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Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise

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Nilsson MI, Greene NP, Dobson JP, Wiggs MP, Gasier HG, Macias BR, Shimkus KL, Fluckey JD. Insulin resistance syndrome blunts the mitochondrial anabolic response following resistance exercise. *Am J Physiol Endocrinol Metab* 299: E466–E474, 2010. First published July 6, 2010; doi:10.1152/ajpendo.00118.2010.—Metabolic risk factors associated with insulin resistance syndrome may attenuate augmentations in skeletal muscle protein anabolism following contractile activity. The purpose of this study was to investigate whether or not the anabolic response, as defined by an increase in cumulative fractional protein synthesis rates (24-h FSR) following resistance exercise (RE), is blunted in skeletal muscle of a well-established rodent model of insulin resistance syndrome. Four-month-old lean (*Fa/?*) and obese (*fa/fa*) Zucker rats engaged in four lower body RE sessions over 8 days, with the last bout occurring 16 h prior to muscle harvest. A priming dose of deuterium oxide (^2H_2O) and ^2H_2O-enriched drinking water were administered 24 h prior to euthanization for assessment of cumulative FSR. Fractional synthesis rates of mixed (−5%), mitochondrial (−1%), and cytosolic (+15%), but not myofibrillar, proteins (−16%, $P = 0.012$) were normal or elevated in gastrocnemius muscle of unexercised obese rats. No statistical differences were found in the anabolic response of cytosolic and myofibrillar subfractions between phenotypes, but obese rats were not able to augment 24-h FSR of mitochondria to the same extent as lean rats following RE (+14% vs. +28%, respectively). We conclude that the mature obese Zucker rat exhibits a mild, myofibrillar-specific suppression in basal FSR and a blunted mitochondrial response to contractile activity in mixed gastrocnemius muscle. These findings underscore the importance of assessing synthesis rates of specific myocellular subfractions to fully elucidate perturbations in basal protein turnover rates and differential adaptations to exercise stimuli in metabolic disease.

skeletal muscle; protein synthesis; diabetes; ^2H_2O

ALTHOUGH PHYSICAL ACTIVITY is an effective, low-cost, and nonpharmacological alternative to traditional prevention and treatment methods for insulin resistance syndrome (40, 41), insulin-resistant skeletal muscle may be "desensitized" to the anabolic action of exercise (14, 37, 38, 47, 51, 59, 63). Considering the putative role of mitochondria in the development and progression of insulin resistance syndrome (49), the contractile activity-induced response of the organelle in metabolic disease states is of particular interest (34). Chronic endurance exercise augments mitochondrial protein synthesis rates (66) and induces mitochondrial growth in skeletal muscle (11); however, the drive for mitochondrial biogenesis is blunted in insulin-resistant individuals (14), potentially altering the adaptive response of the organelle to contractile activity (47, 63). De Filippis et al. (14) recently reported an impaired ability to activate critical regulators of mitochondrial biogenesis (AMP-activated protein kinase/peroxisome proliferator-activated receptor-γ coactivator-1α/nuclear respiratory factor 1) following acute endurance exercise in skeletal muscle of obese and insulin-resistant subjects, causing a blunted induction of downstream nuclear-encoded mitochondrial genes (cytochrome *c* oxidase subunit VIc). Whether or not these findings can be generalized to other modes of exercise and whether the observed suppression at the transcriptome level is accompanied by a global reduction in mitochondrial protein synthesis rates remain to be elucidated.

Chronic resistance exercise (RE) has well-recognized effects on protein synthesis rates, lean mass, and strength, but there is growing evidence that resistance training can also improve mitochondrial function and oxidative enzyme activity in skeletal muscle (60, 61). Although augmentations of mitochondrial protein synthesis rates appear to dissipate after the initial RE bout in young individuals (66), older adults exhibit a sustained increase in the translational capacity of mitochondrial proteins even after several months of resistance training (46, 61). Despite clinically significant improvements in risk factors associated with insulin resistance syndrome following long-term participation in moderate-intensity resistance exercise (10, 12, 17, 62), several studies indicate that insulin resistance (and/or related comorbidities) may limit gains in muscle mass (51, 59) and strength (35) by altering anabolic signaling events following resistance-type contractile activity (37, 38, 51). Paturi et al. (51) recently reported an attenuated hypertrophic response in soleus muscle following synergistic ablation of gastrocnemius and plantaris in the obese Zucker rat (*fa/fa*), a widely recognized model of genetic obesity and insulin resistance syndrome. These findings are contrary to those of Peterson et al. (52), suggesting that the anabolic response, or lack thereof, is age, muscle group, and/or fiber type specific in this rat strain. Albeit by not measuring protein synthesis rates per se, Katta et al. (37) also demonstrated a blunted activation of Akt/mammalian target of rapamycin (mTOR)/$p70^{S6K}$ 1–3 h following high-force electrical stimulation in tibialis anterior of the obese phenotype, further supporting the notion of a blunted anabolic response postexercise in the insulin-resistant state. Considering the emerging role(s) of the evolutionally conserved mTOR pathway in mitochondrial biogenesis (6, 13, 56), synthesis rates of both nuclear- and mtDNA-encoded mitochondrial proteins may be compromised following contractile stimuli in tissues with insulin resistance.

To our knowledge, cumulative fractional protein synthesis rates [24-h FSR (28)] of cellular subfractions regulating metabolism (mitochondria and cytosol) and contraction/energy utilization (myofibrils) have not been directly assessed in insulin-resistant skeletal muscle following contractile activity.

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For these purposes we employed a stable isotopic method, centering on the use of deuterium oxide (^2H_2O) as a tracer, to assess 24-h FSR of mitochondrial-, cytosolic-, and myofibrillar-rich subfractions in gastrocnemius muscle of sedentary and resistance-exercised lean and obese Zucker rats. The versatility of ^2H_2O as a metabolic label lies in its proven utility for assessing long-term biosynthesis of macromolecules in a variety of species under free-living conditions (for reviews, see Refs. 9 and 15), including obese rodent models (2). Considering the paucity of information on how insulin resistance syndrome affects cumulative turnover rates of proteins in aforementioned compartments, we also sought to characterize basal differences in 24-h protein synthesis rates between phenotypes. Acute RE induces transient changes in protein expression/activation (7), which may be undetectable 16 h following contraction (time of muscle harvest in this study). As such, the objective of our research was not to elucidate the signaling events underlying a potentially divergent anabolic response in insulin-resistant skeletal muscle following contractile activity.

Our global hypothesis was that insulin resistance syndrome would be associated with an attenuated increase in myocellular protein synthesis rates following moderate-intensity RE. In light of aforementioned findings by De Filippis et al. (14), we specifically wanted to test the hypothesis that mitochondria are desensitized to the anabolic effects of RE at the onset of training in the insulin-resistant state. Second, since recent studies indicate that mitochondrial dysfunction per se (e.g., organelle density and/or net lipid oxidation rates) may not underlie insulin resistance syndrome (32), we hypothesized that basal protein synthesis rates of the organelle would not be compromised in the obese Zucker rat.

MATERIALS AND METHODS

Animals. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Texas A & M University. The primary events of insulin resistance syndrome are overeating and inactivity, irrespective of origin(s) (genetic vs. environmental), making the obese Zucker rat, which carries a missense mutation in the leptin receptor gene, a suitable model for studying the effects of insulin resistance syndrome on protein synthesis. Similar to type 2 diabetic patients with metabolic syndrome, these animals exhibit hyperphagia, hyperinsulinemia (see Table 1 for insulin/glucose levels), hyperleptinemia, hypertriglyceridemia, hypercholesterolemia, glucose intolerance, insulin resistance, angiopathy, and neuropathy (50).

To this end, thirty 4-mo-old male obese (*fa/fa*, Crl: ZUC-Lepr$^{fa/fa}$) and lean (*Fa/?*, Crl: ZUC-Lepr$^{Fa/?}$) Zucker rats (Charles River Laboratories, Wilmington, MA) were housed individually in a climate-controlled environment with a reversed 12:12-h light-dark cycle, having free access to water and standard commercial rat chow (Harlan Teklad 2016 Global). Following 1 wk of acclimation, the rats were matched according to body composition [GE Lunar Prodigy dual-energy X-ray absorptiometry (small animal software); total body, lean, fat, and bone mass] within each phenotype and grouped thereafter into sedentary [fatty-SED (FS; $n = 6$) and lean-SED (LS; $n = 8$)] and resistance-exercised [fatty-RE (FE; $n = 8$) and lean-RE (LE; $n = 8$)] cohorts (Table 1). As expected, obese rats had a significantly higher percent fat and bone mass compared with the lean phenotype ($P < 0.001$) while exhibiting significantly depressed percent lean mass ($P < 0.001$). No differences in dual-energy X-ray absorptiometry-derived parameters were present between experimental conditions within phenotypes.

Experimental protocol. For this study, we employed a well-established (20–23, 25, 26, 28, 29, 48, 65) lower body RE model to augment protein synthesis rates in rat skeletal muscle. Animals assigned to the exercise condition were operantly conditioned (OC) in a

Table 1. *Baseline body composition, endpoint gastrocnemius muscle data, and blood insulin/glucose levels*

	FS ($n = 6$)	FE ($n = 8$)	LS ($n = 8$)	LE ($n = 8$)	Phenotype	RE	Phenotype × RE
DEXA							
Body mass, g	618 ± 24[a]	586 ± 20[a]	384 ± 9[b]	383 ± 6[b]	<0.001	NA	NA
Bone mass, g	18 ± 0.7[a]	17 ± 0.4[a]	11 ± 0.2[b]	11 ± 0.1[b]	<0.001	NA	NA
Lean mass, g	222 ± 7[a]	229 ± 13[a]	313 ± 7[b]	313 ± 5[b]	<0.001	NA	NA
Fat mass, g	376 ± 18[a]	340 ± 8[b]	61 ± 6[c]	60 ± 2[c]	<0.001	NA	NA
%Fat mass	63 ± 1[a]	60 ± 1[a]	16 ± 1[b]	16 ± 1[b]	<0.001	NA	NA
Gastrocnemius							
Wet mass, g	1.47 ± 0.07[a]	1.41 ± 0.04[a]	1.83 ± 0.01[b]	1.79 ± 0.05[b]	<0.001	0.248	0.797
Protein content							
Total, mg	259 ± 13[a]	254 ± 14[a]	330 ± 12[b]	329 ± 22[b]	<0.001	0.872	0.919
Myo, mg	185 ± 13[a]	169 ± 13[a]	237 ± 9[b]	236 ± 17[b]	<0.001	0.547	0.571
Cyto, mg	71 ± 7[a]	82 ± 6[ab]	91 ± 7[b]	91 ± 6b	0.04	0.417	0.366
Protein concentration							
Total, μg/mg	176 ± 10[a]	173 ± 7[a]	180 ± 6[a]	183 ± 10[a]	0.417	0.995	0.725
Myo, μg/mg	126 ± 10[a]	115 ± 5[a]	129 ± 5[a]	131 ± 8[a]	0.194	0.525	0.344
Cyto, μg/mg	48 ± 4[a]	57 ± 5[a]	50 ± 4[a]	50 ± 2[a]	0.552	0.251	0.304
Blood							
Insulin, mU/ml	110 ± 24[a]	126 ± 18[a]	20 ± 5[b]	17 ± 5[b]	<0.001	0.785	0.664
Glucose, mg/dl	159 ± 18[a]	150 ± 19[a]	121 ± 11[a]	129 ± 8[a]	0.248	0.971	0.731

Values are means ± SE. RE, resistance exercised; FS, fatty sedentary; FE, fatty RE; LS, lean sedentary; LE, lean RE; NA, not applicable; DEXA, dual-energy X-ray absorptiometry; Cyto, cytosolic; Myo, myofibrillar. Body composition obtained by DEXA (baseline). Lean and obese Zucker rats ($n = 30$) were matched according to lean body mass within each phenotype and grouped into sedentary [LS ($n = 8$) and FS ($n = 6$)] and RE [LE ($n = 8$) and FE ($n = 8$)] cohorts. Omnibus P value from 1-way ANOVA is listed under the "Phenotype" column. Wet mass and subfractional protein contents/concentrations (total = mixed) of gastrocnemius muscle are obtained at end point. Main effects (phenotype and RE) and interaction (phenotype × RE) from 2-way ANOVA are noted for each variable when applicable. Group means that are significantly different according to Student-Newman-Keuls post hoc analyses or t-tests do not share the same letter ($P \leq 0.05$). Fasted plasma insulin and glucose concentrations from FE, FS, LS, and LE rats were obtained on the final day of experiments. ANOVA statistics were applied as above. Group means that are significantly different according to Student-Newman-Keuls post hoc analyses or t-tests do not share the same letter ($P \leq 0.05$).

dark room to depress an illuminated bar in a Plexiglas exercise cage while wearing a Velcro vest adjustable for progressive increases in weights. Negative reinforcement via an electrical foot shock (<1 mA, 60 Hz) was used to train the rats to perform the desired movement, which resembles a traditional leg squat as performed by humans and requires full extension of hind legs.

Following six OC sessions over a 2-wk period, entrained rats of both phenotypes underwent a voluntary lower body muscular endurance paradigm consisting of four progressive sessions separated by either 48 (RE1–RE3) or 72 h (RE3–RE4) (Fig. 1). The RE cohorts performed the same number of sets and repetitions (reps) per weight and were allowed 2 s of rest between reps and 1 min of rest between sets (~5 sets × 15 reps/session). Weights were matched between phenotypes, and the work intensity was increased progressively throughout the study period (30–230 g RE1, 80–230 g RE2, 80–230 g RE3, and 80–280 g RE4). In relative terms, lean and obese rats lifted ~0.10–0.75 and ~0.05–0.45 g backpack wt·g body wt^{-1}·$session^{-1}$, respectively, which has previously been described as low to moderate intensity for this exercise model (21). When expressed relative to total muscle mass, lean and obese rats lifted ~0.10–0.90 and ~0.15–1.25 g backpack wt·g lean $mass^{-1}$·$session^{-1}$, respectively. Although it is difficult to engage obese animals in voluntary exercise without compromising the quality of movements or significantly reducing exercise intensities, the obese phenotype handled the same absolute workload at minimal expense to movement quality compared with its lean littermate. To account for possible confounding effects of stress on muscle metabolism, negative reinforcement was matched between groups following each OC/RE session. Each animal received a total of 88 shocks (<1 mA, 60 Hz) regardless of phenotype (lean and obese) and activity (RE vs. sedentary). The duration of shock treatment lasted less than ~0.2 s per negative reinforcement, which amounted to a total of ~18 s of administration over the study period.

To measure protein synthesis rates in skeletal muscle of sedentary and exercised cohorts, an intraperitoneal injection of 99.9% ^2H_2O (20 μl/g body wt; Cambridge Isotopes, Andover, MA) was administered to each animal 24 h prior to euthanization, and 4% ^2H_2O was provided ad libitum in the drinking water for the entire 24-h period. In accord with previously published work (20), the final exercise bout (RE4) was completed 16 h prior to muscle harvest, and rat chow was withdrawn 4 h prior to muscle excision. On the morning of euthanization, animals were anesthetized [ketamine hydrochloride/Ketaset (37.5 mg/kg body wt) and medetomidine/Domitor (0.25 mg/kg body wt)], and 2 ml of whole blood was collected by cardiac puncture. This was followed by quick excision of gastrocnemius muscle and euthanasia. Fat, blood, and connective tissue were removed from muscles before being snap-frozen in liquid nitrogen, pulverized, and stored at −80°C.

^2H_2O enrichment of body water/plasma. Labeling (^2H) of the precursory pool [e.g., body water (E_{BW})] was measured by gas chromatography-mass spectroscopy (GC-MS) (Agilent 7890 GC/5975 VL MSD) following 24-h isotopic exchange between ^2H_2O-enriched plasma samples and acetone, a method allowing for as low as 0.008% ^2H_2O enrichments of body water to be assayed (67). Briefly, 20 μl of calibration standards (0–5% ^2H_2O, prepared by mixing naturally labeled water with 99.9% ^2H_2O) and plasma samples were allowed to react for 24 h at room temperature with 2 μl of 10 N NaOH and 4 μl of a 5% (vol/vol) solution of acetone in acetonitrile. After the 24-h incubation period, the solution was extracted by adding 0.5 g of Na_2SO_4 followed by 600 μl of chloroform and vigorous vortex. A small aliquot (50–100 μl) was transferred to a GC-MS vial and automatically injected (1 μl) into an Agilent 7890A GC system, volatilized, and separated on a capillary column (HP-5 ms, 30 m × 0.25 mm × 0.24 μm), using helium as the carrier gas (1 μl/min) at a 40:1 split ratio. The GC injector temperature was set at 220°C, and the transfer line was held at 220°C. The initial temperature of the column program was 60°C, followed by an increase of 20°C/min to 100°C, which was further increased to 220°C at a rate of 50°C/min and held constant for 1 min. Acetone eluted from the column ~1.7 min postinjection and was ionized with an Agilent 5975C VL MSD operating in electron impact mode using an ionization energy of 70 eV. Selective ion monitoring of mass-to-charge ratios 58 (M) and 59 (M + 1) using a dwell time of 10 ms/ion were conducted after autotune. Peak abundances of ions 58 and 59 were extracted from chromatograms, and M + 1/M ratios were used to calculate percent enrichment of body water based on a linear regression formula generated by deuterium oxide standards (r^2 = 0.999). ^2H-labeling of body water was readily detected in both phenotypes (3.04 ± 0.11%, means ± SE), with an average of 3.05 ± 0.17% in sedentary groups and 3.04 ± 0.15% in resistance-exercised groups. All plasma samples were measured twice with separate preparations, and an average value of the two runs was used for all calculations.

[^2H]alanine enrichment in skeletal muscle. Enrichments of mixed and myofibrillar-, cytosolic-, and mitochondria-rich fractions in gastrocnemius muscle (mixed fiber types) were determined by measuring protein-bound [^2H]alanine (E_A), as described by Dufner et al. (16). Pulverized mixed muscle (30 mg) or isolated subfractions (obtained from 100 mg of mixed muscle) were homogenized in 0.3 ml of a 10% (wt/vol) TCA solution and centrifuged at 800 *g* for 15 min at 4°C. For each sample, the supernatant was discarded and the pellet centrifuged and washed three additional times in TCA to remove unbound amino acids. After the wash steps, the protein-rich pellet was hydrolyzed for 24 h in 6 N HCl at 110°C [~0.13 ml/0.01 g tissue; 400 μl for all fractions except mitochondria (200 μl)]. A 100-μl aliquot of the hydrolysate (50 μl for mitochondria) was dried down for 1 h at 110°C and derivitized thereafter with a 3:2:1 (vol/vol/vol) solution of methyl-8, methanol, and acetonitrile for 1 h at 70°C (1 μl/1 μl hydrolysate). The resulting methyl-8-[^2H]alanine derivative was transferred to a GC-MS vial and analyzed with an Agilent 5975C VL MSD equipped with an Agilent 7890A GC system (HP-5 ms capillary column, 30 m × 0.25 mm × 0.24 μm) to determine ^2H labeling of protein-bound alanine. The initial temperature of the column program was set at 90°C and held for 5 min and increased by 5°C/min to 130°C, which was further

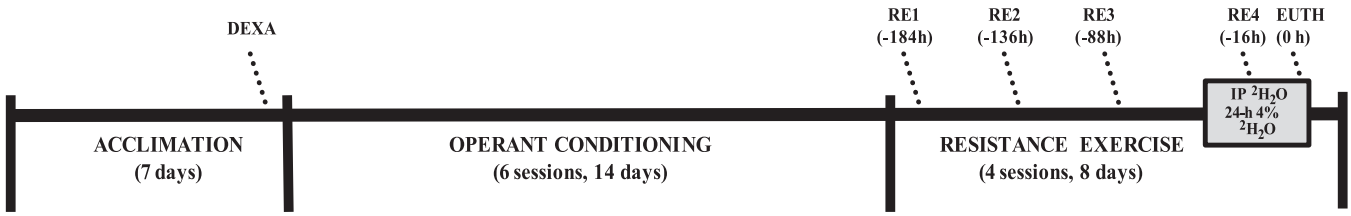

Fig. 1. Schematic display of the study design. After 1 wk of acclimation, rats in resistance exercise (RE) groups were operantly conditioned to perform a squat-like movement while wearing a Velcro vest adjustable for progressive increases in weights. All entrained rats underwent the same resistance exercise paradigm with minimal negative reinforcement, which consisted of 4 progressive sessions separated by either 48 (RE1–RE3) or 72 h (RE3–RE4). The final exercise bout (RE4) was completed 16 h prior to muscle harvest. To measure cumulative muscle protein synthesis, an intraperitoneal (IP) 99.9% ^2H_2O bolus injection was administered 24 h prior to euthanization (EUTH), and 4% ^2H_2O was provided ad libitum in the drinking water throughout the last day. DEXA, dual-energy X-ray absorptiometry.

increased at a rate of 40°C/min to 240°C and held for 5 min, with all steps performed at a constant helium flow of 1 ml/min. Peak abundances of ions 99 and 100 were extracted from chromatograms, and M + 1/M ratios were used to calculate percent enrichment of protein-bound alanine using a regression formula generated by [^{2}H]alanine standards ($r^2 = 0.999$). ^{2}H labeling of alanine was readily detected in all subfractions, using a 20:1 split ratio for mixed proteins, a 10:1 split ratio for myofibrillar and cytosolic proteins, and a 5:1 split ratio for mitochondrial proteins. All samples were analyzed in triplicate.

Calculations. FSR of mixed, myofibrillar, cytosolic, and mitochondrial proteins were calculated using the equation $E_A \times [E_{BW} \times 3.7 \times t\ (h)]^{-1} \times 100$, where E_A represents amount of protein-bound [^{2}H]alanine (mole% excess), E_{BW} is the quantity of ^2H_2O in body water (mole% excess), and 3.7 represents the exchange of ^{2}H between body water and alanine [e.g., 3.7 of 4 carbon-bound hydrogens of alanine exchange with water (16)].

Isolation of skeletal muscle subfractions. The differential centrifugation protocols for the isolation of cytosolic proteins/subsarcolemmal (SS) mitochondria and myofibrillar proteins/intermyofibrillar (IMF) mitochondria were adapted from Rooyackers et al. (54) and Wilkinson et al. (66), respectively. Purity of subfractions was verified with Western blotting (Supplemental Figs. S1 and S2; Supplemental Material for this article can be found on the *AJP-Endocrinology and Metabolism* web site), and protein concentrations/total protein contents were assessed using a commercially available colorimetric assay (58) and calculated as described previously (27).

Cytosolic proteins/SS mitochondria. Briefly, a 10% homogenate using 100 mg of gastrocnemius muscle was prepared in ice-cold mitochondrial isolation *buffer 1* (10 mM HEPES, 200 mM sucrose, 50 mM mannitol, 2 mM EDTA disodium salt, Sigma P8340 protease inhibitor cocktail, pH 7.4) using a glass pestle fitted for round-bottom microcentrifuge tubes (25 compressive "Dounce" strokes). SS mitochondria were isolated by spinning the homogenate at 600 *g* (10 min at 4°C), followed by a 10,000-*g* spin (10 min at 4°C) of the obtained supernatant. We have previously found no differences in basal protein synthesis rates or the contractile activity-induced anabolic response between SS and IMF mitochondria in rat skeletal muscle using the ^2H_2O method (results available upon request). As such, the SS mitochondrial pellet was washed twice in mitochondrial isolation *buffer 2* (50 mM HEPES, 5 mM EGTA, 1 mM ATP, 100 mM KCl, 5 mM $MgSO_4$, Sigma P8340 protease inhibitor cocktail, pH 7.4), once in 95% ethanol, and combined with the IMF population (see below) for assessment of protein synthesis rates. The remaining supernatant, containing cytosolic proteins, was precipitated with 95% ethanol [50% (vol/vol) in total solution] during low-speed centrifugation at 700 *g* (10 min at 4°C) and stored at −80°C for FSR analyses.

Myofibrillar proteins/IMF mitochondria. Briefly, the pellet obtained from the original homogenate was washed in mitochondrial isolation *buffer 3* (100 mM KCl, 50 mM Tris, 5 mM $MgCl_2$ hexahydrate, 1 mM EDTA disodium salt, 10 mM β-glycerophosphate disodium salt, 50 mM NaF, 1.5% BSA, Sigma P8340 protease inhibitor cocktail, pH 7.5) and centrifuged at 650 *g* (3 min at 4°C). IMF mitochondria were isolated from the myofibrillar-rich pellet using mechanical means (50 shear/compressive strokes) rather than proteolysis to avoid possible degradation artifacts (54). The homogenate was spun at 650 *g* (4 min at 4°C) and the supernatant respun at 10,000 *g* (10 min at 4°C) to collect the organelle-rich pellet. IMF mitochondria were washed as described previously and combined with the SS population.

To extract myofibrillar proteins, the remaining pellet was washed in mitochondrial isolation *buffer 4* (same as *buffer 3* but without BSA) and centrifuged twice at 1,200 *g* (10 min at 4°C), followed by the addition of 0.3 M NaOH and heating of the sample (30 min at 37.5°C). Collagen and myofibrils were separated by centrifugation at 4,000 *g* (10 min at 4°C), followed by precipitation of proteins in 1 M PCA at 650 *g* (10 min at 4°C). The myofibrillar precipitate was washed in ethanol and stored at −80°C for FSR analyses.

Statistical analyses. The effects of phenotype (obese vs. lean) and physical activity (sedentary vs. RE) on expression and synthesis of proteins were assessed with 2 × 2 ANOVA using SigmaStat version 3.5. When significant *F* ratios were present, a Student-Newman-Keuls post hoc procedure was used to evaluate differences among group means. If homogeneity of data failed, analyses of variance were conducted on transformed values (square root) and statistical comparisons between two independent group means were completed with Mann-Whitney-Wilcoxon rank sum tests. Statistical significance was set at $P \leq 0.05$ (denoted alphabetically in the figures) for omnibus *F*-tests, post hoc analyses, and a priori tests. *P* values for trending data ($P > 0.05 \leq 0.1$) are included in the figure legends as a convenience to the reader. All data are presented as means ± SE.

RESULTS

Mitochondrial protein content is normal in gastrocnemius muscle of the obese Zucker rat. As expected, gastrocnemius wet mass and total protein content, but not protein concentration, were significantly suppressed in the obese phenotype ($P < 0.05$), and RE did not significantly affect muscle weights or protein contents/concentrations in either cohort (Table 1). The ~22% difference in mixed protein content of gastrocnemius muscle between phenotypes ($P < 0.001$) was attributed primarily to ~25% lower myofibrillar protein content ($P < 0.001$, 176 ± 9 and 236 ± 9 mg in obese vs. lean rats, respectively) and secondarily to ~15% lower cytosolic protein content ($P = 0.04$, 77 ± 5 and 91 ± 5 mg in obese vs. lean rats, respectively) in obese rats. The most interesting and novel finding in this study, with regard to muscle composition, was that total mitochondrial protein content in gastrocnemius muscle was not significantly different between phenotypes (2.73 ± 0.23 and 2.57 ± 0.23 mg in obese vs. lean, respectively; Fig. 2*A*) due to an ~31% higher mitochondrial density/concentration in obese rats ($P = 0.01$, 1.86 ± 0.15 and 1.42 ± 0.07 μg/mg in obese vs. lean, respectively; Fig. 2*B*). Taken altogether, these results are suggestive of an augmented expression of oxidative enzymes [as shown by Holloway et al. (32)] at the expense of expressing contractile proteins in the obese state.

Cumulative synthesis rates of mitochondrial and cytosolic proteins are normal or augmented in gastrocnemius muscle of the obese Zucker rat. The obese phenotype displayed a mild, but significant, suppression of 24-h protein synthesis rates in mixed and myofibrillar subfractions (−11%, $P = 0.015$; and −12%, $P = 0.004$, respectively), whereas cytosolic and mitochondrial FSR were significantly higher (+12%, $P = 0.045$) and not statistically different (−7%, $P = 0.37$), respectively, from lean rats (Figs. 3 and 4). Since there was no statistical difference in mixed FSR between sedentary groups ($P = 0.437$, FS vs. LS), the observed phenotypic suppression was attributed partly to a modest exercise effect in the lean cohort ($P = 0.165$, 4.62 ± 0.22 and 5.00 ± 0.18%/day in LS vs. LE, respectively) (Fig. 3). Myofibrillar FSR, on the other hand, was significantly repressed regardless of experimental condition in the obese phenotype (2.80 ± 0.07 and 3.18 ± 0.10%/day in fatty vs. lean, respectively; Fig. 4*A*), further supporting the notion of a preferential downregulation in the manufacture of contractile proteins to maintain/augment production of metabolic enzymes.

The anabolic response of mitochondria is blunted in gastrocnemius muscle of the obese Zucker rat. Synthesis rates of pooled mitochondrial populations were significantly higher in

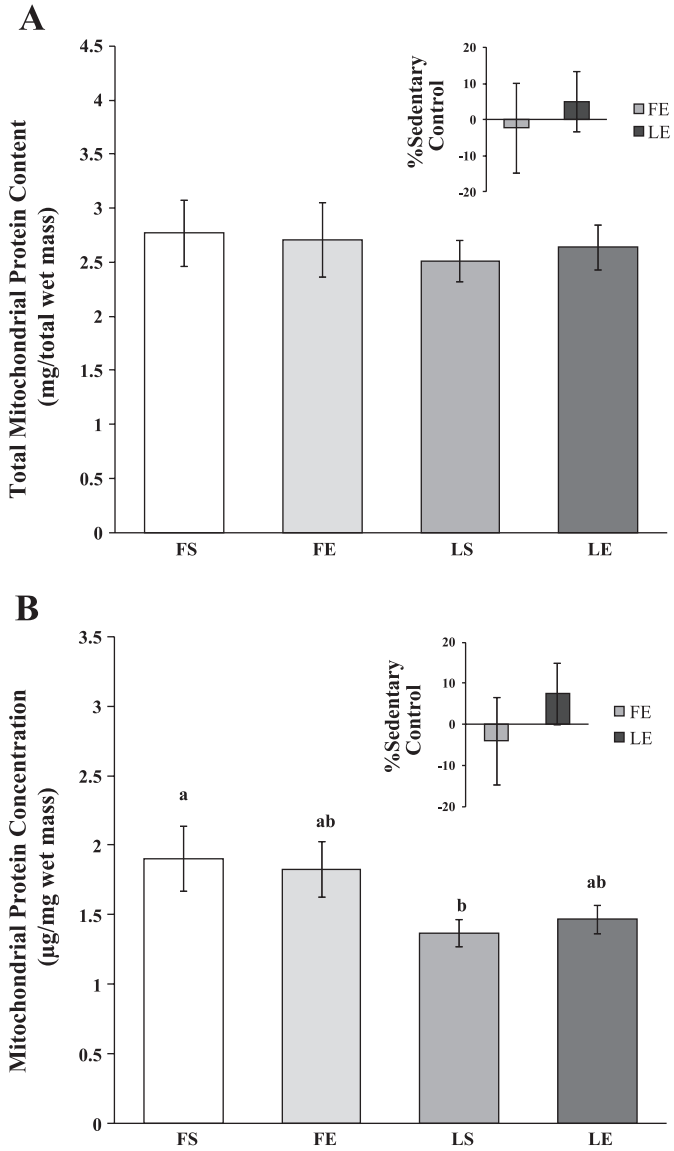

Fig. 2. Mitochondrial protein content (*A*) and protein concentration (*B*) of mixed gastrocnemius muscle in lean and obese Zucker rats. *A*: mitochondrial protein content. No main effects of phenotype ($P = 0.556$), RE ($P = 0.908$), or interaction (phenotype × RE; $P = 0.727$). *B*: mitochondrial protein concentration. Main effect of phenotype ($P = 0.01$), but not RE ($P = 0.948$), or interaction (phenotype × RE; $P = 0.582$). Fatty-sedentary (FS) vs. lean-sedentary (LS) rats, $P = 0.032$; FS vs. lean-RE (LE) rats, $P = 0.084$; fatty-RE (FE) vs. LS, $P = 0.083$; FE vs. LE, $P = 0.115$. Values (means ± SE) are expressed in mg/total wet mass (*A*) and μg/mg wet mass (*B*). Main effects and interaction were evaluated with 2-way ANOVA. Group means that are significantly different according to Student-Newman-Keuls (SNK) post hoc analyses or *t*-tests do not share the same letter ($P \leq 0.05$). *Insets*: RE vs. sedentary control within phenotype (%difference).

exercised vs. sedentary lean rats (+28%, $P = 0.032$, 4.81 ± 0.36 and 6.16 ± 0.52%/day in LS vs. LE, respectively) but not statistically different between corresponding obese groups (+14%, $P = 0.25$, 4.77 ± 0.35 and 5.44 ± 0.32%/day in FS vs. FE, respectively), indicative of an impaired anabolic response of mitochondria in the obese state. Although there was no statistically significant effect of RE on mixed, cytosolic, or myofibrillar protein pools in either phenotype (mixed fiber type gastrocnemius), the current exercise paradigm induced a potent anabolic response in fast fiber type gastrocnemius (mixed FSR) and in mixed fiber type quadriceps (myofibrillar and cytosolic FSR). Consistent with our findings in pooled mitochondria, exercised obese rats were unable to augment protein synthesis rates to the same extent as lean rats in aforementioned subfractions (Supplemental Fig. S3, *A–C*).

DISCUSSION

Although the long-term therapeutic benefits of regular physical activity are well documented in metabolic disease conditions, surprisingly few studies, if any, have compared the protein-synthetic response between insulin-sensitive and insulin-resistant skeletal muscle following exercise at the onset of training. To our knowledge, this is the first study to address whether or not insulin resistance syndrome alters the adaptive response of subcellular compartments that regulate metabolism and energy utilization in skeletal muscle following contractile activity. Our findings support the contention that insulin resistance syndrome is associated with a "desensitization" of skeletal muscle to the anabolic effects of contractile activity, as evidenced by a blunted increase in cumulative synthesis rates (24-h FSR) of mitochondrial (gastrocnemius), cytosolic (quadriceps), and myofibrillar (quadriceps) proteins of resistance-exercised obese Zucker rats. We demonstrate further that mitochondrial protein synthesis rates and total mitochondrial content are normal in unexercised skeletal muscle of the same rat strain.

Insulin resistance syndrome blunts the contractile activity-induced anabolic response of mitochondria in the obese Zucker rat. Although the current exercise protocol augmented protein synthesis rates of all myocellular subfractions, the exercise response, or lack thereof, was not necessarily uniform between muscle groups or detectable in mixed muscle homogenates. An effort should be made to assess turnover rates of specific myocellular protein pools (or individual proteins) since significant perturbations may be present in these subfractions despite

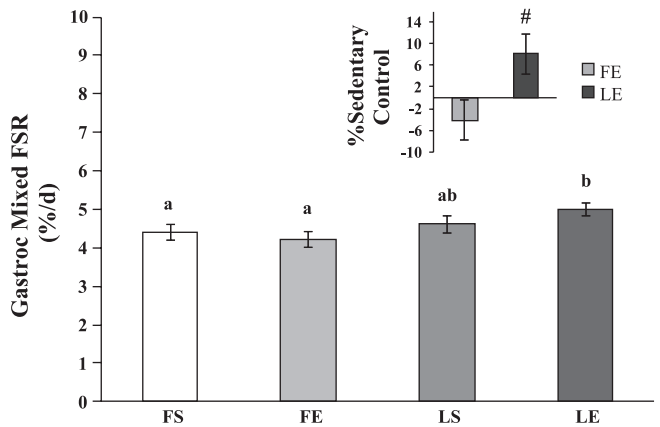

Fig. 3. Mixed fractional protein synthesis rates (FSR) of gastrocnemius muscle (cumulative 24-h FSR) in lean and obese Zucker rats. Main effect of phenotype ($P = 0.015$); FE vs. LE, $P = 0.006$; FE vs. LS, $P = 0.153$; FS vs. LE, $P = 0.049$; LS vs. LE, $P = 0.165$. Two-way analyses of variance indicated no main effect of RE ($P = 0.624$) or interaction between RE and phenotype ($P = 0.16$). Bars/groups sharing the same letter are not significantly different ($P > 0.05$) according to SNK post hoc analyses or *t*-tests. Data are expressed as %/day and presented as means ± SE. *Inset*: RE vs. sedentary control within phenotype (%difference). #Significantly higher compared with exercised group in other phenotype.

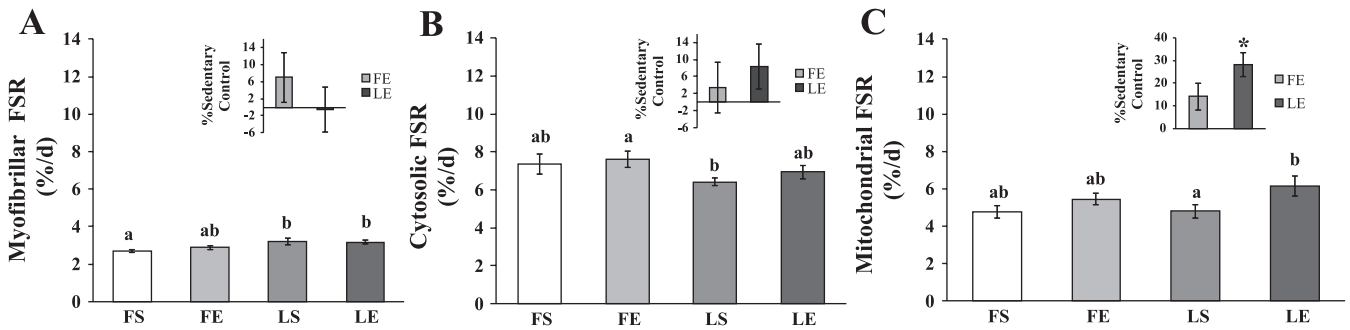

Fig. 4. Subfractional protein synthesis rates of gastrocnemius muscle (cumulative 24-h FSR) in lean and obese Zucker rats. *A*: myofibrillar FSR. Main effect of phenotype ($P = 0.004$); FS vs. LS, $P = 0.012$; FS vs. LE, $P = 0.007$; FE vs. LS, $P = 0.130$; FE vs. LE, $P = 0.100$. *B*: cytosolic FSR. Main effect of phenotype ($P = 0.045$); FS vs. LS, $P = 0.100$; FE vs. LS, $P = 0.027$. *C*: mitochondrial FSR. Main effect of RE ($P = 0.022$); FS vs. LE, $P = 0.065$; FE vs. LE, $P = 0.198$; FS vs. FE, $P = 0.248$; LS vs. LE, $P = 0.032$. Bars/groups sharing the same letter are not significantly different ($P > 0.05$) according to SNK post hoc analyses or *t*-tests. Data are expressed as %/day and presented as means ± SE. *Insets*: RE vs. sedentary control within phenotype (%difference). *Significantly higher compared with unexercised group within phenotype.

subtle changes in a mixed protein pool. Considering the putative role of mitochondria in the development and progression of metabolic disease, the most interesting finding of this study was that the mitochondrial anabolic response was blunted in the insulin-resistant state. Long-standing metabolic disease is associated with morphological changes of mitochondria in humans (39) and obese Zucker rats (4), which may alter the organelle's typical adaptive response to exercise (34). De Filippis et al. (14) reported recently that human obesity is associated with an attenuated increase in nuclear-encoded mitochondrial genes following acute endurance exercise, suggestive of an impairment in the manufacturing process of mitochondrial proteins. Since mitochondrial FSR, as measured in our study, represents [^{2}H]alanine incorporation in both nuclear- and mtDNA-encoded proteins over a 24-h period, any defects in the replication, transcription, or translation of either genome could theoretically have attributed to a blunted anabolic response in the obese phenotype. Considering that the vast majority of mitochondrial matrix and membrane proteins are encoded by nuclear DNA [only 13 of ~1,000–2,000 proteins are encoded by the organelle's own genome (55)], in addition to the fact that replication, transcription, and translation of mtDNA are controlled ultimately by nuclear-encoded proteins (19), we believe that insulin resistance syndrome is associated with multiple dysfunctions in the manufacture and/or import of nuclear proteins, leading to an attenuated anabolic response of mitochondria following acute contractile activity. Because of the necessity of a 24-h ^2H_2O enrichment period, the signal transduction events underlying this phenomenon remain to be elucidated. Emerging evidence for the role of the evolutionary conserved TOR pathway in the regulation of mitochondrial biogenesis (6, 13, 56) indicates that raptor-mediated activation of mTOR is imperative to maintain mitochondrial morphology and function. Previous research has shown that the obese Zucker rat exhibits a blunted activation of the Akt/mTOR/p70^{S6K} pathway 1–3 h following high-force electrical stimulation, suggesting that global synthesis rates, including mitochondrial FSR, are attenuated compared with the lean phenotype (37). Considering that translation of the vast majority of mitochondrial proteins is ultimately under the control of mTOR signaling, it is likely that an alteration in this pathway could have a fundamental impact on mitochondrial FSR following exercise.

Insulin resistance syndrome does not impair synthesis rates of mitochondrial or cytosolic proteins in the obese Zucker rat. Many of the metabolic aberrations associated with insulin resistance syndrome would be expected to cause an abnormal protein turnover, but few studies have elucidated the effect(s) of insulin resistance syndrome on intracellular mechanisms governing skeletal muscle growth. The atrophic phenotype of the obese Zucker rat is consistent with an altered lean mass distribution in type 2 diabetics (upper and lower body) (11, 31) and has been attributed to an increased rate of protein degradation (3), reduced rate of protein synthesis (18), and suppressed satellite cell function (52). Since the phenotype is well established at 21–27 days postpartum (53), we believe that aforementioned regulatory mechanisms should be preferably assessed in weanling rats. Granted that differences in muscle mass may become greater between phenotypes with advancing age, acquired deficits in lean mass appear to remain relatively constant once animals reach maturity, indicating that the rate of muscle loss stabilizes/diminishes in adulthood. Therefore, processes that regulate muscle growth do not necessarily continue to deteriorate at the same rate as those observed in weanling or juvenile obese rats (53). Our results, along with previous reports on protein turnover in this rat strain, indicate that phenotypic differences in protein synthesis rates become less with age (53) and may normalize (44) or even supercompensate (for defects in other regulatory processes) (25) in maturity. Although satellite cell proliferation/differentiation and protein degradation rates were not assessed directly in this study, we observed a substantial increase in atrogin-1 expression (E3 ligase of the ubiquitin-proteasome proteolytic pathway) in the obese vs. lean phenotype (Supplemental Fig. S4), suggesting that other mechanisms "drive" the loss of skeletal muscle in obese rats in adulthood.

Most of the current knowledge pertaining to protein metabolism in the obese phenotype is limited to the mixed protein pool. As such, an important goal of our study was to elucidate whether insulin resistance syndrome is associated with a uniform perturbation in FSR or whether specific subfractions are preferentially affected over others depending upon biological function [e.g., substrate metabolism (cytosol/mitochondria) vs. contractile activity (myofibrils)]. Although the results generally corroborate earlier work that assessed protein synthesis in

this rat strain (18, 24, 53), we have extended previous observations by demonstrating that the suppression is significantly less than reported previously (in younger rats) and isolated to subfractions involved in contractile function rather than substrate metabolism. Our findings are consistent with those of Fluckey et al. (25), who showed an augmentation in mixed muscle protein synthesis rates in 3-mo-old obese rats in situ, and those of Bell et al. (5), who reported an increased protein turnover in type 2 diabetic humans. We speculate that manufacture of metabolic proteins is maintained or augmented to deal with an increased nutrient uptake in the obese state, potentially at the expense of synthesizing contractile proteins (in selected muscle groups).

Any discrepancies between our results and those reported previously in the same rat strain are more than likely attributed to differences in the age/developmental stage of animals and the methods to assess protein synthesis rates. In this study, the average synthesis rates of mixed (4.56%/day), myofibrillar (2.99%/day), and cytosolic (7.07%/day) subfractions in gastrocnemius muscle were qualitatively similar to previous observations in humans and rats (i.e., Myo FSR $<$ mixed FSR $<$ cyto FSR). An approximately two- to threefold difference between cytosolic and myofibrillar protein synthesis rates is viewed as normal in the postabsorptive state, and mixed FSR is typically 30–40% higher compared with myofibrillar FSR. As assessed with ^2H_2O over a 24-h period, FSR of pooled mitochondria was ~16% higher compared with mixed proteins (5.30 vs. 4.56%/day) but ~25% lower than cytosolic proteins, indicating that mitochondrial FSR may not be as high as reported previously in rat muscle (36).

Insulin resistance syndrome is associated with an augmented mitochondrial density in the obese Zucker rat. Our findings, along with those of others (4, 32), show unequivocally that the obese phenotype exhibits a robust augmentation in mitochondrial protein concentrations (e.g., density), which normalizes total mitochondrial protein content compared with its lean counterpart, despite having significantly smaller muscles. From a biological standpoint, it makes sense that this rat strain, which displays an elevated intracellular lipid level due to an upregulation of plasmalemmal and mitochondrial FAT/CD36 (32), has more mitochondria per unit of muscle to handle an excess fat uptake. Although we have extended previous findings by showing that 24-h synthesis rates of mitochondrial proteins, which ultimately are rate limiting for biogenesis of the organelle, are not impaired in the obese phenotype, it is paradoxical that an increased organelle density is not accompanied by an augmentation in mitochondrial FSR. The acquisition and maintenance of mitochondrial polypeptide complexes is based on a coordinated interplay between biosynthesis and degradation, with the latter governed by inner membrane proteases m-AAA (facing matrix) and i-AAA (facing intermembrane space) and "chambered" ATP-dependent matrix proteases Lon and mtClpP (for review, see Ref. 64). It is tempting to speculate that the activity/expression of one or more of these proteases is decreased in the obese phenotype, causing an accumulation of aggregation-prone proteins and free radicals within the organelle. Indeed, oxidative stress has been implicated in the etiology of metabolic disease, and previous research has shown that mitochondrial H_2O_2 emission and cellular redox state link excess fat intake to insulin resistance in both humans and rodents (1). Another plausible explanation is that mitochondrial autophagy (mitophagy) is suppressed in the presence of prevailing hyperaminoacidemia in skeletal muscle of the obese Zucker rat (30, 43). Recent findings in Atg-7 knockout mice indicate that a reduction in autophagy may lead to a compensatory FoxO1-mediated upregulation in the ubiquitin-proteasome system (45), which is consistent with an increased expression of atrogenes in the obese phenotype (see Supplemental Fig. S4 and Ref. 3). Although organelle function may be augmented during early developmental stages and/or young adulthood in the obese Zucker rat (32), more research is needed to discern whether or not mitochondrial dysfunction is present in adulthood following several months of inactivity/overconsumption of nutrients, which is the case in other rodent models (8).

The ultrastructural data obtained in the current study are in agreement with previous observations showing no differences in mixed muscle protein concentrations between phenotypes (18) but a significantly lower total protein content attributed to a loss of contractile and cytosolic proteins in obese rats (57). Although the relative contribution of myofibrillar (~55–65%), stromal (~10–15%), and cytosolic fractions (~20–30%) to the mixed protein pool compares well with other reports (42), the contribution of mitochondrial proteins has not been addressed previously. Values obtained from sedentary obese rats (1.1%) are within the normal range of those reported previously in untrained human skeletal muscle (1–3%) (33), suggesting that our mitochondrial isolation protocol was effective.

Summary and conclusion. Our data strongly suggest that 24-h protein synthesis rates are near normal or augmented in myocellular compartments containing oxidative and glycolytic enzymes (mitochondria and cytosol) but may be relatively repressed in subfractions involved in energy utilization and muscle contraction (myofibrils) in the obese Zucker rat. To our knowledge, this is the first study to demonstrate that insulin resistance syndrome is not associated with a reduction in mitochondrial protein synthesis rates in rat muscle, perhaps at the expense of manufacturing contractile proteins. Although our data do not allow us to describe the underlying mechanism(s) for our findings, results from the present study may indicate that translational capacity (total mRNA and/or ribosome content) and/or translational efficiency (number of transcripts translated for any given mRNA/ribosome) of specific mRNA pools are altered in chronically obese states to cope with nutrient overload. Whether translation of specific mRNA pools is determined exclusively by the availability of transcripts and/or whether there is a preferential regulation of what transcripts are targeted for translation remain to be elucidated.

Although there is growing evidence that chronic resistance exercise is equally effective compared with aerobic training in metabolic disease management, the optimal frequency, intensity, and duration to achieve therapeutic goals are not known. Considering the putative role of mitochondria in the development and progression of insulin resistance syndrome, the finding that the cumulative anabolic response of the organelle is blunted following contractile activity in the insulin-resistant state may be of clinical relevance. Alternative implementation strategies of traditional exercise protocols may be necessary for insulin-resistant individuals to achieve similar improvements in mitochondrial biogenesis compared with healthy subjects. In conclusion, this study extends previous research by showing that mitochondrial protein synthesis

rates, which ultimately regulate biogenesis of the organelle, are not necessarily impaired in chronically obese states but may be desensitized to contractile-induced stimuli.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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