Effects of Vitamin D_3 on *in vivo* Labelling of Chick Skeletal Muscle Proteins with [^{3}H]Leucine

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The effects of the administration of a single oral dose of vitamin D_3 to rachitic chicks on the *in vivo* incorporation of [^{3}H]leucine to proteins of skeletal muscle subcellular fractions was studied. A significant stimulation (50%) of labelling of mitochondrial proteins could be seen. The sterol affected the labelling of sarcoplasmic reticulum (20%) and contractile proteins (10%) to a lesser extent. These results confirm previous observations which implicate vitamin D_3 in the synthesis of mitochondrial proteins.

Introduction

Muscle weakness is a common symptom of nutritional vitamin D deficiency and diseases in which its metabolism is impaired [1–3]. This myopathy may be related to alterations in the mechanisms which regulate sarcoplasmic Ca^{2+} concentration in the muscle cell. Thus, it has been reported that in vitamin D depletion Ca transport by isolated sarcoplasmic reticulum (SR) and sarcolemma is diminished [4, 5]. In addition, the ability of mitochondria to accumulate Ca *in vivo* is impaired [6]. The changes observed in SR and sarcolemma may be related to modifications in phospholipid content and/or composition [5, 7]. In mitochondria, however, changes in protein profiles obtained by polyacrylamide gel electrophoresis were predominant [7]. In order to provide sufficient evidence about the involvement of vitamin D_3 in mitochondrial protein synthesis *in vivo* the effects of the sterol on the incorporation of radioactive aminoacid were studied.

Materials and Methods

One day-old chicks were raised 3–4 weeks on a vitamin D-deficient diet prepared as described previously [8]. Ca and P levels were 1.6% and 0.16% respectively. Treated animals were given a single oral dose of vitamin D_3 (10 µg) while the control group received placebo (propylene glycol). 8 h later both groups were injected i.p. with 100 µCi of L-[4,5-^{3}H]leucine (60 Ci/mmol; New England Nuclear Corporation). The animals were sacrificed 24 h after injection of the radioactive precursor. Leg skeletal muscles were quickly dissected, made free of connective tissue and fat and homogenized with 0.1 M KCl, 0.3 M sucrose, 0.01 M histidine pH 7.4 (4 vol/g muscle) during 1 min using a Virtis homogenizer. Myofibrils, mitochondria, sarcoplasmic reticulum and cytosolic fraction were isolated by differential centrifugation as published elsewhere [7]. Fractions were precipitated and repeatedly washed with 5% trichloro-acetic acid to remove nonprotein radioactivity. They were then solubilized by heating in 1 N NaOH. Aliquots were taken for protein measurement [9] and determination of radioactivity in a Beckman liquid scintillation spectrometer using Aquasol (New England Nuclear) as scintillation fluid. Specific activities of subcellular fractions from vitamin D-deficient and vitamin D-treated animals were corrected for differences in free leucine pool size. An aliquot of the homogenate was taken prior to centrifugation. Proteins were removed by addition of trichloroacetic acid and centrifugation. The supernatant was analyzed for its free leucine content using a Beckman automatic aminoacid analyzer and radioactivity as described above. The significance of the results was evaluated by Student's t test [10].

Results and Discussion

Labelling with [^{3}H]leucine of skeletal muscle subcellular fractions was compared in vitamin D-deficient chicks and chicks treated with an acute dose of vitamin D_3. The results are shown in Table I. Myofibrils and cytosol contained 50% and 48%, respectively, of the total protein bound radioactivity recovered, whereas a small percentage of [^{3}H]leucine was incorporated to sarcoplasmic reticulum and mitochondrial proteins. Specific activity of labelled proteins (expressed in nmol leucine/mg protein when corrected for differences in free leucine pool size) was highest in cytosol followed by mitochondria and SR. Myofibrils were markedly less

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Table I. Effects of vitamin D_3 on the incorporation of [^{3}H]leucine to proteins of chick skeletal muscle subcellular fractions. Treatment with vitamin D_3 and labelling of animals are described under Materials and Methods. Percentages of total radioactivity recovered in fractions are given. Data represent the mean ± SD; $n = 4$. *p < 0.10, **p < 0.010, ***p < 0.005, with respect to vitamin D-deficient controls.

	− Vitamin D		+ Vitamin D_3	
	%	nmol/leu/mg prot	%	nmol/leu/mg prot
Myofibrils	50.20 ± 4.43	1.95 ± 0.15	50.46 ± 3.86	2.13 ± 0.17
Mitochondria	0.26 ± 0.05	6.50 ± 0.81	0.42 ± 0.07**	9.74 ± 0.92***
S. reticulum	1.47 ± 0.12	4.64 ± 0.62	1.53 ± 0.19	5.56 ± 0.60*
Cytosol	48.10 ± 4.73	26.13 ± 3.13	47.60 ± 4.76	27.73 ± 3.05

labelled. This may be related to differences in turnover rates of skeletal muscle proteins [11–13].

A differential action of vitamin D_3 on incorporation of [^{3}H]leucine to muscle fractions was observed. The sterol markedly increased labelling of mitochondrial proteins (50%) and to a lesser extent SR proteins (20%). The incorporation of radioactive precursor to the myofibrillar fraction was only slightly stimulated (10%) whereas labelling of soluble cytosolic proteins was practically unchanged.

Increased labelling of muscle mitochondrial proteins in chicks treated with vitamin D_3 may reflect an effect of the sterol on mitochondrial protein synthesis. It could be previously shown that administration of the sterol to rachitic chicks during one week induced marked changes in the relative amounts of mitochondrial inner membrane proteins as indicated by electrophoretic profiles. No changes in the composition of sarcoplasmic reticulum and cytosolic proteins were detected. However, treatment with vitamin D_3 modified the distribution pattern of components of the actomyosin contractile complex [7]. Lack of pronounced changes in labelling of the myofibrillar fraction in this study may be explained by the fact that treatment time with vitamin D_3 may have not been of sufficient duration considering the lower turnover rates of contractile proteins [11–13].

Previous studies have implied vitamin D_3 and/or derived metabolites in muscle mitochondrial function. It has been reported that vitamin D_3 increases the ability of vitamin D-deficient chick skeletal muscle mitochondria to accumulate Ca *in vivo* [6]. In rat heart muscle changes in oxidative phosphorylation were, in addition, observed [14]. The involvement of 25 OHD_3 and 1.25 $(OH)_2D_3$ in muscle mitochondrial Ca fluxes has been recently shown in cultured muscle [15]. These changes may be connected to the effects of vitamin D_3 on mitochondrial protein synthesis. Identification of the proteins affected by vitamin D_3 is required, however, to establish such correlation. The information may be of potential interest in understanding the biochemical basis of the myopathy associated to vitamin D deficiency and perturbations of its metabolism.

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