Endurance-Type Exercise Increases Bulk and Individual Mitochondrial Protein Synthesis Rates in Rats

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Endurance-Type Exercise Increases Bulk and Individual Mitochondrial Protein Synthesis Rates in Rats

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Physical activity increases muscle protein synthesis rates. However, the impact of exercise on the coordinated up- and/or downregulation of individual protein synthesis rates in skeletal muscle tissue remains unclear. The authors assessed the impact of exercise on mixed muscle, myofibrillar, and mitochondrial protein synthesis rates as well as individual protein synthesis rates in vivo in rats. Adult Lewis rats either remained sedentary ($n = 3$) or had access to a running wheel ($n = 3$) for the last 2 weeks of a 3-week experimental period. Deuterated water was injected and subsequently administered in drinking water over the experimental period. Blood and soleus muscle were collected and used to assess bulk mixed muscle, myofibrillar, and mitochondrial protein synthesis rates using gas chromatography–mass spectrometry and individual muscle protein synthesis rates using liquid chromatography–mass spectrometry (i.e., dynamic proteomic profiling). Wheel running resulted in greater myofibrillar (3.94 ± 0.26 vs. $3.03 \pm 0.15\%/day$; $p < .01$) and mitochondrial (4.64 ± 0.24 vs. $3.97 \pm 0.26\%/day$; $p < .05$), but not mixed muscle (2.64 ± 0.96 vs. $2.38 \pm 0.62\%/day$; $p = .71$) protein synthesis rates, when compared with the sedentary condition. Exercise impacted the synthesis rates of 80 proteins, with the difference from the sedentary condition ranging between -64% and $+420\%$. Significantly greater synthesis rates were detected for F1-ATP synthase, ATP synthase subunit alpha, hemoglobin, myosin light chain-6, and synaptopodin-2 ($p < .05$). The skeletal muscle protein adaptive response to endurance-type exercise involves upregulation of mitochondrial protein synthesis rates, but it is highly coordinated as reflected by the up- and downregulation of various individual proteins across different bulk subcellular protein fractions.

Keywords: deuterium oxide, dynamic proteomic profiling, muscle, muscle protein synthesis, physical activity

Skeletal muscle adaptation is regulated by the balance between protein synthesis and protein breakdown rates. Muscle protein fractional synthesis rates (FSRs) can be determined by administration of stable isotope labeled amino acids and the subsequent measurement of their incorporation into muscle protein. In the 1990s, investigators first applied basic extraction techniques to show differences between bulk myofibrillar and mitochondrial protein synthesis rates in resting skeletal muscle tissue (Rooyackers et al., 1996). This approach was subsequently applied to demonstrate that, for example, endurance-type exercise more robustly increases mitochondrial protein synthesis rates (Wilkinson et al., 2008), whereas resistance-type exercise strongly increases myofibrillar protein synthesis rates (Burd

et al., 2010a, 2010b; Moore et al., 2009). Even more detailed insight into individual protein translational responses has been obtained by performing two-dimensional gel electrophoresis before measuring labeled amino acid incorporation (Balagopal et al., 1994, 1997a). Using this approach, synthesis rates of key myofibrillar (Balagopal et al., 1997a, 1997b; Hasten et al., 1998) and several mitochondrial proteins (Hesketh et al., 2016; Jaleel et al., 2008) have been determined. However, the separation technique is labor intensive (13–15 hr for one sample), technically challenging, and label quantification can require relatively large tissue samples. These limitations have restricted wide-spread application of individual protein FSR measurements.

We and others have applied deuterated water ($^2\text{H}_2\text{O}$) to assess in vivo muscle protein synthesis rates over several days or weeks (Holwerda et al., 2018a, 2018b; Murphy et al., 2018; Robinson et al., 2011; Wilkinson et al., 2014). The endogenous labeling of nearly all (nonessential) amino acids increases total label incorporation into newly synthesized proteins, which improves analytical detectability. Recently, investigators have combined $^2\text{H}_2\text{O}$ administration with high-throughput analytical techniques of liquid chromatography–mass spectrometry (LC-MS) to determine deuterium enrichment in hundreds of tissue-derived peptides (Hesketh

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et al., 2016; Kasumov et al., 2011; Price et al., 2012; Shankaran et al., 2016b; Wang et al., 2014; Zangarelli et al., 2006). Based upon shifts in mass spectra over time, the synthesis rates of several proteins may be assessed simultaneously (Price et al., 2012; Shankaran et al., 2016a). Within the last couple of years, this *dynamic proteome profiling* (DPP) approach has been applied in human and rodent studies to assess in vivo synthesis rates of several proteins in skeletal muscle tissue (Camera et al., 2017; Shankaran et al., 2016a). Simultaneous application of DPP and assessment of bulk mixed muscle, myofibrillar, and mitochondrial protein FSR in the same tissue is warranted to reveal individual protein synthetic responses that may be masked when assessing bulk protein synthesis rates. This is apparent from earlier work demonstrating that myosin heavy chain synthesis rates are ~30% lower when compared with mixed muscle protein synthesis rates (Balagopal et al., 1997a). Furthermore, a quantitative comparison between the methods is required to further establish DPP as an effective approach to evaluate skeletal muscle adaptive responses. Therefore, in the present study, we assessed the impact of exercise on bulk mixed muscle, myofibrillar, and mitochondrial protein synthesis rates and applied DPP to assess individual muscle protein synthesis rates over a 3-week period in rat skeletal muscle tissue. We hypothesized that (a) protein synthesis rates of individual proteins will span a broader range in comparison with bulk protein synthesis rates, (b) exercise will induce both negative and positive differences in individual protein synthesis rates, and (c) the grouped average FSR of all individual proteins would not differ from bulk muscle FSR.

Methods

Experimental Animals

Male Lewis rats ($n=7$, 7 ± 1 months, 363 ± 11 g; Charles River Laboratories, Wilmington, MA) were housed in cages and maintained on 12-hr light/12-hr dark cycles. Rats were sacrificed after an intraperitoneal injection of sodium pentobarbital (150 mg/kg body mass). All procedures were approved by the Animal Experiments Committee at Maastricht University and were in accordance with the *Code of Conduct of the Central Animal Experiments Committee*.

Experimental Protocol

Six rats received standard chow diet and water ad libitum and underwent the 3-week $^2\text{H}_2\text{O}$ protocol. During the first week, all rats remained sedentary to acclimatize to the experimental setting, including $^2\text{H}_2\text{O}$ administration. Starting after Week 1, three rats received cage access to a running wheel, whereas the other three rats remained sedentary. Lewis rats voluntarily perform wheel running for distances of 4,500–10,000 m/day (Makatsori et al., 2003; Werme et al., 1999). After the 3-week experimental period, the animals were sacrificed, and blood and muscle samples were collected and stored as previously described (Holwerda et al., 2018b). A separate rat, housed under identical (sedentary) conditions, was sacrificed to provide unlabeled control soleus muscle for LC-MS/MS analysis.

$^2\text{H}_2\text{O}$ Dosing Protocol

The $^2\text{H}_2\text{O}$ dosing protocol was adopted from previous study (Neese et al., 2002) and consisted of one intraperitoneal injection of 70%

deuterium oxide (Cambridge Isotopes Laboratories, Tewksbury, MA) at 0.02 ml/g body mass and access to 4% deuterium-enriched drinking water.

Plasma Free ^2H -Alanine and Body Water ^2H Enrichments

Plasma amino acid enrichments were determined by gas chromatography–mass spectrometry (GC-MS) as described previously (Holwerda et al., 2018b). Body water ^2H enrichments were determined by dividing plasma ^2H -alanine enrichments by 3.7, which is the labeling factor between body water and alanine (Holwerda et al., 2018b; Wilkinson et al., 2014).

Bulk Protein-Bound ^2H -Alanine Enrichment

Bulk mixed muscle, myofibrillar, and mitochondrial protein fractions were isolated from muscle samples, and protein-bound ^2H -alanine enrichments were measured using GC-MS, as described previously (Churchward-Venne et al., 2019; Holwerda et al., 2016, 2018b).

LC-MS Analysis

A separate piece of muscle underwent a trypsin digestion protocol as previously described (Qiao et al., 2019) to cleave peptides. The peptide mixture was loaded onto an LC-MS/MS, configured as described previously (Qiao et al., 2019; Vogel et al., 2019).

Label-Free Quantitation of Protein Abundances

Progenesis QI for proteomics (Waters, Milford, MA) was used to perform label-free quantitation as described previously (Bowden-Davies et al., 2015; Burniston et al., 2014; Camera et al., 2017; Sollanek et al., 2017). Log-transformed MS data were normalized by intersample abundance ratio, and relative protein abundances were calculated using nonconflicting peptides only. Spectra generated from Mass Spectrometer 2 (MS2) were exported in Mascot generic format and searched against the Swiss-Prot database (2018.7) using a locally implemented Mascot server (version 2.2.03; www.matrixscience.com). The Mascot output, restricted to nonhomologous protein identifications (false discovery rate < 1%), was recombined with MS profile data in Progenesis QI (Burniston et al., 2014).

FSR Calculations—Bulk Muscle Protein Fractions

Bulk mixed muscle, myofibrillar, and mitochondrial protein FSRs were calculated over the 3-week deuterium water administration period using a first-order kinetics equation (Shankaran et al., 2016a):

$$\text{FSR}(\%/ \text{day}) = \frac{-\ln(1-f)}{t}$$

We applied a first-order kinetics equation to account for the prolonged labeling period and potential differences in the synthetic rates between protein fractions and individual proteins. f is the cumulative fractional synthesis, which was determined by dividing the protein-bound ^2H -alanine enrichment in the 3-week muscle samples by the free ^2H -alanine enrichment in the 3-week plasma samples. t represents the time (21 days).

FSR Calculations—Dynamic Proteomic Profiling

Protein synthesis rates were calculated from peptide mass isotope abundance data extracted from spectra generated from Mass

Spectrometer 1 (MS1) using Progenesis Q1 (Waters), as described previously (Camera et al., 2017). The rate of decay (k) of the molar fraction (MF0) of the monoisotopic peak (M0) was calculated as a first-order exponential spanning the beginning (t_0) to end (t) of the experimental period.

$$MF0 = \frac{M0}{M0 + M1 + M2 + M3},$$

$$k = \frac{1}{t - t_0} \cdot \ln \left(\frac{MF0_t}{MF0_{t_0}} \right).$$

The FSR (%/day) was derived by dividing k by body water ^2H enrichment (p) multiplied by the number (n) of ^2H exchangeable H-C bonds present in each peptide. n was calculated for each peptide using existing data on amino acid ^2H labeling in humans (Price et al., 2012).

$$FSR(\%/day) = \frac{k}{(n \cdot p)}.$$

Statistical Analysis

Data are expressed as means $\pm$ SDs. Independent two-tailed Student t tests were used to compare plasma ^2H -alanine enrichments, estimated body water ^2H enrichments, average bulk protein synthesis rates, and grouped (i.e., total, mitochondrial, myofibrillar) individual protein synthesis rates. A one-way between-group analysis of variance was used to compare individual protein differences between the sedentary and exercise conditions. Statistical significance was set at $p < .05$. The false discovery rate Q value was calculated to control multiple testing. Unless otherwise stated, calculations were performed using Excel (version 16; Microsoft, Redmond, WA) or SPSS (version 24; IBM, Armonk, NY). Pearson r product moment correlation analysis was used to examine the linear relationship between bulk mixed muscle, myofibrillar, and mitochondrial FSRs with grouped averages of the individual protein FSRs, which were assigned to subcellular protein fraction (i.e., total, myofibrillar, and mitochondrial).

Results

$^2\text{H}_2\text{O}$ Precursor Labeling

The $^2\text{H}_2\text{O}$ dosing protocol resulted in plasma-free ^2H -alanine enrichments of 8.16 ± 0.09 and 8.04 ± 0.05 mole percent excess (MPE) at Day 21 in the sedentary and exercise groups, respectively ($p = .10$). The corresponding body water deuterium enrichments averaged $2.21 \pm 0.02\%$ and $2.17 \pm 0.02\%$ in the sedentary and exercise groups, respectively ($p = .10$).

FSRs of Bulk Mixed Muscle, Myofibrillar, and Mitochondrial Protein

Bulk mixed muscle protein-bound ^2H -alanine measured by GC-MS averaged 3.37 ± 0.93 MPE in the exercise group and did not differ ($p = .79$) from 3.18 ± 0.66 MPE in the sedentary group. Bulk myofibrillar protein-bound ^2H -alanine enrichments averaged 4.52 ± 0.19 MPE in the exercise group, which was significantly ($p < .05$) greater than 3.84 ± 0.18 MPE in the sedentary group. Bulk mitochondrial protein-bound ^2H -alanine enrichments averaged 5.01 ± 0.12 MPE in the exercise group, which was significantly

Figure 1 — Mixed muscle, myofibrillar, and mitochondrial protein fractional synthesis rates (FSRs, %/day) assessed in the soleus muscle of Lewis rats that either had access to a cage running wheel (exercise, $n = 3$) or were restricted to sedentary conditions (sedentary, $n = 3$) for the last 2 weeks of a 3-week assessment period. Values are presented as means $\pm$ SDs. *Significantly different from the sedentary condition ($P < .05$).

($p < .05$) greater than 4.62 ± 0.21 MPE in the sedentary group. Mixed muscle protein FSRs (Figure 1) did not differ between the exercising ($2.64 \pm 0.96\%/day$) and sedentary rats ($2.38 \pm 0.62\%/day$; $p = .71$). Myofibrillar muscle protein FSR (Figure 1) was $\sim 30\%$ greater in the exercise group ($3.94 \pm 0.26\%/day$) when compared with the sedentary group ($3.03 \pm 0.15\%/day$; $p < .01$). Mitochondrial muscle protein FSR (Figure 1) was $\sim 17\%$ greater in the exercise group ($4.64 \pm 0.24\%/day$) when compared with the sedentary group ($3.97 \pm 0.26\%/day$; $p < .05$).

Individual Muscle Protein Abundances

Label-free profiling was performed on 256 proteins that had at least one unique peptide detected in each animal. The dynamic range of the label-free analysis spanned six orders of magnitude. The most abundant muscle protein was myosin light chain 3 (MYL3; relative protein abundances = $9.89\text{E}+08$), and the least abundant muscle protein detected was myosin heavy chain 9 (MYH9; relative protein abundances = $3.61\text{E}+04$). The normalized abundances of three proteins (musculoskeletal embryonic nuclear protein 1 [MUSTN1], adenylate kinase isoenzyme 1 [KAD1], and 60S ribosomal protein L32 [RPL32]) were statistically ($p < .05$) greater in the exercise compared with sedentary control samples, though with a high false discovery rate (0.48).

FSRs of Individual Muscle Proteins

Based on our selection criteria, we were able to assess the FSRs of 108 out of 256 identified proteins (42%), which resided in the muscle cytosolic, mitochondrial, myofibrillar, and nuclear fractions, as well as in residual blood (Table 1). The weighted average FSR of all individual proteins in both groups was $2.59 \pm 0.69\%/day$, which was not statistically different from bulk mixed muscle protein FSR measured by GC-MS analysis ($p = .91$). The rank order of the synthesis rates for these 108 proteins is displayed in Figure 2. FSR of the detected individual proteins was lowest in histone H2A type 1-C (H2A1C; $0.48 \pm 0.09\%/day$) and highest in alpha-2-HS-glycoprotein (FETUA; $9.25 \pm 0.38\%/day$). The average FSR across individual mitochondrial proteins demonstrated a strong, positive correlation with bulk mitochondrial FSR ($p = .03$, $r = .73$, Figure 3c).

Table 1 Protein Enrichments and FSRs of 108 Individual Proteins Across Blood, Sarcoplasmic, Mitochondrial, Myofibrillar, and Nuclear Subcellular Protein Fractions in Soleus Muscle of Sedentary and Exercising Rats

Subcellular protein fraction	Accession	Protein description	Unique peptide sequences identified (n)	FSR (%/day)		Difference (%)	p value	Q value
				Sedentary	Exercise			
Blood	AI13	Alpha-1-inhibitor 3	2	3.364 ± 0.394	3.052 ± 0.695	-9	.535	0.781
Blood	ALBU	Serum albumin	28	4.507 ± 0.357	5.024 ± 0.531	11	.235	0.643
Blood	ANXA2	Annexin A2	3	1.922 ± 0.797	2.430 ± 0.540	26	.412	0.665
Blood	ANXA5	Annexin A5	1	3.684 ± 0.577	3.883 ± 0.126	5	.592	0.818
Blood	CALR	Calreticulin	2	6.725 ± 0.449	6.780 ± 2.005	1	.965	0.993
Blood	CAVNI	Caveolae-associated protein 1	2	3.143 ± 0.831	2.526 ± 0.425	-20	.316	0.643
Blood	FETUA	Alpha-2-HS-glycoprotein	2	8.514 ± 0.445	9.253 ± 0.379	9	.094	0.643
Blood	HBA	Hemoglobin subunit alpha-1/2	7	1.754 ± 0.351	1.921 ± 0.665	10	.720	0.907
Blood	HBB2	Hemoglobin subunit beta-2	5	4.740 ± 0.701	1.716 ± 1.456	-64	.032	0.643
Blood	HEMO	Hemopexin	1	2.296 ± 0.391	2.470 ± 0.971	8	.787	0.933
Blood	KACB	Ig kappa chain C region, B allele	1	1.471 ± 0.480	2.252 ± 0.669	53	.176	0.643
Blood	PDLI5	PDZ and LIM domain protein 5	6	0.892 ± 0.388	3.136 ± 3.098	252	.281	0.643
Blood	PGS2	Decorin	3	3.338 ± 0.213	4.079 ± 0.937	22	.252	0.643
Blood	PTMS	Parathymosin	1	2.153 ± 0.448	3.310 ± 1.095	54	.166	0.643
Blood	TAGL2	Transgelin-2	2	4.904 ± 2.697	4.731 ± 5.561	-4	.963	0.993
Blood	TRFE	Serotransferrin	5	0.773 ± 0.520	1.263 ± 1.391	63	.598	0.818
Blood	VTDB	Vitamin D-binding protein	1	8.584 ± 2.013	8.566 ± 6.576	0	.997	0.997
Average values, blood proteins (n = 17)								
Cytosolic	1433E	14-3-3 protein epsilon	1	3.692 ± 0.709	3.905 ± 1.595	6	.140	-
Cytosolic	AATC	Aspartate aminotransferase, cytoplasmic	7	2.228 ± 0.432	3.231 ± 0.727	45	.109	0.643
Cytosolic	ALDOA	Fructose-bisphosphate aldolase A	21	2.107 ± 0.107	1.926 ± 0.748	-9	.700	0.907
Cytosolic	ANXA6	Annexin A6	4	2.234 ± 0.186	2.647 ± 0.194	18	.056	0.643
Cytosolic	CAH3	Carbonic anhydrase 3	24	3.504 ± 0.051	4.162 ± 1.321	19	.438	0.685
Cytosolic	DOPD	D-dopachrome decarboxylase	3	1.933 ± 0.087	2.542 ± 0.828	32	.274	0.643
Cytosolic	DPYL2	Dihydropyrimidinase-related protein 2	2	3.014 ± 1.690	2.409 ± 0.383	-20	.578	0.818
Cytosolic	EF2	Elongation factor 2	5	5.524 ± 4.594	5.116 ± 2.265	-7	.897	0.993
Cytosolic	ENOB	Beta-enolase	17	1.911 ± 0.243	2.538 ± 1.138	33	.403	0.665
Cytosolic	ESTD	S-formylglutathione hydrolase	2	1.276 ± 0.258	1.887 ± 0.542	48	.153	0.643
Cytosolic	FABP4	Fatty acid-binding protein, adipocyte	5	1.824 ± 1.361	3.829 ± 3.017	110	.353	0.645
Cytosolic	FKB1A	Peptidyl-prolyl cis-trans isomerase	1	5.161 ± 0.601	5.050 ± 2.468	-2	.943	0.993
Cytosolic	G3P	Glyceraldehyde-3-phosphate dehydrogenase	20	2.099 ± 0.902	2.704 ± 0.261	29	.327	0.643
Cytosolic	G6PI	Glucose-6-phosphate isomerase	5	1.714 ± 0.297	2.896 ± 1.680	69	.296	0.643
Cytosolic	GPDA	Glycerol-3-phosphate dehydrogenase	3	0.756 ± 0.271	2.919 ± 3.102	286	.295	0.643
Cytosolic	GPX1	Glutathione peroxidase 1	1	2.382 ± 1.000	3.366 ± 1.854	41	.464	0.696
Cytosolic	GSTA3	Glutathione S-transferase alpha-3	2	1.747 ± 0.409	2.394 ± 0.236	37	.077	0.643
Cytosolic	HS90B	Heat shock protein 90-beta	2	2.877 ± 0.481	3.229 ± 1.178	12	.657	0.865
				4.498 ± 0.767	3.531 ± 1.868	-21	.453	0.689

(continued)

Table 1 (continued)

Subcellular protein fraction	Accession	Protein description	Unique peptide sequences identified (n)	FSR (%/day)		Difference (%)	p value	Q value
				Sedentary	Exercise			
Cytosolic	HSP7C	Heat shock cognate 71 kDa protein	11	3.287 ± 0.304	4.001 ± 0.623	22	.149	0.643
Cytosolic	HSPB6	Heat shock protein beta-6	4	2.175 ± 0.089	3.346 ± 1.366	54	.212	0.643
Cytosolic	KAD1	Adenylate kinase isoenzyme I	9	1.174 ± 0.035	2.232 ± 0.767	90	.075	0.643
Cytosolic	KCRB	Creatine kinase B-type	2	4.195 ± 1.085	4.250 ± 0.899	1	.950	0.993
Cytosolic	KCRM	Creatine kinase M-type	28	1.632 ± 0.311	1.533 ± 0.533	-6	.794	0.933
Cytosolic	KPYM	Pyruvate kinase PKM	19	2.501 ± 0.445	2.634 ± 0.558	5	.763	0.926
Cytosolic	LDHA	L-lactate dehydrogenase A chain	3	2.713 ± 0.146	4.233 ± 1.049	56	.068	0.643
Cytosolic	MDHC	Malate dehydrogenase, cytoplasmic	8	1.964 ± 0.076	3.951 ± 3.133	101	.334	0.643
Cytosolic	MYG	Myoglobin	8	1.820 ± 0.084	1.782 ± 0.378	-2	.872	0.992
Cytosolic	NTF2	Nuclear transport factor 2	1	1.129 ± 0.919	5.875 ± 7.078	420	.314	0.643
Cytosolic	PARK7	Protein/nucleic acid deglycase DJ-1	3	1.338 ± 0.686	2.029 ± 0.428	52	.213	0.643
Cytosolic	PDL1	PDZ and LIM domain protein 1	7	3.944 ± 0.314	5.129 ± 1.140	30	.157	0.643
Cytosolic	PFKAM	ATP-dependent 6-phosphofructokinase	2	1.946 ± 0.717	1.484 ± 1.174	-24	.592	0.818
Cytosolic	PGAM1	Phosphoglycerate mutase 1	1	2.617 ± 0.458	3.128 ± 0.629	20	.318	0.643
Cytosolic	PGAM2	Phosphoglycerate mutase 2	6	1.224 ± 0.270	1.943 ± 0.720	59	.181	0.643
Cytosolic	PGK1	Phosphoglycerate kinase 1	14	1.826 ± 0.037	2.866 ± 1.492	57	.294	0.643
Cytosolic	PGM1	Phosphoglucomutase-1	5	1.707 ± 0.535	2.575 ± 2.129	51	.531	0.781
Cytosolic	PIMT	Protein-L-isoaspartate	1	1.382 ± 0.394	2.312 ± 0.445	67	.053	0.643
Cytosolic	PRDX2	Peroxioredoxin-2	3	4.360 ± 1.726	3.298 ± 0.744	-24	.384	0.665
Cytosolic	PRDX6	Peroxioredoxin-6	1	1.593 ± 0.337	1.636 ± 0.195	3	.857	0.985
Cytosolic	PROF1	Profilin-1	3	3.127 ± 0.291	3.271 ± 1.765	5	.897	0.993
Cytosolic	PYGM	Glycogen phosphorylase	7	3.055 ± 0.801	4.734 ± 1.905	55	.232	0.643
Cytosolic	RLA1	60S acidic ribosomal protein P1	1	1.958 ± 0.763	2.392 ± 0.148	22	.388	0.665
Cytosolic	SODC	Superoxide dismutase [Cu-Zn]	2	1.730 ± 0.152	2.156 ± 0.623	25	.313	0.643
Cytosolic	TERA	Transitional endoplasmic reticulum ATPase	1	3.884 ± 0.895	3.609 ± 0.987	-7	.739	0.907
Cytosolic	TPIS	Triosephosphate isomerase	11	1.416 ± 0.240	2.303 ± 1.012	63	.213	0.643
Average values, cytosolic proteins (n = 44)								
Mitochondrial	ACADL	Long-chain specific acyl-CoA dehydrogenase	6	2.420 ± 0.587	3.070 ± 1.267	27	.200	-
Mitochondrial	ACADV	Very long-chain specific acyl-CoA dehydrogenase	2	2.520 ± 0.432	2.876 ± 0.533	14	.419	0.665
Mitochondrial	ALDH2	Aldehyde dehydrogenase	1	2.902 ± 0.664	3.785 ± 1.321	30	.359	0.645
Mitochondrial	AT5F1	ATP synthase F1	2	7.085 ± 1.031	6.859 ± 0.238	-3	.730	0.907
Mitochondrial	ATP5H	ATP synthase subunit d	2	2.155 ± 0.581	3.522 ± 0.579	63	.045	0.643
Mitochondrial	ATPA	ATP synthase subunit alpha	21	0.791 ± 0.510	2.181 ± 1.309	176	.162	0.643
Mitochondrial	ATPD	ATP synthase subunit delta	2	1.856 ± 0.074	2.380 ± 0.303	28	.044	0.643
Mitochondrial	CATA	Catalase	2	1.867 ± 0.282	2.322 ± 0.402	24	.184	0.643
Mitochondrial	CH60	60 kDa heat shock protein	2	5.002 ± 0.700	4.967 ± 0.664	-1	.953	0.993
Mitochondrial	COQ8A	Atypical kinase COQ8A	7	3.443 ± 0.212	3.412 ± 0.733	-1	.948	0.993
Mitochondrial	COQ8A	Atypical kinase COQ8A	1	3.468 ± 1.453	3.516 ± 0.903	1	.964	0.993

(continued)

Table 1 (continued)

Subcellular protein fraction	Accession	Protein description	Unique peptide sequences identified (n)	FSR (%/day)		Difference (%)	p value	Q value
				Sedentary	Exercise			
Mitochondrial	COX5B	Cytochrome c oxidase subunit 5B	1	2.768 ± 0.397	5.035 ± 3.379	82	.313	0.643
Mitochondrial	DLDH	Dihydrolipoyl dehydrogenase	5	1.820 ± 0.817	2.290 ± 0.264	26	.397	0.665
Mitochondrial	ECHA	Trifunctional enzyme subunit alpha	8	1.419 ± 0.208	1.805 ± 0.211	27	.087	0.643
Mitochondrial	ECHM	Enoyl-CoA hydratase	2	3.024 ± 0.718	3.094 ± 0.586	2	.903	0.993
Mitochondrial	ESI	ESI protein homolog	2	1.277 ± 0.195	1.285 ± 0.367	1	.974	0.993
Mitochondrial	ETFA	Electron transfer flavoprotein subunit alpha	7	2.295 ± 0.344	3.572 ± 1.354	56	.189	0.643
Mitochondrial	FUMH	Fumarate hydratase	4	0.777 ± 0.134	1.031 ± 0.889	33	.650	0.865
Mitochondrial	MDHM	Malate dehydrogenase	6	1.914 ± 0.077	2.899 ± 0.619	51	.052	0.643
Mitochondrial	NDUS1	NADH-ubiquinone oxidoreductase 75 kDa subunit	3	3.380 ± 0.804	4.845 ± 0.754	43	.083	0.643
Mitochondrial	NDUS6	NADH dehydrogenase iron-sulfur protein 6	1	3.270 ± 0.418	3.596 ± 0.530	10	.450	0.689
Mitochondrial	ODPB	Pyruvate dehydrogenase E1 component subunit beta	2	1.676 ± 0.266	2.569 ± 0.685	53	.103	0.643
Mitochondrial	PHB2	Prohibitin-2	2	2.086 ± 1.412	3.685 ± 1.282	77	.220	0.643
Mitochondrial	PRDX5	Peroxiredoxin-5	4	1.253 ± 0.428	3.658 ± 4.048	192	.364	0.645
Mitochondrial	QCR2	Cytochrome b-c1 complex subunit 2	2	0.927 ± 0.603	2.498 ± 2.371	169	.328	0.643
Mitochondrial	QCR6	Cytochrome b-c1 complex subunit 6	1	3.018 ± 0.628	4.188 ± 1.109	39	.187	0.643
Mitochondrial	SDHA	Succinate dehydrogenase flavoprotein subunit	4	3.298 ± 0.353	4.467 ± 1.138	35	.164	0.643
Mitochondrial	THIL	Acetyl-CoA acetyltransferase	7	2.338 ± 0.267	3.732 ± 1.750	60	.244	0.643
Average values, mitochondrial proteins (n = 27)								
Myofibrillar	ACTC	Actin, alpha cardiac muscle 1	2	2.505 ± 0.519	3.336 ± 1.049	33	.068	–
Myofibrillar	ACTN1	Alpha-actinin-1	7	1.002 ± 0.499	1.61 ± 0.463	61	.197	0.643
Myofibrillar	ACTS	Actin, alpha skeletal muscle	5	1.554 ± 0.109	3.603 ± 2.365	132	.208	0.643
Myofibrillar	CALD1	Nonmuscle caldesmon	2	0.765 ± 0.28	1.884 ± 1.428	146	.254	0.643
Myofibrillar	CALM1	Calmodulin-1	1	3.078 ± 0.971	3.090 ± 0.641	0	.987	0.996
Myofibrillar	CAPZB	F-actin-capping protein subunit beta	5	6.185 ± 2.286	5.372 ± 1.449	–13	.630	0.851
Myofibrillar	ENOB	Beta-enolase	17	2.887 ± 0.238	3.522 ± 0.604	22	.165	0.643
Myofibrillar	MYL1	Myosin light chain 1/3, skeletal muscle isoform	14	1.276 ± 0.258	1.887 ± 0.542	48	.153	0.643
Myofibrillar	MYL6	Myosin light polypeptide 6	3	3.676 ± 2.090	4.110 ± 1.324	12	.776	0.932
Myofibrillar	PROF1	Profilin-1	3	2.514 ± 0.329	3.319 ± 0.379	32	.050	0.643
Myofibrillar	TBB4B	Tubulin beta-4B chain	5	3.127 ± 0.291	3.271 ± 1.765	5	.897	0.993
Myofibrillar	TNNT3	Troponin T, fast skeletal muscle	2	1.989 ± 0.271	2.218 ± 0.558	12	.556	0.801
Myofibrillar	TPM1	Tropomyosin alpha-1 chain	7	3.056 ± 1.607	1.858 ± 0.943	–39	.328	0.643
Myofibrillar	TPM3	Tropomyosin alpha-3 chain	1	1.249 ± 0.230	2.136 ± 1.041	71	.223	0.643
Average values, myofibrillar proteins (n = 14)								
Nuclear	CAPG	Macrophage-capping protein	2	2.249 ± 0.101	3.664 ± 1.452	63	.167	0.643
Nuclear	CSRPI	Cysteine and glycine-rich protein 1	2	2.967 ± 1.068	2.472 ± 0.683	20	.168	–
Nuclear	H2A1C	Histone H2A type 1-C	2	3.137 ± 2.015	5.558 ± 3.239	77	.333	0.643
Nuclear	H2B1	Histone H2B type 1	2	4.957 ± 1.699	5.251 ± 1.987	6	.855	0.985
				0.476 ± 0.093	0.676 ± 0.32	42	.358	0.645
				0.946 ± 0.275	1.136 ± 0.798	20	.716	0.907

(continued)

Table 1 (continued)

Subcellular protein fraction	Accession	Protein description	Unique peptide sequences identified (<i>n</i>)	FSR (%/day)		Difference (%)	<i>p</i> value	<i>Q</i> value
				Sedentary	Exercise			
Nuclear	HMGB1	High mobility group protein B1	1	0.749 ± 0.296	0.828 ± 0.249	11	.739	0.907
Nuclear	ROA2	Heterogeneous nuclear ribonucleoproteins A2/B1	1	6.819 ± 0.731	7.335 ± 0.64	8	.410	0.665
Nuclear	ROA3	Heterogeneous nuclear ribonucleoprotein A3	1	3.469 ± 0.282	3.856 ± 0.55	11	.340	0.643
Nuclear	SYNP2	Synaptopodin-2	3	3.074 ± 0.277	3.665 ± 0.111	19	.027	0.643
Average values, nuclear proteins (<i>n</i> = 8)				2.953 ± 0.709	3.538 ± 0.987	20	.349	–
Average values, total proteins (<i>n</i> = 108)				2.692 ± 0.617	3.298 ± 1.220	23	.091	–

Note. *n* = 3 for sedentary rats and *n* = 3 for exercising rats. Data were analyzed using between-group analysis of variance on each protein. Statistical significance was set at *p* < .05. Average values for each protein subfraction are expressed as means ± SDs. FSRs = fractional synthesis rates.

Figure 2 — Rank order of individual protein synthesis rates in rat soleus muscle. Panel (a) displays the mean rate constant (*k*) for the synthesis of each protein ranked from lowest to highest during the 3-week experimental period. Labels indicate the UniProt Knowledgebase identifiers for the detected proteins. Panel (b) displays box plots presenting the average (median and interquartile range) rate constant of synthesis of proteins that were analyzed using DPP and the BULK during the 3-week experimental period. Independent two-tailed Student *t* test found no significant (*P* = .91) difference in the average rate of synthesis of the grouped individual proteins (*n* = 108) when compared with BULK. DPP = dynamic proteome profiling; BULK = bulk mixed muscle protein fraction.

Figure 3 — Correlations between the averages of grouped individual protein fractional synthesis rates (FSRs) and the corresponding bulk mixed muscle (a), myofibrillar (b), and mitochondrial (c) protein fractions FSRs. The solid line indicates the linear regression line of best fit, and the dashed lines indicate the 95% confidence interval. A significant positive correlation was observed between methods for mitochondrial proteins (*p* = .03, *R*² = .73).

Individual Protein Synthetic Responses to Exercise

Exercise appeared to impact the synthesis rates of the majority of identified proteins. Eighty proteins demonstrated a difference in FSR greater than +10% or less than −10% between muscles of sedentary and exercising animals, and the percentage difference of myofibrillar and mitochondrial proteins relative to sedentary conditions ranged from −64% to +420% (Figure 4). When all detected proteins were averaged, exercise did not result in significantly higher protein synthesis rates when compared with sedentary (3.30 ± 1.22 vs. $2.69 \pm 0.62\%$ /day, respectively, *p* = .09). When proteins were grouped into subcellular protein fraction, exercise did not result in significantly higher mitochondrial protein synthesis rates when compared with sedentary (3.34 ± 1.05 vs. $2.51 \pm 0.52\%$ /day, respectively, *p* = .07). At the individual protein level, exercise resulted in greater synthesis rates

(*p* < .05) of F1-ATP synthase (AT5F1), ATP synthase subunit alpha (ATPA), hemoglobin (HBB2), myosin light chain 6 (MYL6), and synaptopodin-2 (SYNP2) (Figure 5).

Discussion

We observed that bulk myofibrillar and mitochondrial protein synthesis rates were greater in the soleus muscle tissue of exercising rats when compared with their sedentary littermates. Exercise impacted the synthesis rates of 80 out of 108 detected proteins, with the response ranging between −64% to +420% when expressed relative to the sedentary condition. Bulk myofibrillar and mitochondrial protein synthesis rates were, respectively, ~30% higher and ~17% higher in the exercising rats when compared with the sedentary rats (Figure 1). Our findings align with previous studies in rodents that have applied a

Figure 4 — Percentage difference in fractional synthesis rates (FSRs) of individual mitochondrial (a) and myofibrillar (b) proteins assessed in the soleus muscle of Lewis rats that either had access to a cage running wheel (exercise, $n = 3$) or were restricted to sedentary conditions (sedentary, $n = 3$) over a 3-week experimental period. Presented proteins demonstrated a difference in FSR greater than +10% or less than -10% between sedentary and exercise rats. Values are presented as the average percentage difference of FSR in the exercise group compared with the sedentary group. *Significantly different in exercise versus sedentary ($P < .05$).

variety of isotope-based methods to demonstrate the robust anabolic impact of chronic and acute exercise on muscle protein synthesis rates (Gasier et al., 2010; Kubica et al., 2005; Mosoni et al., 1995; Munoz et al., 1994; Wong & Booth, 1990). However, exercise did not result in significantly higher mixed muscle protein synthesis rates when compared with the sedentary rats (Figure 1). This finding is in contrast with some (Gasier et al., 2010; Kubica et al., 2005; Mosoni et al., 1995; Munoz et al., 1994; Ogasawara et al., 2014, 2016; Wong & Booth, 1990), but not all (Katzeff et al., 1995; Wang et al., 2017), studies that have assessed the impact of exercise on mixed muscle protein synthesis rates in rats. The absence of a response may be explained by a relatively low (or negative) synthetic response of proteins residing outside of the myofibrillar and mitochondrial subfractions. Nonmyofibrillar and mitochondrial proteins have been estimated to comprise 30–40% of all muscle proteins (Balagopal et al., 1996). It has also been

demonstrated that synthesis of myosin heavy chain, one of the most abundant muscle proteins (~25%), only contributes ~18% to mixed muscle protein synthesis rates (Balagopal et al., 1997a). Theoretically, a ~30% increase in myosin heavy-chain synthesis would only increase mixed muscle protein synthesis by ~5–6%, which could be masked by the co-occurring responses of other proteins (Balagopal et al., 1997a).

We performed DPP by measuring the mass spectra of hundreds of muscle-derived peptides using high-throughput tandem MS. Individual protein synthesis rates in the present study align well with absolute individual protein synthesis rates reported in a recent study that applied a comparable DPP approach in rat muscle (Shankaran et al., 2016b). The individual protein synthesis rates spanned a broader range than the absolute bulk protein synthesis rates (Figure 2b). We observed that the synthetic rates of key mitochondrial proteins, such as cytochrome-c oxidase ($2.77 \pm 0.40\%/day$) and

Figure 5 — Volcano plot presenting the average fold changes in synthesis rates of individual proteins of the exercise group compared with the sedentary group. *p* values were calculated using between-subject analysis of variance. Proteins are represented in black if they exhibited a statistically significant ($p < .05$, all false discovery rate = 0.64) change when compared with the sedentary condition. AT5F1 = F1-ATP synthase; ATPA = ATP synthase subunit alpha; HBB2 = hemoglobin; MYL6 = myosin light chain-6; SYN2 = synaptotagmin-2.

succinate dehydrogenase ($3.30 \pm 0.35\%$ /day), were fourfold higher when compared with key myofibrillar proteins, such as α -actin ($0.77 \pm 0.28\%$ /day) and α -actinin-1 ($1.55 \pm 0.11\%$ /day), which align with previous literature (Jaleel et al., 2008). The FSR of proteins assigned to “mitochondrion” had a strong, positive correlation with bulk mitochondrial protein FSR (Figure 3c). However, we did not detect a correlation in the mixed muscle or myofibrillar protein fractions (Figure 3a and 3b). The discrepancy may be explained by the greater detection of mitochondrial proteins ($n = 27$) in comparison with myofibrillar proteins ($n = 14$). Furthermore, certain highly abundant proteins (e.g., collagen) were not detected using DPP, but were likely captured in the bulk mixed protein isolation. Strict categorization of some proteins known to reside in more than one cellular compartment or translocate between compartments (e.g., heat shock proteins) may have also contributed to the absence of a correlation between methods for myofibrillar proteins.

Exercise seemed to impact the synthesis rates of the majority of detected proteins (80/108), with the response ranging from -64% to $+420\%$ relative to sedentary conditions. Notably, we reveal that exercise resulted in significantly greater synthesis rates of F1-ATP synthase and ATP synthase subunit alpha when compared with the sedentary group (Table 1 and Figure 5). Furthermore, several proteins within the tricarboxylic acid cycle and electron transport system demonstrated 35–140% higher protein synthesis rates in the exercise group when compared with the sedentary group (Figure 4a). Increased synthesis rates of these proteins, and ATP synthase in particular, likely contribute to the increase in muscle oxidative capacity observed over more prolonged endurance-type exercise training (Burniston & Hoffman, 2011; Hesketh et al., 2016; Holloszy et al., 1970). However, we did not detect significantly greater

abundances of these proteins in the present study, which is likely due to low subject number or the mere 2-week exercise intervention. We chose to assess protein synthetic responses in the soleus muscle primarily due to the high Type I muscle fiber content and aerobic nature of wheel running exercise. As soleus muscle may possess a greater sensitivity to induce transcriptional and/or protein synthetic responses to physical (in)activity when compared with other muscles (e.g., plantaris or tibialis anterior; Miller et al., 2019), we should be careful when translating these findings to other muscle groups.

Bulk protein FSR assessment and DPP provide distinct advantages and disadvantages and can complement each other. For example, DPP allows identification and assessment of protein synthesis rates within subcellular protein fractions, which is valuable for resolving more specific physiological issues, such as characterizing how key mitochondrial proteins (e.g., ATP synthase) respond to physical (in)activity, aging, and/or disease. Characterizing the coordinated regulation of individual mitochondrial proteins is of particular relevance given the gap in our understanding of mitochondrial protein adaptation due to the challenges of isolating a pure mitochondrial fraction from muscle tissue (Burd et al., 2015). Despite this advantage, we and others have been able to assess the synthesis rates of only 100–150 individual proteins in skeletal muscle tissue. This number is equivalent to merely 2% of the approximately 5,500 proteins present in the skeletal muscle proteome (Gonzalez-Freire et al., 2017). Although the top 100 detectable muscle proteins have been suggested to represent 85% of total muscle protein (Geiger et al., 2013), the detectable proteins cannot currently be localized to the different muscle fiber types or to nonmuscle tissue (e.g., fat, connective tissue), which likely respond differently to stimuli. The methodological approach (e.g., sample preparation, instrument sensitivity) may be modified to detect targeted peptide/protein families, which would be useful for resolving some of these issues in the future. On the other hand, bulk protein FSR assessment provides an average representation of all proteins residing in the isolated fraction. Therefore, bulk protein FSR assessment can be applied to reveal the regulation of bulk muscle protein abundance. Acquiring this information can deepen our understanding of skeletal muscle mass regulation to physical (in)activity, nutrition, aging, and disease. Considering the high cost of DPP and the rather limited number of proteins detected in muscle tissue, it is evident that, in most cases, the assessment of bulk protein FSR provides sufficient insight into the muscle protein synthetic response to various interventions.

Limitations of the current study must be acknowledged. First, the relatively low subject numbers may have resulted in Type II errors among comparisons between bulk mixed muscle protein FSR and grouped individual protein FSR data, as well as the lack of a difference in mixed muscle protein FSR between muscle tissues collected from the sedentary and exercising rats. Second, we allowed the exercising rats to run with no restriction but did not quantitate exercise duration or intensity.

In conclusion, bulk mitochondrial, but not mixed muscle or myofibrillar, protein synthesis rates align well with the averages of grouped individual protein FSRs from the same subcellular fraction. The impact of exercise on individual muscle proteins is highly coordinated as reflected by the simultaneous up- and downregulation of several individual proteins residing in different muscle protein fractions.

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designed the research; A.M. Holwerda and M. Nabben conducted the research; F.G. Bouwman, J.G. Burniston, P. Wang, J. van Kranenburg, and A.P. Gijzen analyzed the data; A.M. Holwerda and J.G. Burniston performed the statistical analysis; all authors contributed to data interpretation; and A.M. Holwerda, F.G. Bouwman, J.G. Burniston, E.C.M. Mariman, and L.J.C. van Loon wrote the paper and hold primary responsibility for the final content. All authors read and approved the final manuscript.

References

- Balagopal, P., Ford, G.C., Ebenstein, D.B., Nadeau, D.A., & Nair, K.S. (1996). Mass spectrometric methods for determination of [^{13}C]Leucine enrichment in human muscle protein. *Analytical Biochemistry*, 239(1), 77–85. PubMed ID: 8660628 doi:10.1006/abio.1996.0293
- Balagopal, P., Ljungqvist, O., & Nair, K.S. (1997a). Skeletal muscle myosin heavy-chain synthesis rate in healthy humans. *American Journal of Physiology*, 272(1, Pt. 1), E45–E50.
- Balagopal, P., Nair, K.S., & Stirewalt, W.S. (1994). Isolation of myosin heavy chain from small skeletal muscle samples by preparative continuous elution gel electrophoresis: Application to measurement of synthesis rate in human and animal tissue. *Analytical Biochemistry*, 221(1), 72–77. PubMed ID: 7985807 doi:10.1006/abio.1994.1381
- Balagopal, P., Rooyackers, O.E., Adey, D.B., Ades, P.A., & Nair, K.S. (1997b). Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *American Journal of Physiology*, 273(4, Pt. 1), E790–E800.
- Bowden-Davies, K., Connolly, J., Burghardt, P., Koch, L.G., Britton, S.L., & Burniston, J.G. (2015). Label-free profiling of white adipose tissue of rats exhibiting high or low levels of intrinsic exercise capacity. *Proteomics*, 15(13), 2342–2349. PubMed ID: 25758023 doi:10.1002/pmic.201400537
- Burd, N.A., Holwerda, A.M., Selby, K.C., West, D.W., Staples, A.W., Cain, N.E., . . . Phillips, S.M. (2010a). Resistance exercise volume affects myofibrillar protein synthesis and anabolic signalling molecule phosphorylation in young men. *The Journal of Physiology*, 588(Pt. 16), 3119–3130. doi:10.1113/jphysiol.2010.192856
- Burd, N.A., Tardif, N., Rooyackers, O., & van Loon, L.J.C. (2015). Optimizing the measurement of mitochondrial protein synthesis in human skeletal muscle. *Applied Physiology, Nutrition, and Metabolism*, 40(1), 1–9. PubMed ID: 25494678 doi:10.1139/apnm-2014-0211
- Burd, N.A., West, D.W., Staples, A.W., Atherton, P.J., Baker, J.M., Moore, D.R., . . . Phillips, S.M. (2010b). Low-load high volume resistance exercise stimulates muscle protein synthesis more than high-load low volume resistance exercise in young men. *PLoS One*, 5(8), e12033. doi:10.1371/journal.pone.0012033
- Burniston, J.G., Connolly, J., Kainulainen, H., Britton, S.L., & Koch, L.G. (2014). Label-free profiling of skeletal muscle using high-definition mass spectrometry. *Proteomics*, 14(20), 2339–2344. PubMed ID: 25065561 doi:10.1002/pmic.201400118
- Burniston, J.G., & Hoffman, E.P. (2011). Proteomic responses of skeletal and cardiac muscle to exercise. *Expert Review Proteomics*, 8(3), 361–377. doi:10.1586/ep.11.17
- Camera, D.M., Burniston, J.G., Pogson, M.A., Smiles, W.J., & Hawley, J.A. (2017). Dynamic proteome profiling of individual proteins in human skeletal muscle after a high-fat diet and resistance exercise. *The FASEB Journal*, 31(12), 5478–5494. doi:10.1096/fj.201700531R
- Churchward-Venne, T.A., Pinckaers, P.J.M., Smeets, J.S.J., Peeters, W.M., Zorenc, A.H., Schierbeek, H., . . . van Loon, L.J.C. (2019). Myofibrillar and mitochondrial protein synthesis rates do not differ in young men following the ingestion of carbohydrate with whey, soy, or leucine-enriched soy protein after concurrent resistance- and endurance-type exercise. *The Journal of Nutrition*, 149(2), 210–220. doi:10.1093/jn/nxy251
- Gazier, H.G., Fluckey, J.D., & Previs, S.F. (2010). The application of $^2\text{H}_2\text{O}$ to measure skeletal muscle protein synthesis. *Nutrition & Metabolism*, 7, 31. PubMed ID: 20409307 doi:10.1186/1743-7075-7-31
- Geiger, T., Velic, A., Macek, B., Lundberg, E., Kampf, C., Nagaraj, N., . . . Mann, M. (2013). Initial quantitative proteomic map of 28 mouse tissues using the SILAC mouse. *Molecular & Cellular Proteomics*, 12(6), 1709–1722. PubMed ID: 23436904 doi:10.1074/mcp.M112.024919
- Gonzalez-Freire, M., Semba, R.D., Ubaida-Mohien, C., Fabbri, E., Scalzo, P., Højlund, K., . . . Ferrucci, L. (2017). The human skeletal muscle proteome project: A reappraisal of the current literature. *Journal of Cachexia, Sarcopenia and Muscle*, 8(1), 5–18. doi:10.1002/jcsm.12121
- Hasten, D.L., Morris, G.S., Ramanadham, S., & Yarasheski, K.E. (1998). Isolation of human skeletal muscle myosin heavy chain and actin for measurement of fractional synthesis rates. *American Journal of Physiology*, 275(6), E1092–E1099. PubMed ID: 9843753.
- Hesketh, S., Srisawat, K., Sutherland, H., Jarvis, J., & Burniston, J. (2016). On the rate of synthesis of individual proteins within and between different striated muscles of the rat. *Proteomes*, 4(1), 12. doi:10.3390/proteomes4010012
- Holloszy, J.O., Oscai, L.B., Don, I.J., & Molé, P.A. (1970). Mitochondrial citric acid cycle and related enzymes: Adaptive response to exercise. *Biochemical and Biophysical Research Communications*, 40(6), 1368–1373. PubMed ID: 4327015 doi:10.1016/0006-291X(70)90017-3
- Holwerda, A.M., Kouw, I.W., Trommelen, J., Halson, S.L., Wodzig, W.K., Verdijk, L.B., & van Loon, L.J. (2016). Physical activity performed in the evening increases the overnight muscle protein synthetic response to presleep protein ingestion in older men. *The Journal of Nutrition*, 146(7), 1307–1314. doi:10.3945/jn.116.230086
- Holwerda, A.M., Overkamp, M., Paulussen, K.J.M., Smeets, J.S.J., van Kranenburg, J., Backx, E.M.P., . . . van Loon, L.J.C. (2018a). Protein supplementation after exercise and before sleep does not further augment muscle mass and strength gains during resistance exercise training in active older men. *The Journal of Nutrition*, 148(11), 1723–1732. doi:10.1093/jn/nxy169
- Holwerda, A.M., Paulussen, K.J.M., Overkamp, M., Smeets, J.S.J., Gijzen, A.P., Goessens, J.P.B., . . . van Loon, L.J.C. (2018b). Daily resistance-type exercise stimulates muscle protein synthesis in vivo in young men. *Journal of Applied Physiology*, 124(1), 66–75. doi:10.1152/jappphysiol.00610.2017
- Jaleel, A., Short, K.R., Asmann, Y.W., Klaus, K.A., Morse, D.M., Ford, G.C., & Nair, K.S. (2008). In vivo measurement of synthesis rate of individual skeletal muscle mitochondrial proteins. *American Journal of Physiology—Endocrinology and Metabolism*, 295(5), E1255–E1268.
- Kasumov, T., Ilchenko, S., Li, L., Rachdaoui, N., Sadygov, R.G., Willard, B., . . . Previs, S. (2011). Measuring protein synthesis using metabolic ^2H labeling, high-resolution mass spectrometry, and an algorithm. *Analytical Biochemistry*, 412(1), 47–55. PubMed ID: 21256107 doi:10.1016/j.ab.2011.01.021
- Katzeff, H.L., Ojamaa, K.M., & Klein, I. (1995). The effects of long-term aerobic exercise and energy restriction on protein synthesis. *Metabolism*, 44(2), 188–192. PubMed ID: 7869914 doi:10.1016/0026-0495(95)90263-5
- Kubica, N., Bolster, D.R., Farrell, P.A., Kimball, S.R., & Jefferson, L.S. (2005). Resistance exercise increases muscle protein synthesis and translation of eukaryotic initiation factor 2B epsilon mRNA in a mammalian target of rapamycin-dependent manner. *Journal of*

- Biological Chemistry*, 280(9), 7570–7580. doi:[10.1074/jbc.M413732200](https://doi.org/10.1074/jbc.M413732200)
- Makatsori, A., Duncko, R., Schwendt, M., Moncek, F., Johansson, B.B., & Jezova, D. (2003). Voluntary wheel running modulates glutamate receptor subunit gene expression and stress hormone release in Lewis rats. *Psychoneuroendocrinology*, 28(5), 702–714. PubMed ID: [12727136](https://pubmed.ncbi.nlm.nih.gov/12727136/) doi:[10.1016/S0306-4530\(02\)00062-8](https://doi.org/10.1016/S0306-4530(02)00062-8)
- Miller, B.F., Baehr, L.M., Musci, R.V., Reid, J.J., Peelor, F.F., Hamilton, K.L., & Bodine, S.C. (2019). Muscle-specific changes in protein synthesis with aging and reloading after disuse atrophy. *Journal of Cachexia, Sarcopenia and Muscle*, 10(6), 1195–1209.
- Moore, D.R., Tang, J.E., Burd, N.A., Rerich, T., Tarnopolsky, M.A., & Phillips, S.M. (2009). Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. *Journal of Physiology*, 587(Pt. 4), 897–904. doi:[10.1113/jphysiol.2008.164087](https://doi.org/10.1113/jphysiol.2008.164087)
- Mosoni, L., Valluy, M.C., Serrurier, B., Prugnaud, J., Obled, C., Guezennec, C.Y., & Mirand, P.P. (1995). Altered response of protein synthesis to nutritional state and endurance training in old rats. *American Journal of Physiology*, 268(2, Pt. 1), E328–E335. PubMed ID: [7864110](https://pubmed.ncbi.nlm.nih.gov/7864110/)
- Munoz, K.A., Aannestad, A., Tischler, M.E., & Henriksen, E.J. (1994). Skeletal muscle protein content and synthesis after voluntary running and subsequent unweighting. *Metabolism*, 43(8), 994–999. PubMed ID: [8052157](https://pubmed.ncbi.nlm.nih.gov/8052157/) doi:[10.1016/0026-0495\(94\)90179-1](https://doi.org/10.1016/0026-0495(94)90179-1)
- Murphy, C.H., Shankaran, M., Churchward-Venne, T.A., Mitchell, C.J., Kolar, N.M., Burke, L.M., . . . Phillips, S.M. (2018). Effect of resistance training and protein intake pattern on myofibrillar protein synthesis and proteome kinetics in older men in energy restriction. *Journal of Physiology*, 596(11), 2091–2120. doi:[10.1113/JP275246](https://doi.org/10.1113/JP275246)
- Neese, R.A., Misell, L.M., Turner, S., Chu, A., Kim, J., Cesar, D., . . . Hellerstein, M.K. (2002). Measurement in vivo of proliferation rates of slow turnover cells by $^2\text{H}_2\text{O}$ labeling of the deoxyribose moiety of DNA. *Proceedings of the National Academy of Sciences of the United States of America*, 99(24), 15345–15350. PubMed ID: [12424339](https://pubmed.ncbi.nlm.nih.gov/12424339/) doi:[10.1073/pnas.232551499](https://doi.org/10.1073/pnas.232551499)
- Ogasawara, R., Fujita, S., Hornberger, T.A., Kitaoka, Y., Makanae, Y., Nakazato, K., & Naokata, I. (2016). The role of mTOR signalling in the regulation of skeletal muscle mass in a rodent model of resistance exercise. *Scientific Reports*, 6, 31142. doi:[10.1038/srep31142](https://doi.org/10.1038/srep31142)
- Ogasawara, R., Sato, K., Matsutani, K., Nakazato, K., & Fujita, S. (2014). The order of concurrent endurance and resistance exercise modifies mTOR signaling and protein synthesis in rat skeletal muscle. *American Journal of Physiology—Endocrinology and Metabolism*, 306(10), E1155–E1162. PubMed ID: [24691029](https://pubmed.ncbi.nlm.nih.gov/24691029/) doi:[10.1152/ajpendo.00647.2013](https://doi.org/10.1152/ajpendo.00647.2013)
- Price, J.C., Khambatta, C.F., Li, K.W., Bruss, M.D., Shankaran, M., Dalidd, M., . . . Hellerstein, M.K. (2012). The effect of long term calorie restriction on in vivo hepatic proteostasis: A novel combination of dynamic and quantitative proteomics. *Molecular & Cellular Proteomics*, 11(12), 1801–1814. PubMed ID: [22984287](https://pubmed.ncbi.nlm.nih.gov/22984287/) doi:[10.1074/mcp.M112.021204](https://doi.org/10.1074/mcp.M112.021204)
- Qiao, Q., Bouwman, F.G., Baak, M.A.V., Renes, J., & Mariman, E.C.M. (2019). Glucose restriction plus refeeding in vitro induce changes of the human adipocyte secretome with an impact on complement factors and cathepsins. *International Journal of Molecular Sciences*, 20(16), 4055. doi:[10.3390/ijms20164055](https://doi.org/10.3390/ijms20164055)
- Robinson, M.M., Turner, S.M., Hellerstein, M.K., Hamilton, K.L., & Miller, B.F. (2011). Long-term synthesis rates of skeletal muscle DNA and protein are higher during aerobic training in older humans than in sedentary young subjects but are not altered by protein supplementation. *The FASEB Journal*, 25(9), 3240–3249. doi:[10.1096/fj.11-186437](https://doi.org/10.1096/fj.11-186437)
- Rooyackers, O.E., Adey, D.B., Ades, P.A., & Nair, K.S. (1996). Effect of age on in vivo rates of mitochondrial protein synthesis in human skeletal muscle. *Proceedings of the National Academy of Sciences of the United States of America*, 93(26), 15364–15369. PubMed ID: [8986817](https://pubmed.ncbi.nlm.nih.gov/8986817/) doi:[10.1073/pnas.93.26.15364](https://doi.org/10.1073/pnas.93.26.15364)
- Shankaran, M., King, C.L., Angel, T.E., Holmes, W.E., Li, K.W., Colangelo, M., . . . Hellerstein, M.K. (2016a). Circulating protein synthesis rates reveal skeletal muscle proteome dynamics. *Journal of Clinical Investigation*, 126(1), 288–302. doi:[10.1172/JCI79639](https://doi.org/10.1172/JCI79639)
- Shankaran, M., Shearer, T.W., Stimpson, S.A., Turner, S.M., King, C., Wong, P.-Y., . . . Evans, W.J. (2016b). Proteome-wide muscle protein fractional synthesis rates predict muscle mass gain in response to a selective androgen receptor modulator in rats. *American Journal of Physiology—Endocrinology and Metabolism*, 310(6), E405–E417. doi:[10.1152/ajpendo.00257.2015](https://doi.org/10.1152/ajpendo.00257.2015)
- Sollanek, K.J., Burniston, J.G., Kavazis, A.N., Morton, A.B., Wiggs, M.P., Ahn, B., . . . Powers, S.K. (2017). Global proteome changes in the rat diaphragm induced by endurance exercise training. *PLoS One*, 12(1), e0171007. PubMed ID: [28135290](https://pubmed.ncbi.nlm.nih.gov/28135290/) doi:[10.1371/journal.pone.0171007](https://doi.org/10.1371/journal.pone.0171007)
- Vogel, M.A.A., Wang, P., Bouwman, F.G., Hoebers, N., Blaak, E.E., Renes, J., . . . Goossens, G.H. (2019). A comparison between the abdominal and femoral adipose tissue proteome of overweight and obese women. *Scientific Reports*, 9(1), 4202. PubMed ID: [30862933](https://pubmed.ncbi.nlm.nih.gov/30862933/) doi:[10.1038/s41598-019-40992-x](https://doi.org/10.1038/s41598-019-40992-x)
- Wang, D., Liem, D.A., Lau, E., Ng, D.C., Bleakley, B.J., Cadeiras, M., . . . Ping, P. (2014). Characterization of human plasma proteome dynamics using deuterium oxide. *Proteomics Clinical Applications*, 8(7–8), 610–619.
- Wang, W., Ding, Z., Solares, G.J., Choi, S.-M., Wang, B., Yoon, A., . . . Ivy, J.L. (2017). Co-ingestion of carbohydrate and whey protein increases fasted rates of muscle protein synthesis immediately after resistance exercise in rats. *PLoS One*, 12(3), e0173809. PubMed ID: [28296942](https://pubmed.ncbi.nlm.nih.gov/28296942/) doi:[10.1371/journal.pone.0173809](https://doi.org/10.1371/journal.pone.0173809)
- Werme, M., Thorén, P., Olson, L., & Brené, S. (1999). Addiction-prone Lewis but not Fischer rats develop compulsive running that coincides with downregulation of nerve growth factor inducible-B and neuron-derived orphan receptor 1. *The Journal of Neuroscience*, 19(14), 6169–6174. PubMed ID: [10407052](https://pubmed.ncbi.nlm.nih.gov/10407052/) doi:[10.1523/JNEUROSCI.19-14-06169.1999](https://doi.org/10.1523/JNEUROSCI.19-14-06169.1999)
- Wilkinson, D.J., Franchi, M.V., Brook, M.S., Narici, M.V., Williams, J.P., Mitchell, W.K., . . . Smith, K. (2014). A validation of the application of D₂O stable isotope tracer techniques for monitoring day-to-day changes in muscle protein subfraction synthesis in humans. *American Journal of Physiology—Endocrinology and Metabolism*, 306(5), E571–E579. PubMed ID: [24381002](https://pubmed.ncbi.nlm.nih.gov/24381002/) doi:[10.1152/ajpendo.00650.2013](https://doi.org/10.1152/ajpendo.00650.2013)
- Wilkinson, S.B., Phillips, S.M., Atherton, P.J., Patel, R., Yarasheski, K.E., Tarnopolsky, M.A., & Rennie, M.J. (2008). Differential effects of resistance and endurance exercise in the fed state on signalling molecule phosphorylation and protein synthesis in human muscle. *The Journal of Physiology*, 586(15), 3701–3717. PubMed ID: [18556367](https://pubmed.ncbi.nlm.nih.gov/18556367/) doi:[10.1113/jphysiol.2008.153916](https://doi.org/10.1113/jphysiol.2008.153916)
- Wong, T.S., & Booth, F.W. (1990). Protein metabolism in rat gastrocnemius muscle after stimulated chronic concentric exercise. *Journal of Applied Physiology*, 69(5), 1709–1717. doi:[10.1152/jappl.1990.69.5.1709](https://doi.org/10.1152/jappl.1990.69.5.1709)
- Zangarelli, A., Chanseaux, E., Morio, B., Brugère, C., Mosoni, L., Rousset, P., . . . Walrand, S. (2006). Synergistic effects of caloric restriction with maintained protein intake on skeletal muscle performance in 21-month-old rats: A mitochondria-mediated pathway. *The FASEB Journal*, 20(14), 2439–2450. doi:[10.1096/fj.05-4544com](https://doi.org/10.1096/fj.05-4544com)