The effect of exercise on protein turnover in isolated soleus and extensor digitorium longus muscles

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Summary. The rate of protein degradation was found to be increased in isolated soleus and extensor digitorum muscles of 60–80 g rats after exercise consisting of running for 120 min. These findings support the hypothesis that exercise causes an increase in skeletal muscle protein degradation, and that both red and white muscles are affected similarly.
Key words. Rat muscle; exercise; protein degradation; protein synthesis; tyrosine release.

Recently there has been considerable interest in muscle protein degradation during exercise[1]. Although several studies have been conducted, the effect of exercise on muscle protein degradation remains unclear. Muscle protein degradation during exercise has been reported to be increased[2,3], decreased[4,5], or unchanged[6,7]. In most of these studies the rate of protein degradation was determined by measuring the urinary excretion of 3-methylhistidine or the 3-methylhistidine/creatinine ratio. 3-Methylhistidine is an amino acid found only in contractile protein and is quantitatively excreted when these proteins are degraded. Since most of the contractile proteins are found in skeletal muscle, 3-methylhistidine excretion has been used as a measure of skeletal muscle protein degradation. Recently the validity of 3-methylhistidine excretion as an indicator of skeletal muscle protein degradation has been questioned, because a considerable portion of the excreted 3-methylhistidine was found to originate from the degradation of gut protein[8,9]. Thus, even though the proportion of nonmuscle 3-methylhistidine is low, its contribution to excretion is considerable because the turnover rate of these nonmuscle proteins is greater than that of skeletal muscle proteins. On the other hand, the continued use of 3-methylhistidine excretion as a measure of skeletal muscle protein degradation has been advocated[10]. Comparable rates of protein synthesis and degradation were found when the 3-methylhistidine excretion and constant infusion techniques were used[11], which showed that under some conditions 3-methylhistidine excretion was a valid method for the protein degradation studies. However, because of the inconsistent findings of the effect of exercise on muscle protein degradation using the 3-methylhistidine method, we measured the rate of muscle protein degradation after exercise in an entirely independent manner. In the experiments reported here the rate of protein synthesis and degradation was measured in vitro in isolated soleus and extensor digitorum longus muscles of rats that were exercised eccentrically by downgrade running.

Materials and methods. Male Sprague-Dawley rats weighing 60–80 g were obtained from the East Carolina University School of Medicine animal facilities. The rats were individually caged in a temperature-controlled room (20–23 °C) with lights on from 07.00 to 19.00 h, and were allowed free access to food and water. The rats in the exercised group were run downgrade at 16 $m \cdot min^{-1}$ (−16° grade) for 120 min on a motor driven treadmill. The controls remained sedentary in their cages. The exercised rats were sacrificed immediately after the exercise bout and the soleus and extensor digitorum longus removed for the measurement of the rates of protein synthesis and degradation. The rates of protein synthesis and degradation were measured using a modification[12] of Goldberg's in vitro method[13]. The soleus or extensor digitorum longus was preincubated at 37 °C for 30 min in Krebs-Henseleit buffer equilibrated with 95% O_2:5% CO_2 and containing 5 mM glucose and plasma amino acids at concentrations found in fed rats (except tyrosine and phenylalanine which were omitted). The muscles were then transferred to flasks containing fresh buffer which was the same as the preincubation buffer except that it also contained [^{14}C] phenylalanine (0.5 mM, 0.1 $mCi \cdot mmol^{-1}$). After a 2-h incubation in a shaking water bath the muscle was removed and homogenized in 10% trichloroacetic acid. The rate of protein synthesis was determined by measuring the incorporation of radiolabeled phenylalanine into protein. The rate of protein synthesis was then converted to tyrosine units by multiplying the rate of phenylalanine incorporation by 0.77, the ratio of tyrosine/phenylalanine in muscle protein. Net protein breakdown was determined by measuring the release of tyrosine into the medium fluorometrically[14]. The rate of protein degradation was calculated as the sum of the rate of protein synthesis and net protein breakdown. This method gave linear rates of protein synthesis and degradation for at least 150 min.

Results and discussion. The rates of protein synthesis and degradation were measured in isolated soleus and extensor digitorum longus muscles of rested and exercised rats. These muscles were chosen because they are small enough to allow adequate diffusion of metabolites, and because they are of different fiber types. Running the rats downgrade eccentricly biases the exercise, which can cause muscle damage[15]. However, we have previously shown that muscle damage to the soleus is minimal in the size rats used in this experiment[16], and does not occur in the extensor digitorum longus muscle[17]. Thus the effects observed in this study are likely due to exercise and not muscle damage. The results in the table show that this exercise protocol did not significantly affect the rate of protein synthesis in either the soleus or extensor digitorum longus muscles. Based on the measurement of protein synthesis in the perfused hindlimb, Bylund-Fellenius and co-workers have suggested that muscle contraction causes a decrease in the rate of protein synthesis that is greater in white than red muscles[18]. Our finding of unaltered rates of protein synthesis after exercise in the white extensor digitorum longus was therefore unexpected. This discrepancy suggests that either our exercise protocol was not strenuous enough or this method does not provide an adequate measure of protein synthesis during exercise. On the other hand, our findings of a 22% and 29% increase in the rates of protein degradation in the extensor digitorum longus, and soleus muscles respectively suggest that this method is able to detect changes in protein degradation after exercise. These findings of increased protein degradation after exercise in isolated muscle by a method that does not depend on 3-methylhistidine support, but do not prove the hypothesis that exercise causes an increase in the rate of rat skeletal muscle protein degradation. Absolute proof of this hypothesis awaits the development of a method to directly measure skeletal muscle protein degradation in vivo.

Effect of exercise on protein turnover in isolated rat muscles

Muscle	Protein degradation (pmol tyr released/ mg muscle/2 h)		Protein synthesis (pmol tyr incorporated/ mg muscle/2 h)	
	Control	Exercised	Control	Exercised
EDL[a]	324 ± 16[b]	368 ± 15[c]	108 ± 11	100 ± 7
Soleus	384 ± 23	472 ± 27[c]	116 ± 11	111 ± 6

[a] Extensor digitorum longus. [b] Values are means ± SEM for 10 rats. [c] Statistically ($p < 0.05$) different from control values.

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Absence of blood vessels in the brain parenchyma of hynobiid salamanders[1]

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Summary. The brains of hynobiid salamanders were studied with a light and an electron microscope. Blood vessels were found in the neural lobe, the olfactory bulb, and the meninx. In the bulk of the brain parenchyma, however, blood vessels were not found. This virtual absence of brain vascularization may be unique to hynobiid salamanders among vertebrates.
Key words. Brain; vascularization; salamander; amphibia.

The vertebrate brain demands a high oxygen supply. Indeed, the brain is generally supplied with abundant blood vessels[2,3]. To our knowledge, no vertebrates have been reported to lack blood vessels in the brain parenchyma. In hynobiid salamanders, however, we report here that the bulk of the brain parenchyma is not vascularized. This is an unusual situation and may mean that the general concept that the vertebrate brain must be intensely vascularized for its continuous and complicated activities must be seriously questioned.

Materials and methods. Mature individuals of hynobiid salamanders, *Hynobius nebulosus, Hynobius naevius,* and *Hynobius kimurai* were studied. *H. nebulosus* lives in hills and breeds in stagnant ponds. *H. naevius* and *H. kimurai* live in mountains and breed in rapidly flowing brooks. They were collected in the breeding season (winter and early spring) from their breeding sites in Shimane Prefecture, in western Japan. *H. nebulosus* measured about 9 cm, *H. naevius* about 14 cm, and *H. kimurai* about 17 cm in total length. The brains of these three salamanders were fixed in Bouin's solution and embedded in paraffin. They were serially sectioned transversely at 7 μm and stained with paraldehyde fuchsin and Masson-Goldner's method.

In *H. nebulosus* and *H. kimurai,* India ink was perfused from the heart after heparinization. In some individuals, the perfused brains were immersed in physiological saline and the meningeal surface was observed under a dissecting microscope. In other individuals, the perfused brains were fixed in Bouin's solution and embedded in paraffin. They were serially sectioned at 10 μm and observed without staining.

In *H. nebulosus,* the cerebral hemisphere, the diencephalon, the mesencephalon, and the medulla oblongata were fixed with glutaraldehyde and osmium tetroxide, and embedded in Epon. Semithin sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate.

For comparison, the brain of the Japanese newt, *Cynops pyrrhogaster,* was studied with routine light microscopical histology. This species was chosen for comparison because it is about the same size as *H. nebulosus* and lives in the same habitat as *H. nebulosus*[4]. In addition, the brains of the congo eel, *Amphiuma tridactylum,* and the lesser siren, *Siren intermedia,* were studied histologically, although these urodeles are much larger than hynobiid salamanders.

Results. Paraffin sections. In the brains of hynobiid salamanders, blood vessels are found 1) in the meninx covering the regular brain parenchyma, the median eminence, the paraphysis, and the choroid plexus, 2) in the small neural lobe (pars nervosa), and 3) in the olfactory bulb (fig. 1) and an adjacent small part of the cerebral hemisphere. Ependymal cells of the paraphysis and the choroid plexus are densely covered with blood vessels, some of which appear sinus-like. However, no blood vessels are found in the bulk (caudal five-sixths) of the cerebral hemisphere, the diencephalon, the mesencephalon, the metencephalon, and the medulla oblongata.

The paraphysis and the choroid plexus are large in hynobiid salamanders as in other urodele groups[5]. The telencephalic choroid plexus is located in the lateral ventricle except for its rostral extremity. The diencephalic choroid plexus is located not only in the third ventricle, but also in the mesencephalic ventricle except for its caudal extremity. The myelencephalic choroid plexus covers the fourth ventricle except for the most caudal part, which is covered with the simple posterior tela. In *Cynops pyrrhogaster, Amphiuma tridactylum,* and *Siren intermedia,* the brain parenchyma is well vascularized.

The thickness between the ventricular surface and the meningeal surface of the lateral pallium of *H. nebulosus, H. naevius,* and *H. kimurai* measures about 250 μm, 340 μm, and 390 μm, respectively. In *C. pyrrhogaster,* the thickness of the corresponding region measures about 400 μm. In the living state, the parenchymal wall must be slightly thicker than these values measured on paraffin section.

Perfusion studies. Under a dissecting microscope, a capillary net was seen on the meningeal surface of the brain. Although the shape of meshes of the net is variable, they measure approximately 50–100 μm in size. The sections of the India ink-perfused brain were carefully observed for blood vessels, but the results were the same as those with routine histology (fig. 2).

Epon sections. Both semithin and ultrathin sections were scruti-