Skeletal muscle protein synthesis and the abundance of the mRNA translation initiation repressor PDCD4 are inversely regulated by fasting and refeeding in rats

Sana Zargar,1,2 Tracy S. Moreira,1,2 Helena Samimi-Seisan,1,2 Senthure Jeganathan,1,2 Dhanshri Kakade,1 Nushaba Islam,1 Jonathan Campbell,1 and Olasunkanni A. J. Adegoke1,2

1School of Kinesiology and Health Science and 2Muscle Health Research Centre, York University, Toronto, Ontario, Canada

Submitted 24 November 2010; accepted in final form 9 March 2011

Zargar S, Moreira TS, Samimi-Seisan H, Jeganathan S, Kakade D, Islam N, Campbell J, Adegoke OA. Skeletal muscle protein synthesis and the abundance of the mRNA translation initiation repressor PDCD4 are inversely regulated by fasting and refeeding in rats. Am J Physiol Endocrinol Metab 300: E986–E992, 2011. First published March 15, 2011; doi:10.1152/ajpendo.00642.2010.—Optimal skeletal muscle mass is vital to human health, because defects in muscle protein metabolism underlie or exacerbate human diseases. The mammalian target of rapamycin complex 1 is critical in the regulation of mRNA translation and protein synthesis. These functions are mediated in part by the ribosomal protein S6 kinase 1 (S6K1) through mechanisms that are poorly understood. The tumor suppressor programmed cell death 4 (PDCD4) has been identified as a novel principal substrate, the ribosomal S6 protein kinase 1 (S6K1) (32). Upon activation, this complex phosphorylates two principal substrates, the ribosomal S6 protein kinase 1 (S6K1) and the eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1) (20, 40). Phosphorylation of S6K1 leads to its activation, whereas 4E-BP1, an inhibitor of cap-dependent mRNA translation initiation, is inhibited by phosphorylation. S6K1 mediates the growth-promoting action of mTORC1, whereas inhibition of 4E-BP1 increases cell proliferation (9, 16).

S6K1 is critical to the regulation of skeletal muscle mass because mice lacking this enzyme have reduced muscle mass (3, 38). In several studies, stimulation of skeletal muscle protein synthesis by amino acids and insulin occurs in concert with increasedThr189 phosphorylation of this kinase, whereas the level of phosphorylated S6K1 is reduced by nutrient deprivation (3, 5, 8, 19, 22, 34, 37, 38).

The exact mechanisms by which activated S6K1 promotes skeletal muscle protein metabolism and mass are not clear but appear to include at least two pathways. First, S6K1 can phosphorylate the ribosomal protein S6 (33). This is thought to lead to the translation of a subset of ribosomal proteins critical for protein synthesis. This view has been challenged, because S6 phosphorylation is not reduced in the absence of S6K1, nor does restoration of phosphorylated S6 levels rescue growth defects seen in S6K1-null muscle cells (37, 44). Second, S6K1 may promote protein synthesis by phosphorylating eIF4B (28).

This stimulates the association of the latter with the eukaryotic initiation factor 3 complex, leading to increased mRNA translation.

Recently, programmed cell death 4 (PDCD4) was described as a substrate of S6K1 (15). In its unphosphorylated state, PDCD4 inhibits mRNA translation by binding to and inhibiting eIF4A (25, 27) and -4G (30, 51). Upon growth factor stimulation, PDCD4 is phosphorylated by S6K1. This modification targets it for degradation by the ubiquitin protein ligase β-transducin repeat-containing protein (15). As a result its inhibition of mRNA translation is relieved, and protein synthesis can proceed (15). It is presently unknown whether PDCD4 protein is expressed in skeletal muscle, nor is it clear whether it is regulated by nutrients. These questions are critical because muscle is the single largest contributor to whole body protein metabolism, and the mTORC1/S6K1 pathway that regulates its growth is nutrient sensitive (31).

Overactivation of S6K1 is implicated in experimental and human insulin resistance, obesity, and cancer (9, 17, 48, 49). It is crucial to identify the mechanisms by which this kinase regulates protein metabolism in skeletal muscle. Such an understanding can lead to the dissection of the anabolic effects of this kinase from the undesirable consequences of its activities. Our objectives were to use the feed deprivation refeeding model in the rat as well as in vitro muscle cell culture to examine the regulation of PDCD4 in skeletal muscle and to relate changes in fractional rates of protein synthesis to the regulation of PDCD4. We also investigated whether depleting

OPTIMAL SKELETAL MUSCLE MASS AND METABOLISM are critical to the regulation of whole body substrate homeostasis and health. Indeed, in several diseases, including obesity, diabetes, and cancer, defects in muscle metabolism underlie or exacerbate the metabolic outcomes (21, 24, 41).

The growth factor- and nutrient-sensitive kinase complex mammalian target of rapamycin complex 1 (mTORC1) is now recognized as a master regulator of skeletal muscle mass (6, 7, 42, 43). Upon activation, this complex phosphorylates two principal substrates, the ribosomal S6 protein kinase 1 (S6K1) and the eukaryotic initiation factor (eIF) 4E-binding protein 1 (4E-BP1) (20, 40). Phosphorylation of S6K1 leads to its activation, whereas 4E-BP1, an inhibitor of cap-dependent mRNA translation, is inhibited by phosphorylation. S6K1 mediates the growth-promoting action of mTORC1, whereas inhibition of 4E-BP1 increases cell proliferation (9, 16).

S6K1 is critical to the regulation of skeletal muscle mass because mice lacking this enzyme have reduced muscle mass (3, 38). In several studies, stimulation of skeletal muscle protein synthesis by amino acids and insulin occurs in concert with increased Thr189 phosphorylation of this kinase, whereas the level of phosphorylated S6K1 is reduced by nutrient deprivation (3, 5, 8, 19, 22, 34, 37, 38).

The exact mechanisms by which activated S6K1 promotes skeletal muscle protein metabolism and mass are not clear but appear to include at least two pathways. First, S6K1 can phosphorylate the ribosomal protein S6 (33). This is thought to lead to the translation of a subset of ribosomal proteins critical for protein synthesis. This view has been challenged, because S6 phosphorylation is not reduced in the absence of S6K1, nor does restoration of phosphorylated S6 levels rescue growth defects seen in S6K1-null muscle cells (37, 44). Second, S6K1 may promote protein synthesis by phosphorylating eIF4B (28).

This stimulates the association of the latter with the eukaryotic initiation factor 3 complex, leading to increased mRNA translation.

Recently, programmed cell death 4 (PDCD4) was described as a substrate of S6K1 (15). In its unphosphorylated state, PDCD4 inhibits mRNA translation by binding to and inhibiting eIF4A (25, 27) and -4G (30, 51). Upon growth factor stimulation, PDCD4 is phosphorylated by S6K1. This modification targets it for degradation by the ubiquitin protein ligase β-transducin repeat-containing protein (15). As a result its inhibition of mRNA translation is relieved, and protein synthesis can proceed (15). It is presently unknown whether PDCD4 protein is expressed in skeletal muscle, nor is it clear whether it is regulated by nutrients. These questions are critical because muscle is the single largest contributor to whole body protein metabolism, and the mTORC1/S6K1 pathway that regulates its growth is nutrient sensitive (31).

Overactivation of S6K1 is implicated in experimental and human insulin resistance, obesity, and cancer (9, 17, 48, 49). It is crucial to identify the mechanisms by which this kinase regulates protein metabolism in skeletal muscle. Such an understanding can lead to the dissection of the anabolic effects of this kinase from the undesirable consequences of its activities. Our objectives were to use the feed deprivation refeeding model in the rat as well as in vitro muscle cell culture to examine the regulation of PDCD4 in skeletal muscle and to relate changes in fractional rates of protein synthesis to the regulation of PDCD4. We also investigated whether depleting

Address for reprint requests and other correspondence: O. A. J. Adegoke, School of Kinesiology and Health Science, 4700 Keele St., Toronto, ON, M3J 1P3, Canada (e-mail: oadegoke@yorku.ca).
PDCD4 by RNA interference would modulate phenylalanine incorporation into proteins in myoblasts.

MATERIALS AND METHODS

Chemicals and Reagents

Hors eradish peroxidase chemiluminescence substrate was obtained from Millipore (Billerica, MA), ECL Plus reagents from GE Healthcare (Piscataway, NJ), l-[2,3,4,5,6,3H]phenylalanine from GE Healthcare or American Radiolabeled Chemicals (St. Louis, MO), protease inhibitor cocktail, phosphatase inhibitor cocktail, l-tyrosine decarboxylase, pyridoxal phosphate, benzamidine, l-leucine, l-leucyl-l-alanine, sodium vanadate, ninhydrin, chloroform, and n-heptane from Sigma-Aldrich (St. Louis, MO), Pierce bicinchoninic acid protein assay kit from Thermo Fisher Scientific (Rockford, IL), l-phenylalanine from Calbiochem (San Diego, CA), and α-modification of Eagle’s medium (AMEM), antibiotic and antimitotic preparation, and trypsin from Wisent (St. Bruno, QC, Canada). Other reagents were of analytical grade.

Antibodies

Antibodies to PDCD4, S6K1, Thr389 S6K1, ribosomal protein S6 (S6), Ser235/236 S6, mTOR, Ser2448 mTOR, Ser473 Akt, horseradish peroxidase-conjugated anti-mouse, and anti-rabbit IgG were purchased from Cell Signaling Technology (Danvers, MA). Antibodies to Ser67 PDCD4 and γ-tubulin were obtained from Sigma-Aldrich.

Animals

Male Sprague-Dawley rats ranging from 50 to 70 g were purchased from Charles River Laboratories. We chose rats of this age because in them rates of muscle protein synthesis are high and sensitive to nutrient deprivation/provision (14). Rats were acclimatized in the animal facility at York University while being maintained at the standard 12:12-h light-dark cycle (lights on at 0700). They had free access to a nonpurified rat diet (50) and water. From 6 wk of age, rats were handled daily to minimize handling stress on the day of the experiment. All experiments were approved by the York University Institutional Animal Welfare Committee, and the guidelines of the Canadian Council on Animal Care were followed.

Study Design

Effects of feed deprivation and refeeding on PDCD4 expression and regulation in skeletal muscle. Rats were assigned to one of two groups: 1) fed (CTL) or 2) 48-h feed deprivation (FD). Water was provided at all times. A subgroup of the FD group (n = 6) was studied at the end of deprivation. Other FD rats were refed for 2 h (2-h RFD; n = 6). To determine the fractional rates of protein synthesis, we used the flooding dose technique (23) as modified by Jepson et al. (29) for the intraperitoneal delivery of radioactive phenylalanine. Ten to fifteen minutes later they were euthanized by decapitation, and gastrocnemius muscle samples were processed from Cell Signaling Technology (Danvers, MA). Antibodies to Ser67 PDCD4 and γ-tubulin were obtained from Sigma-Aldrich.

Statistical analysis. Values are presented as means ± SE. Treatment means were compared by one-way ANOVA and differences among means assessed using the Bonferroni multiple comparison test. When analyses revealed that variances were different (as was the case for Fig. 2, C and D), we performed nonparametric analyses (Kruskal-Wallis) and used Dunn’s post hoc test to identify means that differed from one another. For comparison of the effect of PDCD4 depletion on phenylalanine incorporation into proteins, we used an unpaired t-test. Analyses were done using GraphPad (version 3; GraphPad Software, La Jolla, CA). The level of significance was set at P ≤ 0.05.
RESULTS

**PDCD4 expression in skeletal muscle and its regulation during a feed deprivation and refeeding cycle.** PDCD4 is a 464-AA residue protein (35) that migrates with the 55-kDa protein marker (Fig. 1A). Since its expression at the protein level has not been shown in muscle cells, and to confirm that

![Fig. 1. Programmed cell death 4 (PDCD4) expression and regulation in skeletal muscle in response to feed deprivation and refeeding.](image1.png)

Fig. 1. Programmed cell death 4 (PDCD4) expression and regulation in skeletal muscle in response to feed deprivation and refeeding. A: PDCD4 protein level in L6 myoblasts treated with scramble siRNA or 1 of 2 siRNA oligonucleotides designed against PDCD4. B and C: PDCD4 phosphorylation (B) and abundance (C) in rat skeletal muscle in CTL (fed), in response to 48-h feed deprivation (FD), and FD followed by refeeding. Values are means ± SE; n = 6. Means without a common symbol differ; P < 0.05. RFD, refeeding; MW, molecular weight.

![Fig. 2. Mammalian target of rapamycin (mTOR) signaling and fractional rates of protein synthesis in skeletal muscle in response to FD and RFD.](image2.png)

Fig. 2. Mammalian target of rapamycin (mTOR) signaling and fractional rates of protein synthesis in skeletal muscle in response to FD and RFD. Phosphorylation (ph) of mTOR (A and B) and ribosomal S6 protein kinase 1 (S6K1; A and C) and protein synthesis (D) in rat skeletal muscle in CTL, in response to FD, and FD followed by RFD. Values are means ± SE; n = 6. Means without a common symbol differ; P < 0.05.
after administration of AA by gavage. PDCD4 phosphorylation (A) and abundance (B) and S6K1 phosphorylation (C) in rat skeletal muscle in CTL, in response to FD, and in FD followed by amino acid gavage. Values are means ± SE; n = 7–8 for A and B; n = 5 for C. Means without a common symbol differ; P < 0.05.

the detected signal is for the authentic protein, we knocked down PDCD4 using two different siRNA oligonucleotides. PDCD4 levels were lower only in L6 myoblasts treated with siRNA designed against this protein. We reproducibly detected PDCD4 in skeletal (mixed gastrocnemius) muscle (Fig. 1, B and C). FD significantly suppressed its Ser67 phosphorylation (P < 0.05). This was reversed during refeeding. When expressed relative to γ-tubulin, phosphorylation of PDCD4 tended to be lower in FD than in CTL (P = 0.10; data not shown). Upon phosphorylation, PDCD4 is degraded (15). Compared with CTL, FD increased the abundance of PDCD4 by ~60% (P < 0.05), but this was attenuated in 2-h RFD (Fig. 1C). In another experiment with rats that were subjected to an 18-h FD, leucine gavage tended to suppress soleus muscle PDCD4 abundance relative to FD (P = 0.08; Supplemental Fig. S1; Supplemental Material for this article can be found online at the AJP-Endocrinology and Metabolism website).

The observed changes in PDCD4 occurred concurrently with the activation of mTOR (as measured in Ser2448 phosphorylation) and in particular with the activation of S6K1 (Thr389 phosphorylation), the presumed kinase for PDCD4. Indeed, in RFD, increased muscle fractional rates of protein synthesis, as well as the phosphorylation of mTOR, S6K1, and S6, occurred in parallel to one another and to the phosphorylation of PDCD4 (Fig. 1B and 2, A–D, and Supplemental Fig. S2).

Oral amino acid administration can mediate the effect of feeding on PDCD4 abundance. Rather than refeeding feed-deprived rats, we gavaged them with a mixture of essential and nonessential AA. Relative to CTL, FD did not have a significant effect on PDCD4 phosphorylation but significantly increased its abundance (Fig. 3, A and B). Compared with CTL, PDCD4 phosphorylation was lower at 2 h after the AA gavage but was not different from CTL at other time points. Relative to FD, AA gavage, just like refeeding, significantly reduced PDCD4 abundance at 30 min after the gavage. At other time points after the gavage, except at 2 and 6 h, PDCD4 abundance was not different from control. In addition, AA gavage stimulated the phosphorylation of S6K1 and S6, although the response was variable (Fig. 3C and Supplemental Fig. S3). There was no effect of AA gavage on Akt Ser473 phosphorylation (Supplemental Fig. S4). Fractional rates of protein synthesis were suppressed by FD, but the rates at 1 and 2 h after AA gavage were not different from CTL; however, these values were also not different from FD (Table 1).

**Table 1.** Skeletal muscle fractional rates of protein synthesis and plasma total BCAA in CTL and FD rats and in FD rats after administration of AA by gavage

<table>
<thead>
<tr>
<th>Protein synthesis, %/day</th>
<th>CTL</th>
<th>FD</th>
<th>30 min</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.9 ± 1.3*</td>
<td>7.3 ± 1.6†</td>
<td>6.6 ± 1.6†</td>
<td>9.8 ± 1.9†</td>
<td>8.5 ± 1.6†</td>
<td>8.0 ± 2.1†</td>
<td>7.8 ± 1.3†</td>
</tr>
</tbody>
</table>

| BCAA, μmol/l | 447 ± 75† | 469 ± 80† | 1,378 ± 140* | 1,498 ± 204* | 759 ± 59† | 617 ± 40† | 452 ± 109† |

Values are means ± SE. BCAA, branched-chain amino acids; CTL, fed; FD, feed-deprived; AA, amino acids. Skeletal muscle (mixed gastrocnemius) fractional rates of protein synthesis and plasma levels of BCAA were determined as described in MATERIALS AND METHODS. For protein synthesis, n = 7–8, except for 30 min of AA, for which n = 6. For BCAA, n = 5–6, except for 6 h of AA, for which n = 4. Means within a row and without a common symbol differ (P < 0.05).
In muscle cells, PDCD4 abundance is negatively regulated by serum. To show that our observations of the expression and regulation of this protein in skeletal muscle were not due to contamination with nonmuscle cells, we studied L6 myoblasts, a model that has been used by others to examine the regulation of S6K1 and mRNA translation by leucine (32). Serum deprivation for 24 h did not have any significant effect on PDCD4 phosphorylation or abundance (Fig. 4, A–C). Compared with serum deprivation, readmission of serum significantly increased PDCD4 phosphorylation and reduced its abundance in a time-dependent manner (Fig. 4, A–C).

Phenylalanine incorporation into proteins is higher in starved myoblasts depleted of PDCD4. When cultured in growth medium, phenylalanine incorporation into proteins tended to be higher in L6 myoblasts depleted of PDCD4 (by 13%, \( P = 0.08 \)). However, when cells were grown in an AA- and serum-free medium, this measurement more than doubled in cells depleted of PDCD4 (\( P < 0.0001 \); Fig. 4D). Similar results were obtained with a second siRNA oligonucleotide designed against a different region of PDCD4 (Supplemental Fig. S5), indicating the specificity of the effect.

**DISCUSSION**

We have demonstrated that, in rat skeletal muscle, fractional rates of protein synthesis and the levels of PDCD4 were inversely regulated by feed deprivation/refeeding. In rats that were either fed or feed deprived and then refed, fractional rates of protein synthesis and the activation of S6K1 were higher, whereas the amount of PDCD4 was lower. The reverse was the case in feed-deprived rats. Moreover, when L6 cells were cultured in an AA-free medium, the rate of phenylalanine incorporation into proteins was significantly higher in cells depleted of PDCD4 compared with cells with normal levels of the protein. Thus, our data indicate that PDCD4 is a nutrient- and growth factor-sensitive negative regulator of protein synthesis in skeletal muscle and in myoblasts.

That S6K1 is important in regulating muscle mass is incontrovertible (3, 38). What has been unclear is the mechanism by which it acts. This is an important question because S6K1 activities cause muscle growth, mediate changes in insulin-stimulated glucose uptake, and are implicated in insulin resistance, obesity, and cancer (9, 17, 48, 49). Identifying the specific pathways by which S6K1 regulates protein synthesis can permit the isolation of the muscle growth-promoting functions of the kinase from its undesirable effects in insulin resistance. A previous study identified S6K1 as the kinase that, upon mitogen stimulation, phosphorylates PDCD4 and targets it for ubiquitination (15). Our data demonstrating the expression of PDCD4 in skeletal muscle and in muscle cells, and the fact that it is regulated by feed deprivation/refeeding, indicate that this protein likely plays an important role in regulating muscle protein status. We also showed that amino acids, in the absence of elevated systemic insulin, regulated PDCD4. This further highlights the significance of this protein as a possible nutrient-responsive regulator of protein synthesis. However, the overactivation of mTORC1 and S6K1, kinases that are critical in regulating muscle mass, is implicated in cancer (13), and PDCD4 is a tumor suppressor (47). A preferred strategy to increase muscle protein synthesis and mass could involve interventions that selectively promote PDCD4 degradation in skeletal muscle so that muscle protein anabolism is promoted without increased tumor risk in nonskeletal muscle tissues.

Our attempt to delineate the components of food that mediated the changes in PDCD4 and S6K1 regulation and protein synthesis revealed some differences between the effects of refeeding vs. AA gavage. Whereas increased phosphorylation of PDCD4 was seen at 2 h after the start of refeeding, the effect of AA gavage was less clear. However, in both studies, the...
abundance of PDCD4 was suppressed by refeeding or AA gavage. In addition, fractional rates of protein synthesis increased from the feed-deprived state to 2 h postrefeeding. There was some interanimal variability in the amino acid gavage groups. This was not due to differential enrichments of phenylalanine in the tissue free pools in the amino acid gavage groups, because these measurements were not different from CTL (data not shown). The variability was likely due to differential responses of the rats to the amino acid gavage. Nevertheless, fractional rates of protein synthesis at 1 and 2 h after AA gavage were not different from CTL. Thus, refeeding and, to a lesser extent, amino acid gavage induced fractional rate of protein synthesis; both suppressed PDCD4 abundance. Notably, there was no insulin response to the AA gavage, although we did not measure the level of the hormone at 30 min postgavage. The changes in PDCD4 phosphorylation induced by refeeding vs. amino acid gavage suggest a role for Akt (45) and insulin (and perhaps components of the feed other than amino acids) (4) in mediating the effects of feeding on PDCD4 phosphorylation. Our finding that changes in PDCD4 abundance preceded changes in muscle fractional rates of protein synthesis (Fig. 3B and Table 1) suggests that, irrespective of what those other factors might be, they regulate protein synthesis and PDCD4 abundance in tandem.

Although both the phosphorylation status and the abundance of PDCD4 are regulated by mitogen-induced activation of S6K1 (15), it is not clear which is more important. In the case of 4E-BP1, which like PDCD4 inhibits eIF4F formation and cap-dependent mRNA translation, its phosphorylation is enough to relieve its inhibition of eIF4E (40). PDCD4 inhibits eIF4A in a dominant manner, since one molecule of this protein binds 2 and inactivates two molecules of eIF4A. It also prevents the binding of eIF4A to eIF4G (10, 35, 36). The site of S6K1 phosphorylation on PDCD4, Ser67, is not involved in the interaction of the protein with eIF4A (10, 36). In fact, a truncated protein that lacks the NH2-terminal region within which Ser67 is located efficiently binds to and inhibits eIF4A helicase activity (10). Our findings indicate that the abundance of PDCD4, rather than its phosphorylation per se, may be the critical factor in the regulation of mRNA translation. Indeed, in many of the conditions tested, changes in the abundance of PDCD4 mirrored the alterations in fractional rates of protein synthesis and phenylalanine incorporation into proteins more closely than changes in PDCD4 phosphorylation (compare Fig. 1, B and C, with Fig. 2D and Fig. 3B with Table 1; also see Fig. 4).

PDCD4 mRNA is abundant in several tissues, especially liver, lung, and kidney, but only very low levels are detected in skeletal and heart muscles (39, 46). To our knowledge, this is the first report of the expression of PDCD4 protein in skeletal muscle. Although we cannot completely rule out the expression of this protein in other cell types, our data from L6 myoblasts indicate that the expression of PDCD4 seen in rat skeletal muscle could not be ascribed solely to other cell types. We further demonstrated that, in myoblasts cultured in an amino acid- and serum-free medium, phenylalanine incorporation into proteins was significantly higher in cells depleted of PDCD4 compared with cells with normal levels of this protein. Thus, our data indicate that the abundance of PDCD4 is implicated, at least in part, in the suppression of muscle fractional rate of protein synthesis seen during feed deprivation. An extrapolation of our findings in myoblasts to whole muscle and animal protein metabolism is not possible. This is because cell incubation media do not accurately replicate the in vivo environment (18). As such, an assessment of the precise contribution of PDCD4 to skeletal and whole body protein status will require studying protein metabolism in PDCD4 knockout animals. However, our data provide a starting point on which such studies can be based.

In conclusion, our data indicate that the abundance of PDCD4 may be important in regulating skeletal muscle fractional rates of protein synthesis in response to feed deprivation and refeeding. When S6K1 levels are depleted, PDCD4 degradation is delayed, although at later times its levels decrease (15). If our findings in the cell culture experiments are confirmed in animal (or muscle specific) knockouts of PDCD4 or in studies where siRNA/shRNA that targets PDCD4 is transfected into a limb muscle, strategies that diminish the abundance of this protein without a requirement for S6K1 could constitute attractive interventions in enhancing muscle protein anabolism and mass.

ACKNOWLEDGMENTS

We thank Mathew Krause for help with muscle dissection and Drs. David Hood and Michael Riddell for proofreading the manuscript. Present address for T. S. Moreira: Department of Nutritional Sciences, Faculty of Medicine, University of Toronto, Toronto, ON, Canada M5S 3E2. Present address for J. Campbell: Department of Medicine, Mount Sinai Hospital, Toronto, ON, Canada MSG 1X5.

GRANTS

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and by Minor and Junior Faculty Funds from the Faculty of Health, York University, to O. A. J. Adegoke.

DISCLOSURES

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

REFERENCES

PDCD4 AND PROTEIN SYNTHESIS IN SKELETAL MUSCLE


