.028 | +0.025 ± 0.006* | +0.1 ± 0.1 | 5 |
| 20 mM Taur | 99.0 ± 1.1 | 5.965 ± 0.043 | 6.000 ± 0.046 | +0.035 ± 0.011* | -0.1 ± 0.1 | 6 |
| Type II | | | | | | |
| 5 mM Taur | 99.3 ± 0.8 | 5.881 ± 0.039 | 5.893 ± 0.039 | +0.012 ± 0.008 | +0.6 ± 0.4 | 6 |
| 10 mM Taur | 100.0 ± 0.5 | 5.898 ± 0.027 | 5.897 ± 0.033 | -0.001 ± 0.007 | -0.5 ± 0.4 | 4 |
| 20 mM Taur | 101.9 ± 1.4 | 5.874 ± 0.045 | 5.886 ± 0.036 | +0.012 ± 0.009 | -0.4 ± 0.6 | 5 |

Values are means ± SE of the maximum Ca²⁺-activated force (Max) and the change (Δ) in the pCa₅₀ and Hill coefficient (h) with taurine (Taur) present, relative to the bracketing control (Con) responses in absence of Taur [Taur replaced with equimolar sucrose (Sucr)], as in Fig. 1. The mean of the pCa₅₀ values in Taur and for the bracketing Con are also shown. n, No. of fibers examined from 3 subjects. *Response in presence of Taur is significantly different ($P < 0.05$) from bracketing Con level (Student's paired two-tailed t -tests). The mean value of h in Con conditions was 4.2 ± 0.2 in the type I fibers ($n = 6$) and 5.2 ± 0.2 in the type II fibers ($n = 6$).

area (i.e., time integral) with longer Ca²⁺ loading times (see Fig. 2, A and B). The order of examination of different load times was varied to verify that this had no effect on the responses (this was also routinely performed in other experiments in this study). In the four type I fibers examined (from two subjects), the presence of 5 mM taurine potentiated both the peak and area of the force response to the full release solution (see Fig. 2), with the largest degree of potentiation relative to control being seen with the shorter load times (i.e., 10–20 s, see Fig. 2B). Similar results were also found in two type II fibers from two different subjects (not shown). This effect of taurine on Ca²⁺ uptake could be fully reversed by prolonged washout of the taurine (Fig. 2C). These data indicate that the presence of taurine increased the rate at which the SR accumulated Ca²⁺ at pCa 6.7 in both fiber types.

We also explicitly examined whether the presence of taurine affected the maximal SR Ca²⁺ load level attained. In these experiments, the given fiber segment was first preequilibrated with or without 5 mM taurine for 5 min and then placed in a pCa 6.7 load solution at the same taurine concentration (i.e., 0 or 5 mM) for a further 5 min, which should have been ample time to reach maximal SR Ca²⁺ loading (see Fig. 2B), and then the amount of Ca²⁺ accumulated by the SR was assayed with the full release solution. The maximal SR Ca²⁺ content found after loading with 5 mM taurine present was not significantly different from that found in bracketing measurements with loading in the absence of taurine [mean relative force-time integral: 102 ± 6% for type I fibers (*n* = 6) and 100 ± 7% for

type II fibers (*n* = 6), 3 subjects]. Thus the presence of taurine increased the rate at which the SR took up Ca²⁺ at pCa 6.7, but did not alter the maximal amount that could be accumulated.

Comparison of effects of brief or prolonged exposure to taurine. To further verify that taurine's apparent ability to augment SR Ca²⁺ accumulation was not the result of some direct effect of taurine on the contractile apparatus, in other experiments, fibers were preequilibrated with taurine for 10 min, and then the SR was loaded and emptied of Ca²⁺ in the absence of taurine. This procedure, which involved prolonged preexposure to taurine and its removal from the myoplasm before loading, still resulted in increased SR Ca²⁺ accumulation [compare first two traces in Fig. 3B, and mean data (solid bar) in Fig. 3C]. In marked contrast, when the period of exposure to taurine before loading was only relatively brief (e.g., <30 s, Fig. 3A), there was no apparent increase in SR Ca²⁺ accumulation (e.g., Fig. 3A). In the nine fibers examined that were exposed only briefly to taurine (20 mM for 30 s) and then loaded for 30 s with taurine present, there was no significant change in the peak or area of the force response elicited by the full release solution (mean peak and area of response with taurine, relative to bracketing responses without taurine: 102 ± 3 and 104 ± 4%, respectively, in five type I fibers, and 100 ± 4 and 99 ± 6%, respectively, in four type II fibers). These data strongly suggest that taurine exerts its effect on Ca²⁺ accumulation from within the SR lumen and requires substantial time to enter and equilibrate inside the SR (see DISCUSSION). In apparent accord with this, it was found also that

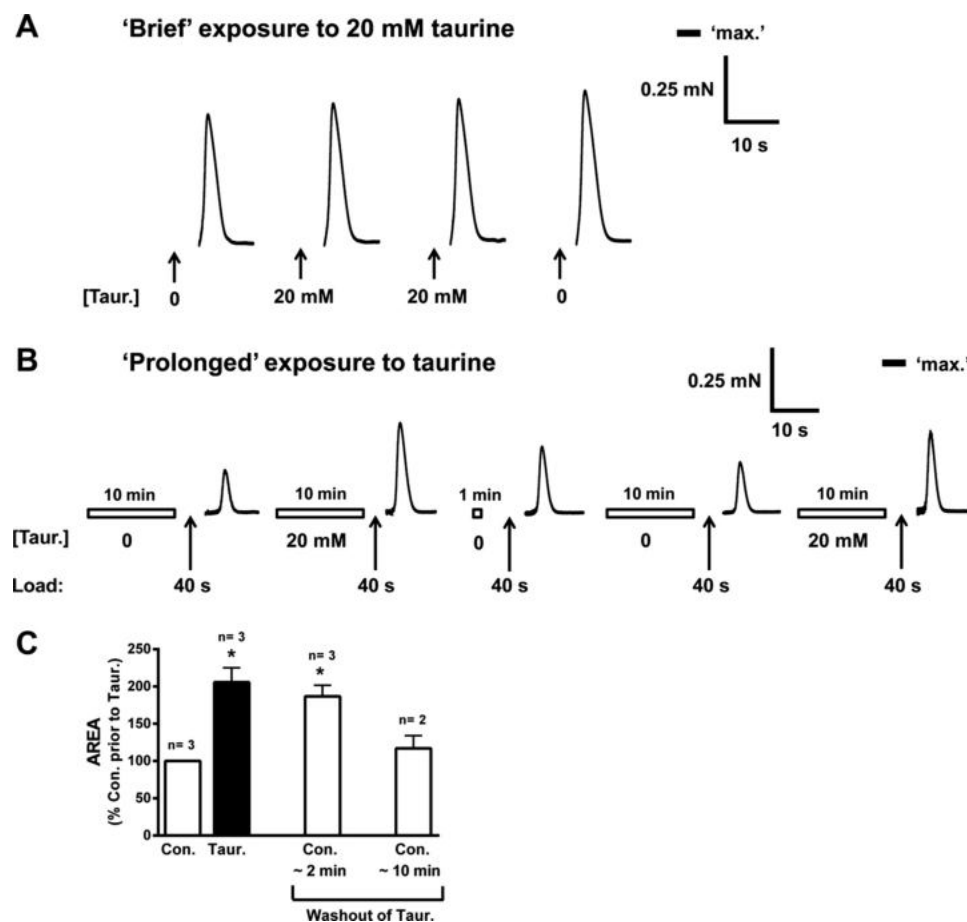


Fig. 3. Only prolonged taurine exposure increases SR Ca²⁺ accumulation. A: SR Ca²⁺ accumulation (i.e., area of full release force response) in a type II fiber was not appreciably altered when SR loading was performed after only a "brief" exposure to 20 mM taurine (taurine present only during 30-s preequilibration and 30-s loading period). Bracketing control responses were obtained with same protocol with taurine absent (replaced by 20 mM sucrose, with >10 min washout of taurine). B: SR Ca²⁺ accumulation was increased in another type II fiber that was preexposed to taurine for 10 min before Ca²⁺ was loaded and released in the absence of taurine (compare first and second responses). The enhanced Ca²⁺ uptake persisted on the following load-release cycle (after <2 min total washout time for taurine), but uptake eventually returned to the initial control level after a further 10-min washout of taurine. C: mean (+SE) area of full release response in 3 other type II fibers when loaded for set period 1) without taurine exposure (Con), 2) after 10 min preexposure to 5 mM taurine (Taur), and 3) after a total of ~2 min and ~10 min washout of taurine.

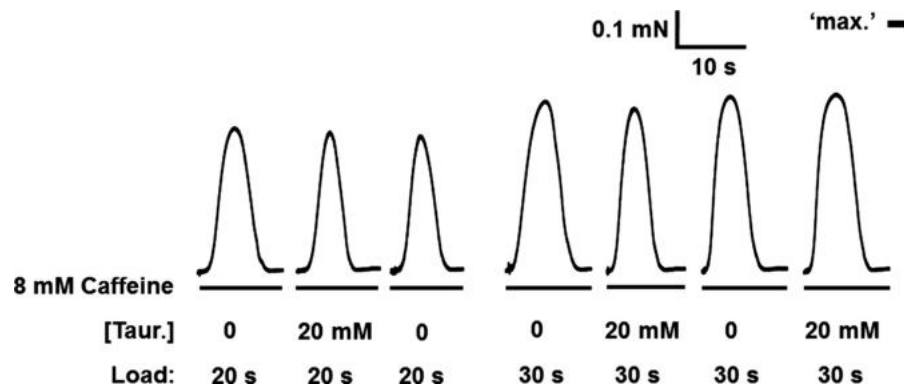


Fig. 4. Taurine has no effect on force response elicited by a submaximal caffeine stimulus. The SR of a skinned type I fiber was loaded with Ca²⁺ for a set time (either 20 or 30 s at pCa 6.7) and then pre-equilibrated in either control conditions (no taurine, 20 mM sucrose) or with 20 mM taurine for only 15 s and then exposed to 8 mM caffeine (see MATERIALS AND METHODS), producing submaximal force responses. After exposure to the 8 mM caffeine solution, the SR was depleted of all remaining releasable Ca²⁺ with the full release solution (not shown, see Table 2 for summarized data). The force response elicited by the 8 mM caffeine solution was unchanged in the presence of taurine (Table 2). Horizontal lines under each force response indicate exposure to the 8 mM caffeine solution. max, Maximum Ca²⁺-activated force level for the fiber.

the effects on SR Ca²⁺ accumulation of a prolonged (>10 min) preexposure to taurine persisted for some minutes after complete removal of taurine from the cytoplasm, as seen in Fig. 3B by the comparatively large size of the third compared with the first full release response and in the mean data in Fig. 3C.

Effect of taurine on submaximal caffeine-induced force responses. We also specifically examined whether the presence of taurine in the myoplasm affected the responsiveness of the RyRs by testing whether taurine altered the force response elicited by a submaximal caffeine concentration in conditions of weak Ca²⁺-buffering (see MATERIALS AND METHODS), which is a sensitive measure of RyR activation (9, 37). In these experiments, the SR was loaded with Ca²⁺ for a set time (20–40 s) in the absence of taurine, and then the fiber was equilibrated with either 0 or 20 mM taurine for 15 s before being exposed to a solution at that taurine level (i.e., 0 or 20 mM) containing 8 mM caffeine, which elicited a submaximal force response (e.g., Fig. 4). After exposure to this submaximal stimulus, the SR was depleted of all remaining releasable Ca²⁺ by exposure to the full release solution before the cycle was repeated. The responses generated by the submaximal caffeine stimulus are sensitive to very small differences in Ca²⁺ release, and yet the presence of 20 mM taurine had no significant effect on either the peak or force-time integral of the response relative to the control level, or on the amount of Ca²⁺ remaining in the SR, in either type I or type II fibers (see Fig. 4 and Table 2). Thus there was no evidence to suggest that the presence of cytoplasmic taurine altered either the responsiveness of the RyRs or the extent of any Ca²⁺ leak through the RyRs.

Effect on SR Ca²⁺ accumulation of varying taurine concentration over physiological range. The normal cytoplasmic concentration of taurine in vastus lateralis muscle fibers in healthy young adults appears to be in the range ~12–14 in type I fibers and ~3–11 mM in type II (see Introduction). The preceding experiments in this study compared the effects of having taurine present or completely absent, the latter being a more extreme case than would be expected to occur in humans, even with diets almost completely lacking any taurine. To examine the effects on SR Ca²⁺ accumulation of varying taurine over its likely physiological range, we directly compared the uptake properties with 5, 10, and 20 mM taurine in

the same fiber (Fig. 5). In these experiments, the given fiber was always equilibrated with the indicated level of taurine for >10 min before making measurements, and the effect of 10 mM taurine was examined both before and after examining each of the other concentrations (5 and 20 mM taurine). The order of presentation of 5 and 20 mM taurine was randomized in different fibers, and the effects of a given taurine level were highly reproducible, regardless of the order of presentation (as in Fig. 2A). It was found that increasing the taurine concentration from 10 to 20 mM had no significant effect on the rate of SR Ca²⁺ accumulation in either type I or type II fibers, whereas lowering the concentration from 10 to 5 mM caused a significant reduction in the uptake rate in both fiber types (Fig. 5).

Comparison of the effects of carnosine and taurine on SR Ca²⁺ accumulation. Finally, we also examined whether a physiological level of carnosine (10 mM, see Ref. 9) affected SR Ca²⁺ accumulation in a similar way as taurine. In these

Table 2. Effect of 20 mM taurine on submaximal caffeine-induced force responses

| Treatment | Peak Force to 8 mM Caffeine, %Con | Area, %Con | SR Ca ²⁺ Remaining, % | n |
|------------------------------|-----------------------------------|------------|----------------------------------|---|
| Type I: load time (20 s) | | | | |
| 8 mM Caff + 20 mM Sucr (Con) | 100 | 100 | 100 | 4 |
| 8 mM Caff + 20 mM Taur | 101.8 ± 8.1 | 96.3 ± 7.1 | 86.3 ± 11.7 | |
| Type II: load time (40 s) | | | | |
| 8 mM Caff + 20 mM Sucr (Con) | 100 | 100 | 100 | 5 |
| 8 mM Caff + 20 mM Taur | 94.6 ± 4.7 | 99.5 ± 7.7 | 94.6 ± 8.8 | |

Values are means ± SE, expressed as a percentage of bracketing Con measurements with no Taur (i.e., with 20 mM Sucr), as in Fig. 4. n, No. of fibers examined in three subjects. Force response to 8 mM caffeine (Caff) was in the presence of 20 mM Taur. Peak force produced by 8 mM Caff under Con conditions was 58.3 ± 6.8 and 30.6 ± 10.6% of Max Ca²⁺-activated force (pCa 4.7) for type I and II fibers, respectively. Relative amount of Ca²⁺ remaining in SR after the 15-s exposure to 8 mM Caff determined from area of force response on subsequent exposure to full release solution. There was no significant change in any response parameter with 20 mM Taur present.

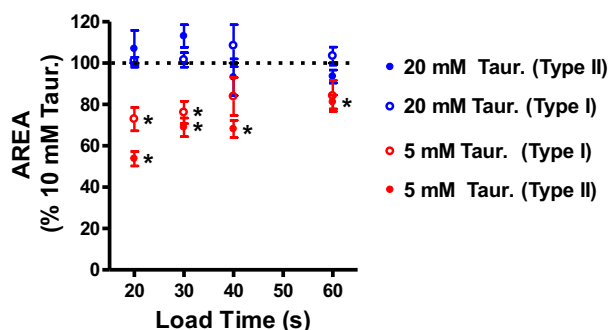


Fig. 5. Low taurine concentration reduces SR Ca²⁺ accumulation. Values are means (\pm SE) of relative area (i.e., time integral) of full release force response in 4 type I fibers and 6 type II fibers (from 2 subjects) after loading SR for indicated time with 5 or 20 mM taurine present; values are expressed relative to that found in same fiber with same loading time and 10 mM taurine present (black dotted line, 100%). Fibers were equilibrated in given taurine concentration for >10 min in all cases, and taurine was replaced isomotically with sucrose as required. SR Ca²⁺ accumulation was similar with either 10 or 20 mM taurine present, but was significantly lower (*) when the taurine concentration was only 5 mM.

experiments, the fiber was preequilibrated for 10 min with either 10 mM carnosine or 10 mM taurine or 10 mM sucrose before loading and releasing Ca²⁺ in standard conditions without either carnosine or taurine. In the one type I fiber and the one type II fiber examined, such preequilibration with carnosine had no apparent effect on Ca²⁺ accumulation, whereas taurine had its usual effect of increasing uptake (Fig. 6).

DISCUSSION

This study demonstrates that the presence of physiological levels of intracellular taurine potentiates the rate of SR Ca²⁺ accumulation at pCa 6.7 in both type I and type II fibers in vastus lateralis muscle of humans, similar to findings reported in rat EDL fibers (2) and cardiac trabeculae (43). The rate of Ca²⁺ accumulation was increased without any change to the maximum amount of Ca²⁺ accumulated by the SR. Furthermore, the increase in Ca²⁺ uptake only became apparent many minutes after adding the taurine to the cytoplasm and persisted for a number of minutes after complete washout of the taurine from the cytoplasm (Fig. 3). In the skinned fiber preparation, the cytoplasmic concentration of a diffusible, small molecular weight substance such as taurine equilibrates (>95%) with the level present in the bathing solution in <1 s (31), as evident from the very rapid response of the contractile apparatus to bath application/removal of Ca²⁺, ATP, or other agents (8, 10). Hence, we conclude that taurine was probably not mediating its

effect on Ca²⁺ uptake via a cytoplasmic action, but instead via an action from inside the SR, with it taking some minutes for the taurine concentration inside the SR to come into equilibrium with the concentration in the cytoplasm. However, at present, there is only indirect evidence to support this suggestion, and the issue needs further investigation, including whether taurine is actively transported across the SR membrane by a specific transporter or by other means (e.g., see Ref. 41).

The increase in the rate of net Ca²⁺ uptake at pCa 6.7 was most likely the result of taurine modifying sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA) function, either by increasing its maximum pumping rate or increasing its affinity for Ca²⁺ (or both). The fact that the effects of taurine were similar in type I and type II fibers might be viewed as somewhat surprising, given that the two fiber types have different SERCA isoforms: type I fibers containing primarily or exclusively SERCA2, and type II fibers containing SERCA1 (28, 29). However, numerous factors influence the Ca²⁺ affinity and maximum pumping rate of the SERCA, including the regulatory proteins phospholamban and sarcolipin (34), as well as pH (49), oxidation, and other cellular and membrane conditions, and it is clear that some factors (e.g., pH changes) exert broadly similar effects on both SERCA isoforms. It is unlikely that taurine increased the net SR Ca²⁺ accumulation by reducing some concurrent level of SR Ca²⁺ leakage because such a decrease in Ca²⁺ leakage would be expected to have had relatively little effect on the net Ca²⁺ uptake at the short loading times (when there was little Ca²⁺ present in the SR to leak out), which is the opposite of the behavior observed (Fig. 2C).

The effect of taurine observed here on the rate of SR Ca²⁺ accumulation at pCa 6.7 (i.e., 200 nM cytoplasmic free Ca²⁺) seems likely to be of physiological relevance because muscle fiber relaxation involves the SR lowering the cytoplasmic [Ca²⁺] from >2 μ M during a tetanic contraction down to the normal resting level of ~50 to 100 nM, and SERCA1 and SERCA2 in human fibers display half-maximal Ca²⁺ pumping at ~pCa 6.6 and pCa 6.8, respectively (29). In human vastus lateralis muscle, there is normally ~12–14 mM taurine in type I fibers and ~3–11 mM in type II fibers (15, 17, 46). It was found here that there was no difference in SR Ca²⁺ accumulation at pCa 6.7 in either fiber type when changing the taurine level from 10 to 20 mM, but that lowering the taurine from 10 to 5 mM resulted in significantly slower Ca²⁺ accumulation in both types (Fig. 5), with even greater effect if taurine was absent entirely (Fig. 2B). Such decreases in the SR Ca²⁺ accumulation rate would be expected to slow muscle relaxation

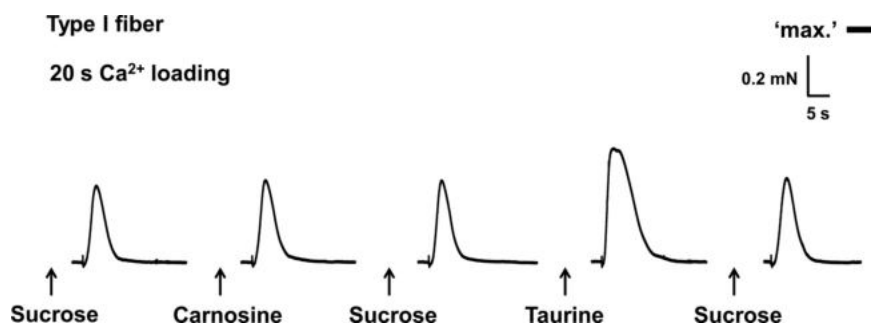


Fig. 6. Carnosine does not increase SR Ca²⁺ accumulation. Force response is shown to full release solution in a type I fiber loaded with Ca²⁺ after preequilibration for 10 min with either carnosine, taurine, or sucrose (each at 10 mM). In each case, the preequilibration solution was washed out for 15 s, the fiber was loaded using the same load solution (pCa 6.7, 1 mM total EGTA with 10 mM sucrose), and release was elicited with the standard full release solution. SR Ca²⁺ accumulation was increased only when the fiber had been preequilibrated with taurine.

to some degree, and, if the Ca²⁺ affinity of the SERCA were decreased, it would likely also result in a reduced resting SR Ca²⁺ content. Thus muscle taurine levels may have a significant regulatory action on intracellular Ca²⁺ movements and contractile function. An implication of this is that, if muscle taurine levels were to drop substantially below normal, as might occur in particular diseases, such as chronic renal failure or in individuals adhering to strict taurine deficient diets (24, 44), it could have significant adverse effects on SR Ca²⁺ uptake properties and muscle function, with this possibly being a greater problem in type II fibers, where the normal taurine level can be comparatively low.

Comparison of effects of taurine and carnosine. It is of interest to compare the effects of taurine on muscle function with those of carnosine, another major intracellular constituent, particularly given that it has been suggested that the muscle concentrations of taurine and carnosine may vary inversely (23), such as when increasing carnosine levels by chronic β-alanine supplementation (11, 16). Certainly, carnosine levels in humans are lower in type I fibers than in type II fibers, whereas, for taurine, levels show the reverse behavior (15, 17, 46). However, β-alanine supplementation in humans was found to increase carnosine levels in both fiber types by 30–50% without significant change in the taurine levels (15). Our laboratory has reported previously (9) that the presence of intracellular carnosine markedly increases the Ca²⁺ sensitivity of the contractile apparatus in both type I and type II human fibers (by ~0.12 and 0.10 pCa units, respectively, with 16 mM carnosine), whereas it was found here that taurine had very much less effect, with the addition of 20 mM taurine causing only a ~0.035 pCa unit increase in Ca²⁺ sensitivity in type I fibers and having no significant effect in type II fibers (Table 1). Consequently, the net effect on the contractile Ca²⁺ sensitivity of increasing carnosine levels by supplementation would be little different even if the muscle taurine levels underwent the opposite change. Furthermore, it was found previously that carnosine enhanced the sensitivity of the RyRs-Ca²⁺ release channels to caffeine, at least in type I fibers (9), whereas the present study found no such sensitizing effect of cytoplasmic taurine (Fig. 4 and Table 2). Finally, it was found here that, unlike taurine, carnosine did not noticeably potentiate the rate of SR Ca²⁺ accumulation (Fig. 6). Hence, it is clear that carnosine and taurine have distinctly different effects on both intracellular Ca²⁺ movements and contractile function in human skeletal muscle.

Speculation on the long-term effect of taurine on SR Ca²⁺ handling proteins. In the present study, we examined the acute effects of manipulating muscle taurine concentration. If changes in taurine muscle content persisted for a prolonged period, it is possible that there could be altered expression of SR Ca²⁺-handling proteins, such as calsequestrin or SERCA. Goodman et al. (14) found that, in rats given taurine supplementation, which increased muscle taurine levels by ~40%, there was an increase in twitch and submaximal tetanic force in the EDL (type II) fibers and also ~50% increase in the amount of calsequestrin 1 present, with no change in SERCA1 levels. Assuming that the increased muscle taurine level in the rat EDL fibers resulted in a chronic increase in SR Ca²⁺ uptake, we speculate that there may have been a compensatory increase in calsequestrin 1 expression to help adequately buffer the free Ca²⁺ concentration within the SR.

Concluding remarks. We report here the effects of taurine on the contractile properties and SR Ca²⁺ movements in human type I and type II fibers using a preparation in which the RyRs and SERCA and other key proteins remain fully functional and in their normal *in situ* locations. Taurine was found to help facilitate SR Ca²⁺ accumulation, probably involving either an increase in the maximum SR Ca²⁺ uptake rate or the SERCA affinity for Ca²⁺. Indirect evidence was presented indicating that taurine possibly exerts its effects via an action from within the SR. Changing muscle taurine levels within the physiological range modulates SR Ca²⁺ uptake, and it appears that reducing taurine levels to below normal may adversely affect muscle function.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.L.D. and G.D.L. conception and design of research; T.L.D., C.R.L., and R.M.M. performed experiments; T.L.D., C.R.L., and R.M.M. analyzed data; T.L.D., C.R.L., R.M.M., and G.D.L. interpreted results of experiments; T.L.D. prepared figures; T.L.D. drafted manuscript; T.L.D. and G.D.L. edited and revised manuscript; T.L.D., C.R.L., R.M.M., and G.D.L. approved final version of manuscript.

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