MUSCLE PROTEIN SYNTHESIS AND PROTEIN DEPOSITION IN RAINBOW TROUT (*Oncorhynchus mykiss* Walbaum)

Ian McCarthy,
Department of Zoology, University of Aberdeen,
Aberdeen, AB24 3TZ, Scotland, UK.
Tel/Fax 44 1224 272867/272396,
email i.d.mccarthy@abdn.ac.uk

Dominic Houlihan,
Department of Zoology, University of Aberdeen,
Aberdeen, AB24 3TZ, Scotland, UK.

Chris Carter,
Department of Aquaculture, University of Tasmania,
PO Box 1214 Launceston, Tasmania 7250, Australia

ABSTRACT

White muscle accounts for 30-60% of the body mass of a fish: this presentation examines the relationship between white muscle and whole body rates of protein synthesis and growth and the contribution that the white muscle makes to whole body protein metabolism in rainbow trout. Linear relationships were found between protein consumption and the rates of protein synthesis and protein growth in the white muscle and whole body. The results indicated that 0.65 and 0.17 g of protein were synthesised and 0.24 and 0.12 g of protein deposited per gramme of protein consumed in the whole body and white muscle respectively. The white muscle accounted for 25% and 45% of daily whole body protein synthesis and deposition respectively.

INTRODUCTION

In all animals there is a continual cycle of synthesis and degradation of protein, with growth occurring when the rate of protein synthesis exceeds the turnover rate (Houlihan *et al.* 1995). Following the development of the flooding dose technique by Garlick *et al.* (1980) to measure rates of protein synthesis, early studies in fish concentrated on examining tissue-specific differences in the rates of protein synthesis, degradation and growth (e.g. Houlihan *et al.* 1988). Recently the relationship between consumption, protein synthesis and growth in fish has been examined, with particular attention being paid to the anabolic stimulation of protein synthesis and the retention efficiency of synthesised proteins as growth (Houlihan *et al.* 1988; Carter *et al.* 1993b, 1994; McCarthy *et al.* 1994). The white muscle accounts for 30-60% of the body mass of a fish (Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1933a; McCarthy unpublished data). In the white muscle the fractional rates of protein synthesis and degradation, the percentage of the protein mass synthesised or broken down per day, are lower compared to other body tissues such as the liver and gill (Houlihan *et al.* 1988). However, as a result of its large mass relative to other body tissues the white muscle makes a major contribution to the total absolute daily rate of protein synthesis and deposition. The aims of this study were, (i) to examine the anabolic stimulation of white muscle and whole body rates of protein synthesis in juvenile rainbow trout, (ii) to examine the contribution made by the white muscle to whole body rates of protein synthesis and growth and, (iii) to compare these results with the available data for ectothermic and endothermic vertebrates.

Methods

The full experimental protocol for this study has been published previously (McCarthy *et al.* 1994) and only a brief description is given here. Three groups of 24 rainbow trout were reared for 73 days in freshwater (350 l tanks; 40 l/h water turnover; 7.7°C, range 5 - 11°C) and fed either a 0.5. 1.0 or 2.0 % Body mass/day meal (Aqualine Trout Fingerling) in a single daily meal and a single group of 20 fish were starved. The initial mass of the experimental animals was 40.8 g (± 0.8 SEM, n = 92). Fish from the same stock group (43.3 g ± 2.3 SEM, n= 20) were killed at the start of the experiment in order to estimate the initial protein content of the experimental animals for the calculation of protein growth rates (see below). Individual food consumption rates were measured on four occasions (days 27, 55, 64 and 72) using X-radiography (McCarthy *et al.* 1993, 1994) and the data used to calculate protein consumption rates (g protein/day).

At the end of the experiment, white muscle and whole body rates of protein synthesis were measured in nine fish from each of the three ration groups and in 10 starved fish using the flooding dose technique (Garlick *et al.* 1980, Houlihan *et al.* 1995). The fish (58.3 g ± 3.3 SEM, n = 37) were fed at 0700 and 3h later were injected via the caudal blood vessels, without anaesthesia, with a solution containing 135 mM L-phenylalanine and [L-2,6-^{3}H]phenylalanine (Specific radioactivity 1250 ± 40 SEM, n=4, dpm/nmole). Following a known incorporation period in freshwater (10°C, mean 278 min ± 5 SEM, n = 37), each fish was killed (by a sharp blow to the head and transection of the spinal cord), frozen in liquid nitrogen and stored at -70°C until analysis. Triplicate white muscle and whole body samples were taken in order to measure the free pool and protein-bound specific radioactivity and protein content as outlined in Houlihan *et al.* (1995) in order to calculate white muscle and whole body absolute rates of protein synthesis (g protein/day). White muscle and whole body protein growth rates (g protein/day) were calculated for each fish using the measured final protein content and the estimated initial protein content as outlined in McCarthy *et al.* (1994).

Results and Discussion

a) Method validation

Due to the range of incorporation times (228 - 345 min), the data were grouped at 30 minute intervals to examine the pattern of flooding of the free pool over time and the incorporation of radiolabel into body protein. Following a single flooding dose injection, the free phenylalanine concentration in the white muscle free pool remained elevated and stable (ANOVA, $p<0.05$) over the course of the incubation (Table 1) and exhibited a 12 fold elevation above basal level (74 nmole phe/g wet weight, Carter *et al.* 1995). The specific radioactivity of the white muscle free pool remained elevated over the course of the incubation. The mean S_a values for each time interval were not significantly different from each other or from the phenylalanine-specific radioactivity of the injection solution (ANOVA, $p<0.05$). The mean white muscle S_a was 1091±101 dpm/nmole or 87.3% (±1.3) of the specific radioactivity of the injection solution. Linear regression analysis revealed that the incorporation of [^{3}H]phenylalanine into both the white muscle and the whole body protein pools was linear over time:

White Muscle: $S_b = 0.01*t - 0.05$ ($R^2=0.835$, $n=5$, $p<0.05$)
Whole Body: $S_b = 0.02*t - 0.18$ ($R^2=0.842$, $n=5$, $p<0.05$)

where t is the time in minutes following injection.

The use of the flooding dose technique is based on several assumptions which have been recently reviewed by Houlihan *et al.* (1995). Briefly, these assumptions are (i) that the flooding dose of phenylalanine does not affect the rate of protein synthesis, (ii) the flooding dose results in a rapid elevation and stabilisation of the phenylalanine-specific radioactivity in the body free amino acid pools to a similar level as the injection solution over the incorporation period and (iii) that the labelling of body protein with the radiolabel is linear over the incorporation period. Previous studies in fish and rats have shown that a single flooding dose injection of $[^3H]$phenylalanine does not affect the rate of protein synthesis (reviewed in Houlihan *et al.* 1995). The time course results of this study meet the second and third assumptions, and are agreement with previous studies (reviewed in Houlihan *et al.* 1995) and therefore validate the synthesis values obtained in this paper.

Table 1. White muscle free pool phenylalanine concentration (WM Phe, nmole/g wet wt) and free pool phenylalanine-specific radioactivity (WM S_a, dpm/nmole) and white muscle (WM S_b, dpm/nmole) and whole body protein-bound phenylalanine-specific radioactivity (WB S_b, dpm/nmole) for 60 g rainbow trout following a single flooding dose injection of $[^3H]$phenylalanine (specific radioactivity, 1250 ± 40 dpm/nmole). The data are grouped for 30 minute intervals and are presented as the mean value ± SEM.

Time	n	WM Phe	WM S_a	WM S_b	WB S_b
210-239	4	883±50	1149±33	1.11±0.05	3.28±0.56
240-269	12	863±46	1132±29	1.50±0.21	4.16±0.45
270-299	13	803±79	1269±23	1.63±0.30	4.93±0.56
300-329	4	856±19	1007±57	1.78±0.19	4.53±0.73
330-359	4	956±73	1172±60	1.81±0.57	5.71±0.92

b) CONSUMPTION-SYNTHESIS-GROWTH RELATIONS

Significant linear correlations were found between protein consumption (A_r, g protein/day) and white muscle and whole body protein synthesis (A_s, g protein/day) and between protein consumption and white muscle and whole body protein growth (A_g, g protein/day) (Figure 1):

Consumption-synthesis
Whole Body: $A_s = 0.65*A_r - 0.07$ ($R^2=0.853$, $n=37$, $p<0.001$)
White muscle: $A_s = 0.17*A_r - 0.01$ ($R^2=0.694$, $n=37$, $p<0.001$)

Consumption-growth
Whole Body: $A_g = 0.24*A_r - 0.01$ ($R^2=0.833$, $n=37$, $p<0.001$)
White muscle: $A_g = 0.12*A_r - 0.01$ ($R^2=0.698$, $n=37$, $p<0.001$)

The regression analysis indicated that for every gramme of protein consumed (i) 0.17 and 0.65 g of protein were synthesised and (ii) 0.12 and 0.24 g of protein were deposited in the white muscle and whole body respectively. The efficiency of retention of synthesised protein as growth (A_g*100/A_s) was 72.3% (±5.9) in the white muscle and 22.0% (±2.7) in the whole body (data not shown).

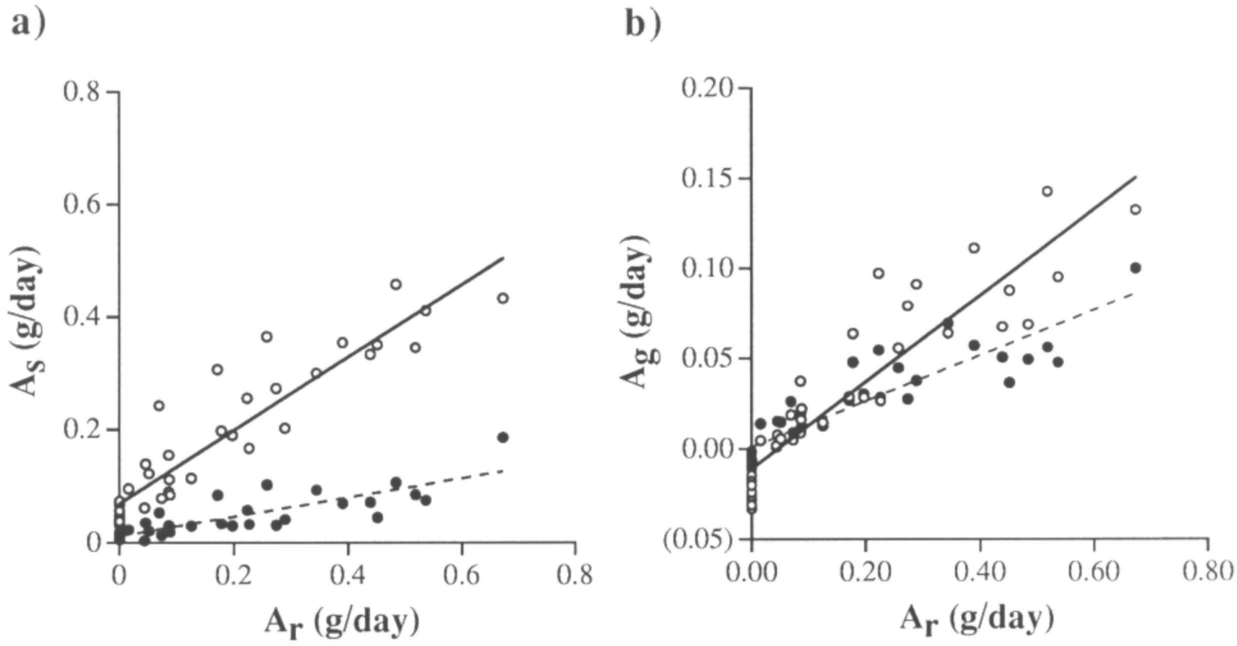

Figure 1. (a) Scatter plot showing the relation between protein consumption (g protein/day) and protein synthesis (g protein/day) in the white muscle (closed circles) and whole body (open circles) of rainbow trout (*O. mykiss* Walbaum). (b) Scatter plot showing the relation between protein consumption (g protein/day) and protein growth (g protein/day) in the white muscle (closed circles) and whole body (open circles) of rainbow trout (*O. mykiss* Walbaum).

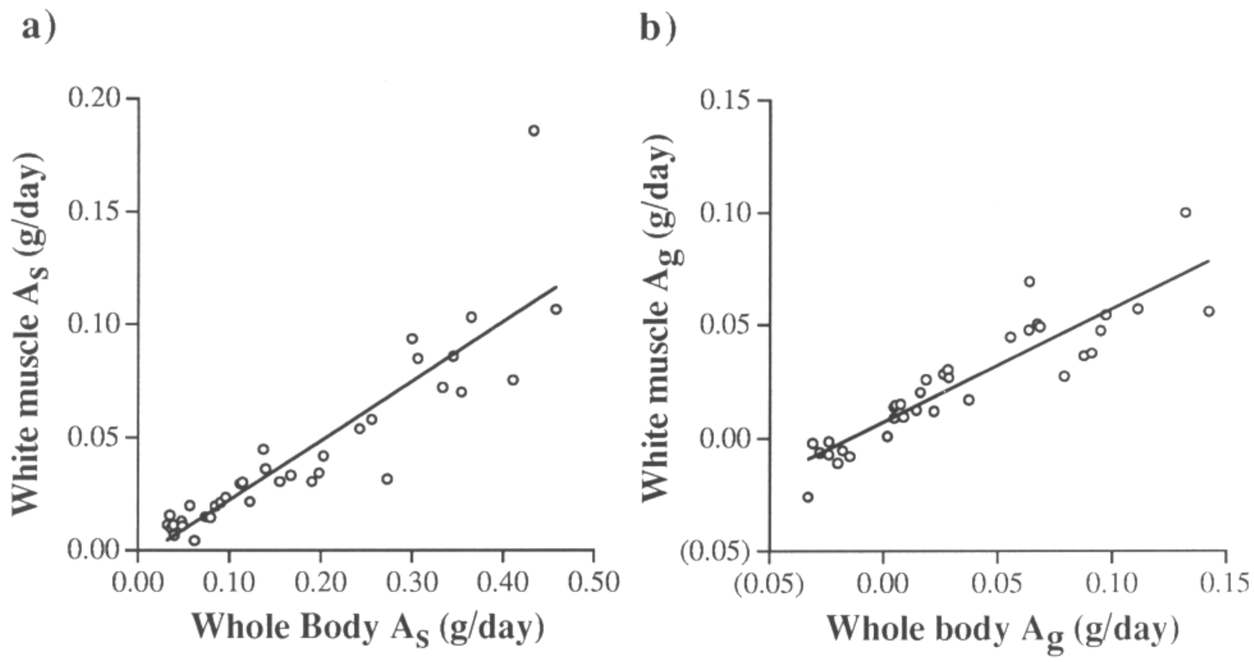

Figure 2. (a) Scatter plot showing the relation between protein synthesis (g protein/day) in the whole body and white muscle of rainbow trout (*O. mykiss* Walbaum). (b) Scatter plot showing the relation between protein growth (g protein/day) in the whole body and white muscle of rainbow trout (*O. mykiss* Walbaum).

The proportion of protein synthesised in the whole body per gramme of protein consumed are in close agreement with published values for rainbow trout of a similar size. Carter *et al.* (1994) report an anabolic stimulation of 0.83g of protein synthesised per gramme of protein consumed in 100 g rainbow trout fed a 2% B.W./day meal of the same commercial diet at 14°C. From the data published by Fauconneau and Arnal (1985) for 98 g rainbow trout at 10°C we have calculated that for every gramme of protein consumed (i) 0.25 and 0.74 g of protein were synthesised and (ii) 0.13 and 0.22 g of protein were deposited in the white muscle and whole body respectively. In fish, the white muscle is characterised by having the highest retention efficiency of synthesised protein of the tissues/organs in the body with efficiencies in the order of 50-70% (this study, Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1993a). The retention efficiency in the whole body is lower and values between 14 and 37 % have been reported for fish (this study, Fauconneau & Arnal 1985; Houlihan *et al.* 1988; Carter *et al.* 1993b, McCarthy *et al.* 1994),

c) WHITE MUSCLE-WHOLE BODY RELATIONS

Significant linear correlations were found between rates of protein synthesis (A_s, g protein/day) in the white muscle and whole body and between rates of protein growth (A_g, g protein/day) in the white muscle and whole body (Figure 2):

Synthesis: WM = 0.25*WB - 0.01 (R^2=0.780, n=37, $p<0.001$)
Growth: WM = 0.45*WB + 0.01 (R^2=0.690, n=37, $p<0.001$)

where WM and WB are the white muscle and whole body rates respectively. The regression analysis indicated that, expressed on an absolute basis (g protein/day), the white muscle accounted for 25 % of daily protein synthesis and 45 % of daily protein growth in this study.

Table 2. (a) Percentage contribution (%) of muscle protein synthesis (g protein/day) to whole body protein synthesis (g protein/day) for several animal species. (* = preruminant lambs, 7-8d old). (b) Percentage contribution (%) of muscle protein growth (g protein/day) to whole body protein growth (g protein/day) for several animal species.

	Species	Body mass	%	Reference
A)	Sea bass	10 g	21	McCarthy *et al.* (unpubl)
	Grass carp	23 g	28	Carter *et al.* (1993a and b)
	Rainbow trout	40-100 g	25	This study
	Rainbow trout	98 g	33	Fauconneau & Arnal (1985)
	Atlantic cod	300 g	26	Houlihan *et al.* (1988)
	Sparrow	25-30 g	32-35	Murphy & Taruscio (1995)
	Lamb*	4.5 kg	29	Attaix *et al.* (1988)
	Rat	100-130 g	25	Preedy *et al.* (1983)
	Rat	100 g	19	Garlick *et al.* (1976)
	Pig	76 kg	42	Garlick *et al.* (1976)
	Cow	376 kg	14	Lobley *et al.* (1980)
B)	Grass carp	23 g	35	Carter *et al.* (1993a and b)
	Rainbow trout	40-100 g	45	This study
	Rainbow trout	98 g	58	Fauconneau & Arnal (1985)
	Atlantic cod	300 g	42	Houlihan *et al.* (1988)

The percentage contribution made by muscle protein synthesis and growth to whole body protein synthesis and growth for a number of animal species are shown in Table 2. The available data suggests that in growing non-ruminant animals reared under excess feeding conditions, the white muscle provides a fairly constant contribution to whole body protein synthesis. The available data on the contribution made by the white muscle to protein deposition in the whole body indicates the substantial contribution (35 - 60 %) made by white muscle to whole body protein deposition.

This work was carried out with funding from the Biotechnology and Biological Sciences Research Council (IDM) and the Ministry of Agriculture, Fisheries and Food (CGC).

References

Attaix, D., Aurousseau, E., Manghebati, A. & Arnal, M. (1988). Contribution of liver, skin and skeletal muscle to whole body protein synthesis in the young lamb. *British Journal of Nutrition*, **60**, 77-84.

Carter, C.G., Houlihan, D.F., Brechin, J., McCarthy, I.D. & Davidson, I. (1993a). Protein synthesis in grass carp, *Ctenopharyngodon idella* (Val.), and its relationship to diet quality. In *Fish nutrition in practice, Proc. IVth Int. Symp. Fish Nutrition and Feeding* (eds. Kaushik, S.J. & Luquet, P.). pp 673-680. Paris: INRA.

Carter, C.G., Houlihan, D.F., Brechin, J., McCarthy, I.D. (1993b). The relationships between protein intake and protein accretion, synthesis and retention efficiency for individual grass carp, *Ctenopharyngodon idella*. *Canadian Journal of Zoology*, **71**, 391-400.

Carter, C.G., Owen, S.F., He, Z-Y., Watt, P.W., Scrimgeour, C., Houlihan, D.F. & Rennie, M.J. (1994). Determination of protein synthesis in rainbow trout, *Oncorhynchus mykiss*, using a stable isotope. *Journal of experimental Biology*, **189**, 279-284.

Carter, C.G., He, Z-Y., Houlihan, D.F., McCarthy, I.D. & Davidson, I. (1995). Effect of feeding on the tissue free amino acid concentrations in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Fish Physiology and Biochemistry*, **14**, 153-164.

Fauconneau, B. & Arnal, M. (1985). *In vivo* protein synthesis in different tissues and the whole body of rainbow trout (*Salmo gairdneri* R.). Influence of environmental temperature. *Comparative Biochemistry and Physiology*, **82A**, 179-187.

Garlick, P.J., Burk, T.L. & Swick, R.W. (1976). Protein synthesis and RNA in tissues of the pig. *American Journal of Physiology*, **230**, 1108-1112.

Garlick, P.J., McNurlan, M.A. & Preedy, V.R. (1980). A rapid and convenient technique for measuring the rate of protein synthesis in tissues by the injection of ^{3}H phenylalanine. *Biochemical Journal*, **217**, 507-516.

Houlihan, D.F., Hall, S.J., Gray, C. & Noble, B.S. (1988). Growth rates and protein turnover in Atlantic cod, *Gadus morhua. canadian Journal of Fisheries and Aquatic Sciences*, **45**, 961-964.

Houlihan, D.F., Carter, C.G. & McCarthy, I.D. (1995). Protein turnover in animals. In *Nitrogen Metabolism and Excretion* (eds. Walsh, P.J. & Wright, P.). pp 1-32. Boca Raton: CRC Press.

Lobley, G.E., Milne, V., Lovie, J.M., Reeds, P.J. & Pennie, K. (1980). Whole body and tissue protein synthesis in cattle. *British Journal of Nutrition*, **43**, 491-502.

McCarthy, I.D., Houlihan, D.F., Carter, C.G. & Moutou, K. (1993). Variation in individual consumption rates of fish and its implications for the study of fish nutrition and physiology. *Proceedings of the Nutrition Society*, **52**, 427-436.

McCarthy, I.D., Houlihan, D.F. & Carter, C.G. (1994). Individual Variation in protein turnover and growth efficiency in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Proceedings of the Royal Society of London Series B*, **257**, 141-147.

Murphy, M.E. & Taruscio, T.G. (1995). Sparrows increase their tissue and whole body rates of protein synthesis during the annual molt. *Comparative Biochemistry and Physiology*, **111A**, 385-396.

Preedy, V.R., Paska, L., Sugden, P.H., Schofield, P.S. & Sugden, M.C. (1983). The effects of surgical stress and short-term fasting on protein synthesis *in vivo* in diverse tissues of the mature rat. *Biochemical Journal*, **250**, 179-188.