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CHAPTER 17

Fasting and starvation

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I. Introduction

Fish can survive for a long time without food and for many species a fasting period forms part of the natural life cycle. Winter months, spawning migration and/or pre-spawning stage can all be naturally non-feeding periods. Thus, numerous species can starve for many months and then recover fully after refeeding. Therefore, these species are well adapted to mobilize their metabolic reserves and even body constituents to survive periods of food deprivation.

Specific effects of starvation on metabolism are dependent on multiple variables; including the species under consideration, the preferential tissues for metabolic stores, the quantity stored and their availability as well as distinct routes of mobilization. In this sense, the pre-fasting diet may exert substantial influence on the

metabolic events initiated by fasting[75]. It is also important to differentiate between experimental and natural fasting, because natural fasting can be accompanied by other, compounding factors, such as gonadal growth, low temperatures, etc. Experimental fasting effects are likely dependent on endogenous and exogenous factors. For instance, the season chosen, temperature and photoperiod, fish age and whether or not the fasting is imposed during a reproductive period will all have substantial bearing on the experimental results. Therefore, all these factors have to be taken into account when comparing fasting responses between species or even within species.

Another important aspect when comparing the experimental results of fasting periods in fishes is the way in which the data are expressed. Machado and coworkers[114], for instance, pointed out that many discrepancies found in the literature can be explained because conclusions are obtained on the basis of changes in the concentrations of tissue metabolites, being more informative than changes in total amounts stored in tissue.

Similarly, Black and Love[15] found a great deal of clear information when data were considered in relation to one another. In the same vein, Foster and Moon[55] indicated the advantage of using body weight and tissue DNA content as reference points for metabolic and enzymatic parameters.

The aim of this chapter is to give a brief overview of gross metabolic and enzymatic changes observed in fasting and starving fish. We have chosen the storage substrate as the organizing principle, before focusing on various parameters of endocrine regulation during fasting and starvation in fishes.

II. Carbohydrate mobilization

1. Liver glycogen

Carbohydrates are stored as glycogen in liver normally amounting to 1–6% of liver weight, although some species, such as carp (*Cyprinus carpio*), can accumulate values exceeding 10%. When required, hepatic glycogen is enzymatically broken down and transported to the extrahepatic tissues as glucose. On arrival at its target cells, glucose is either metabolized or reconverted into glycogen. Unfortunately, liver glycogen determination has a number of inherent problems which may complicate the interpretation of observed changes in this particular parameter under some experimental conditions. First, variation in the amount of liver glycogen is large between individuals; and second, as the example of the carp shows, concentrations may be exceptionally high. Because liver glycogen is normally determined in terminal samples, it is inherently difficult to determine (or detect) small changes in liver glycogen and often even large decreases may be missed due to large individual variability. Usually, glycogen depletion is a continuous process from the beginning of fasting[106]. In most species, liver glycogen is mobilized early in experimental fasting in fish; in fact, partially because of the ease of mobilization, liver glycogen is generally the first substrate to be used during fasting. At 5, 8, 15, 20 days

TABLE 1

Variation of body parameters and liver and muscle components in fed and fasted trout (*Salmo trutta fario*)

	Fed (days)				Fasted (days)		
	1	15	30	50	8	30	50
Body parameters							
Weight (g)	163	175	174	201	135	134	121
Length (cm)	22.7	22.0	24.4	25.3	21.6	23.8	23.7
HSI (%)	1.24	1.09	1.03	1.08	0.99	0.83[a,b]	0.77[a,b]
MLI (g cm^{-1})	3.6	4.2	3.9	4.41	3.2[a]	3.1[a]	2.9[a,b]
VI (%)	8.4	9.1	6.1	9.9	5.0[a,b]	4.5[a,b]	3.6[a,b]
Liver composition							
Glycogen (%)	4.6	3.9	3.9	2.5	0.9[a,b]	0.9[a,b]	0.6[a,b]
Protein (%)	14.3	15.1	15.3	15.4	16.7[a,b]	16.7[a,b]	16.1[a,b]
Lipids (%)	3.7	3.3	4.0	4.3	3.7	3.8	3.6[a]
Water (%)	75.1	75.1	74.6	75.3	76.0[a,b]	75.5[a]	76.2[a,b]
P-DNA (mg 100 g^{-1})	27.8	35.4[b]	36.2[b]	39.1[b]	43.0[a,b]	51.6[a,b]	57.3[a,b]
Muscle composition							
Glycogen (%)	0.24	0.27	0.27	0.30	0.20	0.10[a,b]	0.09[a,b]
Protein (%)	19.7	19.2	19.6	19.3	18.8	18.8	18.2[a,b]
Lipid (%)	1.5	1.6	1.4	1.7	1.5	1.1	1.0[a]
Water (%)	77.8	77.7	77.5	76.9	78.1	77.8	78.5[a]
P-DNA (mg 100 g^{-1})	2.5	2.7	2.2	1.9	3.3	2.4	2.1

Results are means from 10 fish in each group.
[a] Indicates significantly different ($p < 0.05$) from fed fish at the same point (Duncan's test).
[b] Indicates significantly different ($p < 0.05$) from the fed group at day 1 of the experiment (Duncan's test). Abbreviations: HSI = Hepatosomatic index (gram liver weight 100 g^{-1} body weight); MLI = Index of muscle weight (g) over length (cm); VI = Index of viscera weight (g) without liver per 100 g body weight; P-DNA = DNA phosphorous (mg) per 100 g tissue fresh weight. Maximum standard error of individual obervations were below 15%, in most cases below 10% of the mean. Adapted from Navarro et al.[134].

fasting, significant decreases in glycogen have been described in several species of teleostean fishes[65,75,114,115,135,169] or elasmobranchs[47]. Table 1 shows the effects of 15, 30 and 50 days of starvation on liver and muscle glycogen in brown trout (*Salmo trutta fario*).

However, several species undergo prolonged periods of fasting in their natural environment with little depletion of liver glycogen. Pacific sockeye salmon (*Oncorhynchus nerka*) migrate some 1000 km without decrease in this reserve[58]. Fasted European eels (*Anguilla anguilla*) did not show a change in liver glycogen after 95 days fasting[96]. As mentioned above, carp (*C. carpio*) accumulate exceptionally high levels of liver glycogen and after 12 months of starvation still maintain 6% of glycogen in their livers[192]. Nagai and Ikeda[133] indicated that 22 days fasting in carp (*C. carpio*) did not provoke any change in liver glycogen (10.65%) and it was only after 100 days that a clear decrease was observed (1.55%). Such an obvious defence of hepatic glycogen, even after long periods of starvation, may be explained by a compensatory increase in the rate of gluconeogenesis from non-carbohydrate precursors, which has two effects, namely: (1) that hepatocyte glycogen can be spared;

TABLE 2

Variation of body parameters and liver and muscle components in fed and fasted yellow perch (*Perca flavescens*)

	Fed	Fasted for 7 weeks
Body parameters		
Condition factor	1.26	1.13[a]
Hepatosomatic index (%)	1.99	0.85[a]
Plasma		
Glucose (mM/l)	4.27	3.03[a]
Liver composition		
Glycogen (μMol/μg DNA)	253	40.8[a]
Protein (mg/μg DNA)	43.9	28.0[a]
Glucose (μMol/μg DNA)	73.0	98.0
DNA (μg/g tissue weight)	2.4	4.5[a]
Muscle composition		
Glycogen (μMol/μg DNA)	69.4	43.3[a]
Protein (mg/μg DNA)	883	700[a]
Glucose (μMol/μg DNA)	17.4	7.6[a]
DNA (μg/g tissue weight)	0.22	0.25

Values are means of 3 to 12 observations.
[a] Significantly different from fed control fish ($p < 0.05$; Student's t-test).
Condition factor = gram body weight per cm^3 body length. Adapted from Foster and Moon [55].

and (2) that the hepatic production of glucose 6-phosphate can lead to an increased carbon flux into glycogen. Increase in glyconeogenesis was suggested in *A. anguilla* after 95 days fasting from the increase of the hepatic glutamate-oxaloacetate transaminase (aspartate aminotransferase, AspAT, EC 2.6.1.1), together with the maintenance of glycogen. Liver gluconeogenesis was also enhanced in 6- or 7-week fasted rainbow trout[126] (*O. mykiss*) or yellow perch[55] (*Perca flavescens*) (Table 2), respectively. The percentage per wet tissue weight of key gluconeogenic enzymes AspAT, phospho*enol*pyruvate carboxykinase (PEPCK, EC 4.1.1.32) and lactate dehydrogenase (LDH, EC 1.1.1.27) significantly increased in yellow perch[55]. These data are in agreement with the observations of Morata and coworkers[129] who found increases in liver and kidney gluconeogenesis during the second month of starvation in rainbow trout, and with those of French and coworkers[59,58] who determined relative increases in PEPCK and LDH in starving and exercising rainbow trout and increases in PEPCK and glutamate-pyruvate transaminase (alanine aminotransferase, AlaAT, EC 2.6.1.2) in migrating (starving) sockeye salmon.

It is clear that in the face of rapid proteolytic degradation, the activities of key enzymes are somehow defended. However, the mechanism of this defence is not clear until more detailed research, preferably at the molecular level, has been done. For instance, it is not obvious from the descriptive studies mentioned above: (1) whether these enzymes are protected against a generalized proteolytic attack; (2) whether the proteolytic attack, which involves selective activation of cathepsin-like proteases, is general in nature; or (3) whether compensatory increases in enzyme synthesis are responsible for the apparent maintenance of such key enzymes. Thus,

this topic would make an ideal area to apply molecular biological techniques to probe mRNA longevity, transcription rates and possible induction of key enzymes. Of the enzymes mentioned, it is known from other vertebrates that transcription of PEPCK is under multifaceted endocrine control, involving crosstalk between different regulatory pathways. The applicability to piscine systems, however, remains to be elucidated. At any rate, the nature of the key enzymes purportedly preserved, more than hints at key metabolic pathways which retain importance and metabolic flux throughout starvation. Applying a teleological view to intermediary metabolism, all key enzymes support the idea of a drastically changed emphasis on amino acid metabolism, possibly with concurrent increases in the importance of gluconeogenesis. The transaminases can be considered feeder enzymes for carbon into oxidative and gluconeogenic pathways, while PEPCK plays a pivotal role in the flux of carbons from C3, C4 and C5 precursors (which can be derived from most amino acids) into glucose. Nevertheless the often times noticed maintenance of LDH poses a bit of a problem to the picture outlined above.

At any rate, however, given the decrease in liver glycogen, the overall contribution of glycogen to the total energy expenditure is comparatively small considering the limited weight of the liver (hepatosomatic index ranging normally from 1 to 3%; cf. Tables 1 and 2).

2. *Blood glucose*

Although blood glucose in fishes is known to fluctuate widely between species and also within species, within a given, uniform batch of individuals, blood glucose concentrations are maintained at a steady level during long periods of food deprivation. It is thought that this apparent defence of blood glucose against fluctuations or depletion occurs largely at the expense of liver glycogen, at least during the initial stages of fasting. Hochachka and Sinclair[77], for instance, found a decrease in liver glycogen without modification of blood glucose in 14-day fasted rainbow trout. Not surprisingly, glycemia profiles during fasting vary depending on the species considered, and this is one area where other physiological factors mentioned above are likely to exert substantial influence. In juvenile sea bass (*Dicentrarchus labrax*), glycemia is decreased after a 5-day fast, and in juvenile brown trout (*Salmo trutta fario*) after a 10-day fast[65,135]. Zammit and Newsholme[198], working on adults of the same species, failed to observe changes in glycemia after imposing a 40-day fast. It was only after 100 days of food deprivation that blood glucose levels decreased significantly. The hagfish (*Myxine glutinosa*) seems to follow a similar pattern as the teleosts, since this species maintained blood glucose throughout a three-week fast[43]. An entirely different picture emerges for an elasmobranch fish, exemplified by the response of the common dogfish. In these animals (*Scyliorhinus canicula*), whose metabolism is even less 'glucosocentric' than in teleostean fishes, plasma glucose decreases rapidly during food deprivation and reaches a low of 0.52 mg/100 ml (0.03 mM) within eight days of food deprivation; however, later during starvation, blood glucose levels recover and reach pre-fasting levels (12.05 mg/100 ml; 0.67 mM) after 67 days of fasting[47].

In carp and eel, no changes in glycemia were observed after several weeks of fasting[34,131]. Starved *Clarias lazera* (a warm-water catfish) experienced no change in blood glucose after four months, although this parameter decreased to 60% after an additional three months of experimentation[68]. Sluggish fish seem to be able to maintain glucose levels for longer periods than more active species, likely reflecting the comparatively smaller demand and hence turnover of glucose in these species. As shown by Weber and coworkers[188,189], a strong positive correlation exists between glucose turnover and assumed physical activity of teleostean fishes. In the case of the extremely active skipjack tuna (*Katsuwonus pelamis*), for instance, glucose turnover rates approach or even surpass those of many mammals (*cf.* chapter 2, this volume).

In carp (*C. carpio*) an increase in glycemia was observed in early starvation[133]. It is possible to distinguish two phases in the maintenance of blood glucose during fasting and starvation in fishes; one is that glucose sequestered from the plasma pool is replenished by liver glycogenolysis; the other is replenished through the compensatory activation of gluconeogenesis, especially from amino acids mobilized though preferential proteolysis of muscle[114]. However, as the studies mentioned above already reveal, a clear temporal or functional relationship between these phases, as exists in many mammals, cannot be delineated. At any rate, a combination of both phases is clearly involved in the stabilization of blood glucose during starvation.

3. Muscle glycogen

The amount of glycogen stored in fish muscle falls into the range between 0.2 and 1.3% per wet weight and is thus generally assumed to make only a small potential contribution to total energy expenditure[114]. However, it should be kept in mind, that because of its immediate involvement in muscular activity, muscle glycogen is a most volatile entity and its mobilization is more likely related to an increase in muscular activity than to the fasting process. Not surprisingly, the rate of muscle glycogen mobilization is positively correlated with ambient temperatures. In brown trout (*S. trutta fario*) significant decreases in muscle glycogen were found at 8 days fasting in summer, but only at 30 days fasting in winter (Table 1)[135]. Similarly, in rainbow trout (*O. mykiss*) muscle glycogen is lowest in summer[61] when high temperature and likely increased activity of the fish combine to drain the muscle glycogen stores.

In Atlantic cod (*Gadus morhua*), at early stages of starvation, both liver and muscle glycogen were mobilized. But red muscle glycogen remained unchanged during most of the depletion[15].

However, in several fish species glycogen in muscle is not mobilized and it seems likely that this store is maintained at the expense of blood glucose which is supplied by hepatic processes (gluconeogenesis, glycogenolysis). In juvenile sea bass (*Dicentrarchus labrax*) fasted 22 days in summer, muscle glycogen decreased very little[65], while carp fasted 19 or 67 days did not show a depletion in muscle glycogen levels[18]. Similarly, European eels fasted for 164 days maintained unchanged muscle

glycogen concentrations[34]. A comparable pattern seems to hold at least for one species of elasmobranch: 82 days of fasting did not provoke a decrease in muscle glycogen in *S. canicula*[47].

In conclusion, although important differences may exist in carbohydrate metabolism among teleostean species, a tendency to maintain stable glucose values during starvation is observed. Concentration of liver glycogen varies between species, but this is the main tissue for glycogen storage, and liver glycogenolysis accounts for maintenance of glycemia during early starvation (weeks). Liver gluconeogenesis from amino acids (AA) and lactate will supply glucose for later (months) stages of starvation. A different picture emerges for the representative (*S. canicula*) elasmobranch, with lower dependence on glucose and more exaggerated lipid metabolism.

III. Lipid mobilization

Apart from the at times obvious changes in carbohydrates, many authors are convinced that lipids are mobilized first. Lipid depletion during starvation has been demonstrated in many species of fish, marine as well as freshwater, and this literature has been comprehensively reviewed by Sargent and coworkers[161].

An inverse relationship exists between lipid and water contents and catabolized lipids are replaced by an equal volume of water[107,162,191]. Thus, under conditions of moderate starvation and lipid loss, body weight is maintained through the uptake of water. This phenomenon is best exemplified (and exaggerated) in migrating sockeye salmon (*Oncorhynchus nerka*): these animals use up their entire – substantial – storage lipids in the first part of their migration, yet do not change body weight appreciably during this phase[82]. Values exceeding a water content of 81% are reached in fish muscle, when only very low levels of liver lipids can be detected[107].

The strategy of unloading body storage lipids is similar in species classified as fatty and those considered non-fatty, in that the bulk of lipid is used first, before proteins are drawn upon. Lipids can be stored in liver, intestinal fat and muscle, with the quantitative importance of each tissue varying between species. Salmonids generally rely on the deposition of visceral fat, while many gadid species accumulate hepatic storage lipid, and clupeiformes contain high contents of lipids in muscle. Independent of the actual site of deposition, lipids will be mobilized from these sites during starvation.

1. Liver

As mentioned above, the actual decrease of tissue lipid during fasting varies between species depending on the tissue for lipids storage and also depending on the strategy followed in mobilizing other reserves including carbohydrates. In the Atlantic cod (*Gadus morhua*), for instance, liver lipids decreased drastically with fasting; in fact this storage depot was exhausted even in the face of an appreciable quantity of the initial glycogen reserve remaining[15]. Carp could also be included in this group of species that spare glycogen reserves to a certain degree. In carp (*C.*

carpio) after 19 days of fasting, an appreciable depletion on liver lipids occurred, while the fish seemed to protect their entire store of hepatic glycogen throughout this period[17]. In longer term experiments, where carp were fasted for 100 days, liver lipid was exhausted, while glycogen level was retained at about 1.6% (ref.133).

On the other hand, the rest of body fat reserves tends to be depleted before liver lipid is mobilized in salmonids. Nevertheless, substantial decreases in hepatic content of storage lipids can be detected in rainbow trout (*O. mykiss*)[115] or brown trout (*Salmo trutta fario*) with prolonged fasting (Table 1)[135].

In sea bass (*Dicentrarchus labrax*), a species which stores lipid predominantly in mesenteric fat, fasting for 22 days[65] or 130 days[27] did not provoke significant decreases in hepatic lipids. In contrast, Stirling[169] did notice decreases in liver lipids from sea bass that were fasted for 26 and 60 days.

2. *Intestinal fat*

The major lipid storage site of many teleostean fishes is mesenteric fat. For instance, lipid accumulated by rainbow trout (*O. mykiss*) is deposited preferentially in perivisceral adipose tissue[161] and it is this mesenteric fat that is first mobilized in fasted rainbow trout. In this species, during 48 days fasting, more lipid was mobilized from perivisceral depots than either liver or muscle[90]. Species with sustained accumulation of mesenteric fat usually follow the same strategy of mobilizing this lipid reserve preferentially. In *Esox lucius* intestinal lipids are mobilized before any change in liver lipid is apparent[86]. Similarly, in the sea bass (*Dicentrarchus labrax*), mesenteric lipid depots decrease by 41% after 15 days of fasting compared with control fish[65], an observation supporting earlier data in the same species[198].

3. *Muscle lipids*

In species storing lipids in muscle, mobilization of intramuscular lipid is initiated as soon as food intake ceases. An excellent case showing this point is the freshwater teleost *Rhamdia hilarii*. This catfish has no organized or visible adipose tissue in the abdominal cavity and during fasting intramuscular lipid (average of 5% wet weight) plays an important role for the overall energy requirement[114]. In this species, after 30 days of fasting, muscle fat was reduced to one-fifth of fed values, which, considering that muscle constitutes 60–70% of fish body weight, represents an important source of energy. In species devoid of intracellular storage lipid, structural muscle lipid is usually retained until later phases of starvation when protein structures begin to be broken down. In *Gadus morhua*, for instance, a species that has little lipid stored in the skeletal muscle and considerable amounts stored in the liver, this hepatic reserve is used first. Muscle protein is degraded and partial destruction of structural lipids may occur at the final stages of starvation, only when other sources of energy have been nearly consumed.

Muscle lipids in sea bass, which preferentially deposits lipids for storage in mesenteric fat, failed to show significant changes during fasting[65,169], while in starving trout, muscle lipids underwent decreases after a 50-day fast[90,135], but

experienced no changes during a 27-day fast. Muscle lipids in carp (*Cyprinus carpio*) are not easily mobilized in fasting, possibly reflecting the sluggish activity pattern of this species. Working on carp, Takeuchi and colleagues[175] failed to observe variations in muscle lipids as a consequence of fasting. Identical results have been reported for fasting plaice (*Pleuronectes platessa*)[91]. However, it should not be forgotten that the metabolic requirements of fasting species may be adjusted to the amount and availability of storage substances accumulated around the body. Fasting yellow perch (*Perca flavescens*), for instance, were found to be able to enter into a stage of hypometabolism, thus decreasing metabolic output and sparing storage substances[55]. Unfortunately, the potential for such metabolic 'down-regulation' has not been widely appreciated in fishes and is likely overlooked unless stringent controls are incorporated into the experimental protocols. Of course the possibility of behavioral hypometabolic regulation in natural settings should not be overlooked, and likely deserves more attention than it has garnered at this point. For instance, lower rates of oxygen consumption and activities of energy metabolism enzymes in deep-living fishes may reflect a combination of factors: reduced abundance of food; low temperature and water oxygen concentration; and deprived light intensity, which may account for reduced locomotory activity[195]. At any rate, the potential for hypometabolic regulation is well described for other vertebrates (hibernators, estivators) and this is certainly an area of research where fish could add another useful model to the analytical arsenal.

In fishes, red muscle contains more lipids than white muscle; for example, in Atlantic salmon, white muscle has a lipid content of 2%, while red muscle has about 15% (ref. 108). However, dark muscle lipid is not easily mobilized, which may be related to the special properties of these types of tissues. Red muscle is used for sustained swimming and for activities such as maintaining body position against currents, whereas white muscle is used intermittently, for sudden movements in case of pursuit or escape. Some diminution in locomotory capacity during food deprivation may be tolerable[195].

Although generally the muscle of elasmobranchs tends to contain more lipid than that of teleostean fishes, the specific composition may indicate that the bulk of this lipid represents structural rather than storage material and therefore is not easily mobilized. In agreement with this hypothesis, 82 days of starvation did not provoke significant changes on muscle lipids of *S. canicula*[47].

4. Lipid classes

During starvation-induced breakdown of lipids, different phases can be distinguished. The most accessible lipid store appears to be triacylglycerols (TAG, triglycerides), well exemplified in the European eel (*Anguilla anguilla*). In this species, triglycerides were the main energetic substrate during 95 days fasting[96]. Other (structural) lipids, in contrast, are generally entirely spared or used only during the later phases of food deprivation. As a result of focused breakdown of triglycerides, the specific fatty acid composition changes in the course of starvation. Since myristate (14 : 0), palmitoeate (16 : 1), and oleate (18 : 1) are mobilized prefer-

entially, their abundance in the remaining triglyceride declines almost continuously in the liver of *A. anguilla* in the course of starvation[35].

As a rule, triglycerides are mobilized before phospholipids during starvation[161], reflecting the duality of storage *versus* structural lipid. As delineated for the European eel liver above, there also is an apparent selectivity in the fatty acids mobilized. In rainbow trout, saturated fatty acids were mobilized in preference to other types of fatty acids from perivisceral fat deposits. Similarly, the bulk of fatty acids depleted from liver and muscle lipids were 16:1, 18:1 and 20:1 (ref. 90). A marked decrease in the 18:1 content of body and liver of starved rainbow trout was reported[161].

In salmon, spawning depletion resulted in utilization of long-chain mono-unsaturated acids such as 20:1 and 22:1, while those of shorter chains and polyunsaturated acids where consumed later[89]. Levels of 20:5 and 22:6 were maintained or proportionally increased in *Fugu vermicularis porphyreus* during early fasting[72]. In stock fish (*Merluccius capensis*) a relative increase in the degree of unsaturation is noted during starvation[190]. It should be kept in mind that the proportion of unsaturated fatty acids as well as the degree of unsaturation exert pronounced effects on membrane fluidity. Therefore, it is likely that the retention of certain fatty acids at the expense of others is guided not only by the necessity to requisition carbons for oxidation but also by the need to maintain structural integrity and fluidity of the membrane. As a result it can be expected that the mobilization of unsaturated fatty acids from lipids will differ in warm-water- and cold-water-adapted species.

Obviously, there is a natural limit to the amount of structural lipids that must be retained. Below this threshold, essential membrane functions will be compromised and the survival of the fish is in question[107]. In *Perca flavescens*, this limit seems to be reached when only 2.2% of overall lipid is left. Below this critical value the animals die[137].

4.1. Free fatty acids

The fasting-induced pattern of changes in plasma free fatty acids (FFAs) varies between species, but clearly does not lead to the rapid and marked increase of FFA familiar in starving mammals[13]. Researchers noted decreases of FFA in 30- and 90-day starved oyster toadfish (*Opsanus tau*)[176] while rainbow trout experienced increases[166] or no change under the same conditions. In the European eel (*A. anguilla*) no changes in plasma FFA were noticed during the initial 95 days of fast, followed by a marked rise thereafter[96]. A noticeable increase with 3 or 5 days fasting is found in *Limanda limanda*[49]. In sea bass (*D. labrax*) 40 days of starvation caused an increase in plasma FFA concentration of about 65% along with a 3- to 7-fold increase in plasma glycerol concentration[198]. In fasting catfish (*R. hilarii*) plasma FFA increased two-fold during the first 30 days[114]. However, initially FFA levels do not increase, and authors suggested that all FFA produced was first utilized locally, and it is not until later stages of starvation that a spillover of fatty acids into the plasma compartment is noticed.

It is well known that in fasted teleosts, in stark contrast to mammals, the production and utilization of ketone bodies does not play an important role[15,147,198].

In fact, it is under debate whether ketone body metabolism forms an integral part of fish intermediary metabolism at any time. First, some studies showed that 3-hydroxybutyrate dehydrogenase (EC 1.1.1.28), a key enzyme in ketone body production, was lacking in the livers of teleost fish[9,197,198]. Nevertheless, LeBlanch and Ballantyne[101] have demonstrated the presence of that enzyme in tissues of some freshwater species such as goldfish (*Carassius auratus*), brown bullhead (*Ictalurus nebulosus*), pike (*Esox lucius*) and crappie (*Pomoxis nigromaculatus*). 3-Hydroxybutyrate dehydrogenase activity has also been found in marine teleost species: alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), and mummichog (*Fundulus heteroclitus*). The levels of the enzyme in freshwater fishes are highest in the liver and kidney, tissues known to be ketogenic in other vertebrates[9], which could suggest a similar role of ketone bodies in fishes. But the levels of this key enzyme in ketone body formation in the marine species studied did not display the same tissue pattern, since the highest levels in the alewife were found in the brain, while the heart of the smelt had the highest activity. Second, under normal physiological conditions the concentrations of ketone bodies in tissues or plasma and the rate of utilization are very low[48] suggesting the idea of a minor role in teleost metabolism. No data exist showing that ketone bodies make a substantial contribution to the energetic requirements of teleost fishes during starvation.

On the other hand, the presence of the enzyme 3-hydroxybutyrate dehydrogenase in elasmobranchs was observed in earlier studies[128,198]. The levels of this enzyme and plasma levels of ketone bodies are both higher than in teleosts[47,198] and isolated hepatocytes will readily use and convert added ketone bodies[119]. These results indirectly support an earlier contention that plasma transport of fatty acids may be limited in elasmobranchs and other organisms lacking carrier proteins such albumin. Thus, ketone bodies may provide a more soluble lipid transport form[4,5,10]. Nevertheless, the role of ketone bodies in energy production and thus their contribution to energy supply during fasting is doubtful. Contrasting with increments of plasma ketone bodies observed in fasted mammals, plasma levels of these metabolites were not significantly altered in starved skate (*Raja clavata*)[197], but a large increase was observed at 36 days fasting in dogfish (*Scyliorhinus canicula*)[47].

5. *Influence of starvation on lipolysis and lipogenesis*

Generally, information on lipid mobilization in lower vertebrates is scant. Lipolytic activity has been found in salmonid adipose tissue, liver and red (dark) muscle[164]. In trout adipose tissue, the triglyceride lipase is subject to covalent modification through phosphorylation, with activity increasing as the enzyme is phosphorylated (K. Michelsen, J. Harmon and M. Sheridan, unpublished results). Activity of an acid lipase of lysosomal origin was found in rainbow trout dark muscle[14] and adipose tissue[165]. Bilinski and coworkers[14] suggested that lysosomal lipase from rainbow trout lateral line muscle serves mainly in the mobilization of intracellular lipids for internal use. Neutral lipase from rainbow trout adipose tissue was characterized[165] and it is this enzyme which seems to play an important role in lipid mobilization in adipose tissue and thus to supplying fatty acids to peripheral tissues[164].

At the same time, as the lipolytic machinery is activated during starvation, the activity of the lipogenic pathway seems to be curtailed, at least during the later stages of food deprivation. In coho salmon (*O. kisutch*), for instance, the activities of several lipogenic enzymes in liver remain unchanged for the first two days of starvation, while significant decreases were noticeable after 23 days[104]. Usually, cytosolic enzymes supplying reducing power in the form of NADPH are considered 'lipogenic'. These include two enzymes of the pentose phosphate shunt, namely glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, as well as malic enzyme (malate dehydrogenase-decarboxylating) and isocitrate dehydrogenase. The total NADPH available for FA synthesis in liver is lower in American eels (*Anguilla rostrata*) fasted 4–6 months than in fed or freshly caught eels[2]. In rainbow trout liver the activities of the NADP-linked dehydrogenases were substantially depressed after four weeks of food deprivation[8]. As other experiments show, the activities of these NADP-generating enzymes, albeit not always all of them, are governed by the dietary status of fishes[161]. Abundance of fatty material, as in the case of elevated concentrations of dietary lipid, decreases the activity of G6PDH and ME in rainbow trout liver[104]. Conversely, feeding a diet high in carbohydrates to channel catfish (*Ictalurus punctatus*) results in increased hepatic titers of all four NADPH-generating enzymes.

A more direct measure of lipogenic potential would be the analysis of ATP-citrate lyase and particularly of acetyl-coenzyme A carboxylase, the committed step in the synthesis of fatty acids. In the case of ATP-citrate lyase, activity of the enzyme decreases significantly in carp (*Cyprinus carpio*) starved for two months (H. Segner and R. Böhm, unpublished results). Unfortunately, the carboxylase is not well described in piscine systems, and none of the existing studies[60,84,116] analyzed carboxylase activity and phosphorylation status during different physiological stages of the experimental animals. In the rat liver, the enzyme is under stringent control though three different mechanism, all of which are known to be influenced by diet and starvation: (1) allosteric effectors such as citrate; (2) covalent modification through phosphorylation and dephosphorylation; and (3) enzyme concentration.

Liver slices from European eels fasted for 7–39 weeks incorporated ^{14}C-acetate into FA at lower rates (6- to 20-fold) than those found in fed eels[1]. After 95 weeks starvation, eel liver was still able of incorporate ^{14}C-acetate into FA, albeit at a lower rate[1]. *Esox lucius* starved for 2 months, converted ^{14}C-acetate preferentially into unesterified fatty acids and phospholipids[94]. It appears that during starvation, emphasis is on the synthesis of structural lipids, while carbon will be preferentially funnelled into storage lipids (triglycerides) after re-feeding.

In conclusion, lipids are stored as triglycerides in different tissues. But independently of the tissue location, lipid mobilization occurs during fasting, simultaneously or after carbohydrate mobilization, but always before protein degradation. In teleosts, FFA are the main products of lipid mobilization, while in elasmobranchs ketone bodies take their position. In comparison with carbohydrates, lipids represent an important source of energy due to the high depots accumulated and their high caloric value (Table 3).

TABLE 3

Initial energy content in total liver and muscle, relative change and rate of energy loss between different periods of fasting in carp (*Cyprinus carpio*)

	Initial values (kcal)	Relative change (%)	
		Day 1–8	Day 8–19
Liver			
Glycogen	1.12	−57.0	−18.1
Protein	0.84	−18.6	−13.8
Lipids	0.47	−12.4	−29.8
Total	2.42	−35.2	−19.2
Muscle			
Glycogen	0.73	+1.7	−6.5
Protein	21.7	−19.9	−22.4
Lipids	2.0	+23.4	−17.2
Total	24.4	−15.8	−21.2
Rate of energy loss			
Liver (cal/h)		5.07	1.1
Muscle (cal/h)		22.9	16.5

Data are given as means for 8 fish in each group. From Blasco et al.[17].

IV. Protein mobilization

The sequence of mobilization of the different sources of energy during the course of starvation seems to be very similar, in general terms, in the different species of fish. Usually, protein reserves are spared at the beginning of fast, thus proteolysis occurs only when more readily available energy reserves have been widely consumed such as liver glycogen and lipid stores as it does in higher vertebrates. Then, as protein is utilized, water moves in to take its place. Fish present special adaptations for protein mobilization: high level of proteolytic enzymes in muscle, coupled with the generalized ability to excrete excess nitrogen as ammonia or ammonium ion. Only very few species go through the metabolic expense of synthesizing – at appreciable metabolic cost – and excreting urea[123]. The actual contribution protein makes to meeting the overall energy requirements during fasting depends largely on the species[187]. It is trivial to note that the impact of starvation is felt sooner in active than in sluggish fish. The activity of catheptic enzymes is greater and more rapid autolysis is noticed in the muscle of such species as mackerel (*Scomber scombrus*) as compared with carp (*Cyprinus carpio*) or cod (*Gadus morhua*).

Different organs deplete endogenous protein to differing extents during starvation. As detailed below, fish tend to mobilize more protein from white muscle than from dark muscle. While increases in proteolytic activity with starvation have been found in liver, kidney, spleen, and red muscle, not surprisingly, it is the white muscle that experiences the largest increases in proteolytic activity.

In the initial phases of starvation in carp (*Cyprinus carpio*), proteolytic capacity is lowest in muscle, and increasingly larger in spleen, liver, kidney and highest in the intestine. This order in the series is most likely a reflection of normal tissue

turnover and synthetic demand of the tissue. As the period of food deprivation lengthens, this order is almost reversed. Now intestine has the lowest proteolytic activity while activity increases in the order: liver, kidney, spleen and white muscle. Rates of protein turnover must be matched to the energy demands; allowing for the dietary constraints that mark starvation, protein synthesis rates are adjusted to the new requirements. Again, the white muscle appears to be the fish tissue that is most sensitive to fasting and this tissue responds with a reduction in the rate of protein synthesis after food deprivation[71,105]. Protein synthesis rates of other tissues such as the liver and gills have been found to be little affected by starvation[81,154,168].

1. Liver proteins

In general, fasting exerts only limited effects on hepatic protein suggesting that the bulk of this protein has vital functions[31,126]. Observed increases in percentage of liver protein is usually correlated with decreases in liver weight due to the mobilization of other reserves in the course of starvation. These increases are observed in carp[17], eel (*A. rostrata*)[124] or brown trout (*Salmo trutta fario*)[135] after different periods of fasting. Absolute values of the total quantity of the store in the entire organ is a more meaningful physiological measurement, and analysis of data in percentage can lead to erroneous interpretations. Thus, actually, the total quantity of liver protein declines with fasting[17,135].

Fasting exerts little influence on protein synthesis rate in liver, but degradation increases under fasting conditions. Nevertheless, selective destruction of proteins may occur. Effects of starvation on tissue enzyme activities are variable[30]. The activity of gluconeogenic enzymes increases in numerous fasted teleost species[171]. Increases in the liver activities of alanine and aspartate aminotransferases has been reported in many teleost species suggesting a stimulation of gluconeogenic flux from amino acids[59,171]. These changes are accompanied by increased activities of liver gluconeogenic enzymes including phospho*enol*pyruvate carboxykinase (PEPCK)[124,127,171].

Another analytical aspect that has to be taken into account is, again, the manner in which the data are expressed and interpreted. Enzyme activity changes expressed in terms of tissue weight are not always adequate, because the liver mass relative to body size declines with fasting in many fish species, the total potential of the liver for some metabolic pathways would be reduced. This is the case in the perch (*Perca flavescens*) which shows a decrease in overall metabolism including the liver gluconeogenic pathway. It is clear that, particularly with respect to the liver, the use of tissue DNA content as a denominator for various parameters can result in a different interpretation of the fasting response[55].

2. Muscle proteins

Fish are in general well adapted to withstand long periods of depletion and this is probably because the muscle is rich in proteolytic enzymes which can mobilize the body tissues for fuel when required[106].

The main effects of prolonged starvation are increases in muscle proteolysis activity and mobilization of amino acids derived from muscle proteins for utilization by more 'vital tissues'. During periods of extended starvation, catabolism of muscle protein is initiated and the resultant amino acids are the major sources of energy for the fish[107,185]. Muscle protein mobilization is produced after glycogen mobilization when glycemia and lactate are in decline.

In some species, including *Gadus morhua*, muscle protein breakdown increases in the early stages of starvation and maximum values were found 3–4 weeks into a fast. Protein content starts to decline after liver lipid has fallen below a critical value. Muscle protein is the most important body energy store, being used mostly during prolonged starvation[31,187]. During spawning migration of sockeye and chum salmon, catabolism of muscle protein is preceded by 3- to 7-fold increases in the muscular activities of the proteolytic enzymes (cathepsin D and carboxypeptidase A)[118].

In common with mammals, when fish muscle protein is degraded, contractile and soluble proteins are removed preferentially, while connective tissue proteins are used to a lesser extent. The consequence is that the connective fibers rich in collagen are almost entirely spared from degradation. Thus, the proportions of glycine, proline and hydroxyproline (the key amino acids in collagen) in muscle protein increases during fasting.

The changes in protein concentration and composition during depletion may be distinctly different in red and white locomotory muscles[109]. For example, in the flounder (*Pleuronectes platessa*) white muscle loses a significant portion of myofibrillar protein during starvation, but this part of muscle protein was conserved in red muscle. Only mitochondrial and sarcoplasmic proteins were mobilized from red muscle in this species.

The overall balance between synthesis and degradation is altered with fasting. Some regulatory adjustments in protein metabolism are needed to bring about these changes in muscle protein concentration and composition. First, rate of protein turnover needs to be adapted to the new energy demands. Studies on protein synthesis in rainbow trout[105] and barred sand bass[109] have shown that white muscle protein synthesis rate declines to a plateau after 10–14 days without food. Second, and very important, the turnover of specific proteins in each muscle type must be regulated closely to ensure conservation of physiological capacity as we have also mentioned for liver protein. In this context, studies with barred sand bass (*Paralabrax nebulifer*) by Lowery and Somero[109] are illustrative. Also, white muscle myofibrillar protein synthesis rate was more affected by starvation than was the sarcoplasmic protein synthesis rate. Actin, a major myofibrillar component (approximately 10% of total white muscle protein in fish) was very sensitive to fasting (Table 4). That leaves the question of how the synthesis of vital enzymes or other proteins is differentially regulated? For instance, the relative amounts of the different glycolytic enzymes are relatively well conserved during periods of starvation, despite very large decreases in the total activities of the enzymes[118]. The conservation of relative activities could be achieved by different mechanisms. It seems that, at least in barred sand bass, differential effects on synthetic rates may exist. Muscle enzymes present at high concentra-

TABLE 4

Changes in protein and actin concentrations during fasting in *Paralabrax nebulifer*

Days fasting	White muscle		Red muscle	
	protein (mg/g)	% of fed	protein (mg/g)	% of fed
Protein				
0	192 ± 4	100	247 ± 12	100
5	174 ± 9	91	264 ± 12	107
10	177 ± 24	92	263 ± 12	107
16	135 ± 5[a]	71	222 ± 17	90
23	130 ± 3[a]	68	209 ± 11[a]	85
Actin				
0	18.5 ± 1.0	100	13.3 ± 0.3	100
5	15.2 ± 0.5[a]	82	12.9 ± 0.4	97
10	15.6 ± 0.3[a]	84	13.5 ± 0.7	102
16	12.8 ± 0.2[a]	69	12.9 ± 0.5	97
23	13.0 ± 0.4[a]	70	12.7 ± 0.4	95

Values are means ± SEM of four observations.
[a] Significantly different from fed values ($p < 0.025$). Adapted from Lowery and Somero[109].

tions, low specific activities and possessing long half-lives, show a diminution in synthesis rate with fasting much more pronounced than that found in enzymes present in lower concentrations and higher specific activities. In this way, the protein turnover may be regulated such that the relative proportions of specific proteins or enzymes are conserved. Unfortunately, methods of determining specific protein degradation rates present many problems, the most serious of which is the re-incorporation of radiolabeled amino acids[196]. The half-life of a protein, consequence of both degradation and synthesis rates, is likely changed during starvation.

3. *Plasma protein and amino acids*

There is a clear tendency for plasma proteins to decrease in fasting fish. In both brown trout (*S. trutta fario*) and carp (*C. carpio*) the electrophoretic pattern of plasma proteins is substantially altered compared with control fish after 30 days or 6 months fasting, respectively[106]. Plasma protein decreased in fasted mature carp after five days of fasting[17]. In addition, the overall concentration of plasma protein is curtailed. In carp, for instance, a 6-month fast decreases plasma concentration from 3.9 to 2.8% (ref. 106). Apparently, albumins are the first plasma proteins to undergo reduction in concentration, followed by the alpha and beta globulins. Gamma globulins, in contrast, were not utilized; in fact, since the overall protein concentration decreases, the percentage of plasma proteins contributed by gamma globulins is increased. A similar picture was observed in *Gadus morhua* during starvation, while an extreme situation is noticed in sockeye salmon during the spawning migration. The anorexic females of this species build up impressive levels of vitellogenin in their plasma in the course of migration, but at the same time

deplete their plasma store of albumin in its entirety. Some authors have suggested using albumin : globulin ratio as a nutritional indicator because of this sharp decline in albumin during starvation[106]. But in any case, the plasma concentration of different free amino acids is of greater interest than plasma protein levels since it reflects results of whole body protein turnover.

The composition of the free circulating amino acid (AA) pool changes markedly during fasting in all the species studied. In rainbow trout (*O. mykiss*) even a relatively short (24 or 48 h) fasting period lead to a decrease in plasma essential amino acids (EAA) but not in the non-essential AA[186]. The significant positive correlation between the levels of plasma EAA and their respective concentrations in the diet, as well as the lack of such a relationship for NEAA appears to be a general phenomenon[16,32,148]. The decline in EAA observed in plasma of carp or trout at the onset of fasting may respond to the uptake of these AA by tissues without a new contribution from diet, while NEAA levels are metabolized and altered to a greater extent and can undergo extensive interconversions.

Of all the presumed essential AA, histidine dropped most precipitously in fasted migrating salmon[118]. It appears that histidine is utilized by the fish at high rates in the early phases of migration, but the exact metabolic role of this amino acid is still not known. In carp (*Cyprinus carpio*), branched-chain AAs (leucine, isoleucine and valine) were affected most, decreasing by 44% after five days without food (Fig. 1)[16]. This decline suggests an increase of oxidation of these amino acids during fasting[16,199]. Nevertheless, after 19 days fasting a compensatory increase in plasma AA was noticed in this species. This increase is largely due to increases in branched-chain AAs. Of the non-essential AAs, alanine, glutamate and glutamine levels are augmented with the two five-carbon amino acids experiencing the largest

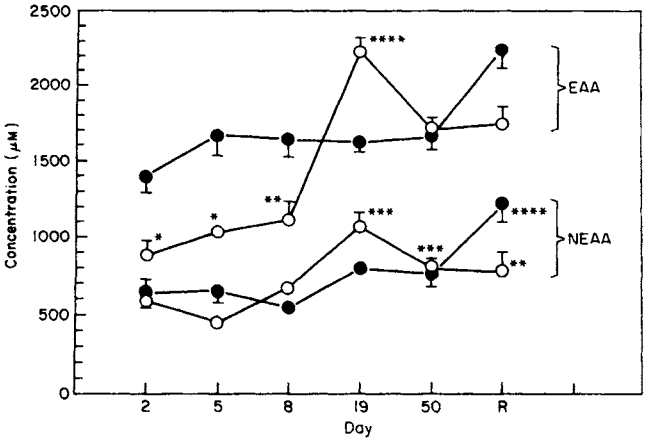

Fig. 1. Variations in plasma essential amino acids (EAA) and non-essential amino acids (NEAA) levels at different periods of starvation and refeeding (12 days) in *Cyprinus carpio*. Control (filled circles), starved (open circles), R = refed (for 12 days). Differences were determined by Duncan's multiple range test. * Different from control (p <0.05); ** different from control (p <0.01); *** different from preceding point (p <0.05); **** different from preceding point (p <0.01). From ref. 16, with permission.

increases (two-fold over control). The increase in plasma amino acids observed in fasting carp after 19 days is a consequence of muscle proteolysis and thus, it may be expected that generalized proteolysis of muscle proteins will provide a balanced cross-section of all the AA. However, this is clearly not the case. In starving dogfish (*Squalus acanthias*) alanine was selectively released into the plasma draining the white muscle[101]. Similarly, Mommsen and coworkers[118] noticed that alanine was the major AA released from white muscle of migrating sockeye salmon for transport of amino acid derived carbon to other tissues. It therefore appears that the main fate of AAs is transdeamination *in situ* to supply carbon precursors for gluconeogenesis mainly as alanine and possibly also as glutamate (which represent 46% of NEAA)[16]. Both alanine and glutamate are excellent gluconeogenic substrates in most teleostean fishes[129,157,171]. While this has been shown directly in isolated fish hepatocytes numerous times, this notion is further supported by the marked depletion of the glutamate/glutamine pool at 50 days fasting in carp, coinciding with tight maintenance of glycemia at this time[16]. Furthermore, other *in vitro* studies have demonstrated that fasting induced an increase in uptake of L-alanine by trout (*Salmo trutta*) hepatocytes that showed an inverse relationship with L-alanine plasma levels[20]. Thus, all the above studies support the metabolic model that glutamate and mainly alanine form fundamental precursors for gluconeogenic pathway in fish tissues under fasting conditions.

In summary, during starvation, proteins are mobilized at the final stage. Different tissues can contribute to the available pool of AAs, however, skeletal muscle represents the largest store of protein and, as consequence of the percent of muscle weight (with respect to body weight) it constitutes the main energy source during prolonged starvation (Table 3). Blood AA from tissue proteolysis will be transported to gluconeogenic tissues to maintain the necessary glycemic level.

V. Endocrine regulation

1. Insulin

Although the role of insulin in mammals under fasting conditions has been widely analyzed, comparative studies on the regulation of fish metabolism by insulin during food deprivation are relatively sparse. Nevertheless, of all the pancreatic hormones, insulin has received the most attention in fish studies[149]. Initial studies on the role of insulin in fish have concentrated on the direct effects of exogenous insulin on blood metabolites and tissue energy reserves. The regulatory function of insulin in fish during different nutritional conditions has been more clearly elucidated when measurements of circulating levels became available. Initial data on plasma insulin levels were successfully obtained by some authors in different teleost species[141,176]. These difficulties in measuring fish insulin levels can be explained by the fact that the mammalian radioimmunoassay (RIA) components proved to be useless tools in measuring fish insulins except in a few isolated cases. Cross-reactivities between many fish insulins and antibodies to mammalian insulins are very weak, in spite of

the fact that piscine insulins are structurally remarkably close to other vertebrate insulins[42]. Thus, the development of RIAs with fish components or homologous RIAs has been an essential step towards understanding the unique role of insulin during fasting in fish. Antibodies raised against insulins isolated from species such as scorpion fish, bonito, cod and anglerfish, together with their respective radiolabeled equivalents, can be successfully used in RIAs to accurately determine insulin levels of other fish species[63,141,178]. Nevertheless, the development of a homologous RIA for coho salmon (*Oncorhynchus kisutch*) has been described[151]. Clearly such homologous systems are to be favored, since they are less prone to erroneous data and require less effort to verify concentration estimates.

The general actions of insulin in fish seem to be similar to other vertebrates, in that insulin is a hypoglycemic hormone, lipogenic and promotes protein synthesis[122,132]. Just as in mammals, the circulating levels of insulin diminish during fasting in fish. Table 5 shows the changes in plasma insulin levels in starved elasmobranch and different teleost species.

An interesting phenomenon has been described for the cyclostomata. Working on the Atlantic hagfish (*Myxine glutinosa*), Emdin[41] found that plasma insulin levels declined within 1 month of starvation from 12 to 6 ng/ml. Concomitantly, a decline of blood glucose and an almost complete exhaustion of glycogen reserves in liver and skeletal muscle were observed. Surprisingly, in this species, insulin lacked metabolic effects on the liver whereas the hormone had the expected, but rather small, effect in muscle, where it stimulates the synthesis of glycogen, protein and lipids. It appears that the physiological role of insulin regulation of skeletal muscle metabolism is similar to that in mammals while its effects in liver are weak or absent. In fact, Emdin[41] considered liver of little significance in fasting or other catabolic situations in this cyclostome. In contrast, more recently, studies on hepatocytes isolated from this species of hagfish[57] reveal unusual effects of insulin in liver carbohydrate metabolism, such as increases in gluconeogenesis and total glucose production. Since it seems that glucagon is absent in the hagfish islet[42] an ancient catabolic function of this hormone may still be present in this primitive group of Agnathans. Similar contradictory insulin effects have also been described in two species of teleosts[53,54]. Thus, it may be necessary to design appropriate fasting studies since agnathan tissue metabolism and its regulation have been poorly studied, and important evolutionary aspects could be elucidated from these studies.

Little work has been done on selachian physiology. The studies on the effects of insulin in the dogfish shark (*Squalus acanthias*) suggest that the dogfish uses ketone bodies as a primary fuel at rest. Glucose would apparently be synthesized by gluconeogenesis to maintain muscle glycogen reserves. These carbohydrate stores would be used for anaerobic glycolysis in situations where vigorous swimming is needed. This hypothesis agrees with the opportunistic food habits of this carnivorous fish altering between consumption of large amounts of food and relatively long periods (about two weeks) of fasting[159].

Artificial food deprivation in an elasmobranch (*Scyliorhinus canicula*) leads to plasma insulin decreases similarly to what is described in the more evolved teleosts. Plasma insulin decrease progressively after 15 and 36 days of food deprivation

with a maintenance of low levels with prolonged fasting[62]. Plasma glucose levels decreased initially, and recovered during the period of declining insulin titers. It is interesting that the glucose decrease was paralleled by changes in plasma amino acid levels at 36 days of fasting. A similar decrease in total plasma amino acid levels has been also seen in other species of chondrichthyes such as *Squalus acanthias*[101], suggesting an increase of gluconeogenic processes would be favored by the diminished plasma insulin levels. More studies are needed to identify the tissue destination and metabolic pathway of the supposed *de novo* synthesized glucose and possible regulation by insulin and other hormones.

Comparatively more studies have been performed in teleosts (Table 5). The general picture emerging from these studies is a clear diminution of insulin plasma levels in fish after just a few days of fasting. Plasma insulin decreased during fasting in goldfish (*Carassius auratus*)[141]. Serum insulin levels of fed animals were nearly twice those found in 5-day fasted goldfish using codfish insulin as standard and guinea pig anti-cod insulin serum and ^{125}I-labeled cod insulin as other components. The same authors measured insulin secretion in islet incubations of oyster toadfish (*Opsanus tau*) obtaining values very similar to those determined earlier in the same species by Tashima and Cahill[176] using scombroid (bonito) insulin standards and guinea pig anti-bovine insulin antibody. In fact, these were the first studies describing a decline of insulin levels caused by fasting in teleosts. Subsequently, Thorpe and Ince[177] observed a very strong decline in plasma insulin in the rainbow trout fasted for 7 days, a diminution of 76% in relation to fed animals, and in only 4 days fasted cod a decline of 61% was observed. Furthermore, upon refeeding, the animals showed a clear recovery of the insulin levels after one week of food administration. Plisetskaya and coworkers[151] developed a homologous radioimmunoassay to determine plasma insulin in salmonids. These authors measured significant decreases (in excess of 50%) in insulin levels in blood of coho salmon (*O. kisutch*) after one or two weeks of fasting. The observed decreases are entirely reversed after refeeding.

Long-term experiments of food deprivation in salmonids always induce a decline in insulin plasma levels. In rainbow trout, a 6-week fast produced a decline in insulin plasma levels. This fact, together with the relative changes in other pancreatic hormones such glucagon and GLP, enhanced the gluconeogenic pathways in the liver, that are activated after this period of fasting[126]. This fact is also in concordance with the observations that, after injection of insulin in starved rainbow trout, glucose synthesis from alanine was drastically depressed, when compared with control fish[28]. The role of insulin in the regulation of gluconeogenic processes is corroborated in salmonids by *in vitro* experiments. In isolated rainbow trout hepatocytes, insulin curtailed the rate of gluconeogenesis from lactate[146]. This gluconeogenic inhibition by insulin is accompanied by activation of pyruvate kinase activity. However, this pattern does not seem to be universal since in isolated hepatocytes of sea raven (*Hemitripterus americanus*) porcine and fish insulins, contrary to expectations, increased the flux rate of amino acids into glucose[52]. More recent studies show that this insulin-stimulated increase in gluconeogenesis in sea raven may be at least partially related to an inhibition of the fructose-6-phosphatase activity ratio of 6-

TABLE 5

Plasma insulin levels of fed or fasted elasmobranch and teleostean fishes

	State		Insulin (ng/ml ± SE)
Scyliorhinus canicula[62]	Fasted	1 d	0.77 ± 0.04
	Fasted	15 d	0.64 ± 0.04
	Fasted	36 d	0.25 ± 0.03
	Fasted	82 d	0.30 ± 0.04
Carassius auratus[140]	Fed		3.50 ± 0.30
	Fasted	5 d	1.90 ± 0.50
Cyprinus carpio[17]	Fasted	1 d	5.21 ± 0.68
	Fasted	5 d	2.27 ± 0.27
	Fasted	50 d	2.23 ± 0.29
	Refed	12 d	5.93 ± 0.26
Cyprinus carpio[18]	Fasted	1 d	10.25 ± 0.71
	Fasted	16 d	8.28 ± 0.96
Gadus morhua[176]	Fed		8.43 ± 0.90
	Fasted	4 d	3.28 ± 0.20
Gadus morhua[73]	Fed		3.30 ± 0.73
	Fasted	7 d	1.60 ± 0.44
	Fasted	21 d	0.20 ± 0.13
Gadus morhua[73]	Fed		4.90 ± 0.82
	Fasted	7 d	1.90 ± 0.82
	Fasted	21 d	0.50 ± 0.12
Dicentrarchus labrax	Fasted	1 d	10.91 ± 0.25
	Fasted	22 d	8.68 ± 0.22
Oncorhynchus mykiss[176]	Fed		5.65 ± 0.30
(*Salmo gairdneri*)	Fasted	7 d	2.20 ± 0.30
Oncorhynchus mykiss[176]	Fed		6.80 ± 0.60
(*Salmo gairdneri*)	Fasted	7 d	1.60 ± 0.30
Oncorhynchus mykiss[125]	Fed		12.10 ± 1.10
	Fasted	42 d	2.00 ± 0.10
	Fed		13.20 ± 1.50
	Fasted	42 d	3.00 ± 0.10
Oncorhynchus kisutch[150]	Fed		4.50 ± 0.80
	Fasted	7 d	1.40 ± 0.20
Oncorhynchus kisutch[150]	Fed		4.30 ± 0.90
	Fasted	21 d	0.90 ± 0.10
Salmo trutta (summer)[134]	Fasted	1 d	4.85 ± 0.27
	Fasted	3 d	3.85 ± 0.21
	Fasted	30 d	3.12 ± 0.11
	Fasted	50 d	1.62 ± 0.14
	Refed	8 d	3.69 ± 0.32
Salmo trutta (winter)[134]	Fasted	1 d	5.48 ± 0.20
	Fasted	8 d	2.53 ± 0.18
	Fasted	30 d	1.29 ± 0.23
	Fasted	50 d	1.17 ± 0.23
	Refed	8 d	4.94 ± 0.23

phosphofructo-1-kinase (PFK-1 EC 2.7.1.11). Phosphoenol-pyruvate carboxykinase and pyruvate kinase (PK, EC 2.7.1.40) were not affected by insulin. PK is the most probable point for critical hormonal regulation in the gluconeogenic process in mammals. But caution must be used in interpreting these results as the relative importance of the various enzymes in the regulation of these pathways in fish is not yet clearly understood. A variable pattern is revealed in American eel (*Anguilla rostrata*) hepatocytes, where insulin induced either increases or decreases in alanine gluconeogenesis depending on the time of the year[53].

Other fasting experiments, with more dynamic measurements of insulin levels, have been performed in teleosts. In juveniles of Pyrenean brown trout, plasma insulin levels showed a progressive decrease (between 20 and 30%) after short-term fasting (3, 8 or 15 days) or after a more prolonged starvation (a decrease of 67% after 50 days). The decline of insulin titers was more rapid in summer than in winter[135]. This diminution of insulin levels was correlated with the mobilization of tissue energy reserves. Thus low levels of insulin could favor a degradation of liver glycogen during short-term fasting, together with the action of other hormones. Knowing the anabolic function of insulin in protein and lipid metabolism in fish[116,144], a decline in plasma insulin may contribute to the decrease in fat and protein reserves under fasting conditions in this species. A progressive decline of insulin plasma levels was also associated with the mobilization of energy reserves in sea bass, although, in that case, the role of glucagon seems to be more relevant[65].

Reduced plasma insulin levels following fasting are common in other species of teleosts, including cod[173,177] and carp[17]. In carp, insulin plasma levels always decrease with fasting experiments, although the response of glucagon to fasting is not so uniform in this species of teleost. Blasco and coworkers[17] found a two-fold decrease in insulin with respect to control values, after 8 days of fasting. This rapid decrease correlated nicely with the early mobilization of protein observed in that study. In carp, as well as in trout fasting experiments, the levels of insulin returned to control values after short periods (about 10 days) of refeeding. This fact suggests a rapid and high adaptability of fish to the recovery from fasting periods[76,135].

Unfortunately, the above-mentioned changes in insulin concentration and their correlation with metabolic output of fishes, are even less clear-cut under natural, rather than experimentally imposed, periods of food deprivation. For example, during the (anorexic) spawning migration of the pink salmon (*Oncorhynchus gorbusha*), plasma levels of insulin remained stable or, if any changes were noted, plasma levels were elevated. The elevation of plasma insulin, opposed to the expected decline, in this unique situation may preserve the energy stores instead of an early mobilization, thus enabling the fish to preserve sufficient metabolic reserves for final maturation of gonadal products and the exhausting spawning upon arrival at the spawning grounds[122]. Another explanation for the elevated insulin titers in pre-spawning non-feeding salmon or lamprey is a possible role of insulin as a gonadotropic hormone similar to the one described for mammals[155].

To ultimately make the connection between changing insulin titers in plasma during starvation and the altered metabolic status of fishes, it has become important to examine the interactions of insulin with its receptor in fish tissues. While the

first studies on insulin receptor in fishes elegantly, if not conclusively, dealt with evolutionary aspects of both the peptide and its receptor[102,130], more recent studies have focused on functional receptor characterization and the varied influence of different physiological states on insulin receptors in fish tissues. Specific insulin binding to fish liver or muscle[66,67] appears to be lower than that reported in mammals and birds[92,130,167]. In rainbow trout, a fast of 40 days caused a decline in plasma insulin levels and an increase in the binding capacity (specific number sites) in liver membranes, a situation reminiscent of increasing binding capacity for insulin in mammals and birds after a short fast[3,167]. However, at the same time, a decrease in the binding affinity in relation to control fish was noted, and as a result, specific insulin binding remained unchanged. This could be explained as a way to reduce the anabolic fluxes in the liver. Refeeding of fasted fish for 15 days restored plasma insulin levels and increased binding affinity with the presumed receptors attaining a higher specific activity than in control fish. Nevertheless during short-term fasting experiments (a few days) the number of binding sites seems to be a major regulating factor: after 3–6 days of fasting no changes in insulin titers were observed, but specific binding of insulin to the liver plasma membranes increased from 5.0 to 9.3% (ref. 66). We conclude that at the onset of fasting, insulin binding increases in fish liver, possibly as a compensatory mechanism, until all the metabolic fluxes are shifted towards catabolic and gluconeogenic processes, while a prolonged fast leads to a stabilization of the binding of insulin to its receptors. Since hepatic metabolism is strictly linked to the ratio of insulin to glucagon, the analysis of binding of other hormones such glucagon to the liver will be a prerequisite to understand metabolic regulation in the liver.

Insulin binding to muscle in fish and its regulation in response to fasting seem to vary between species[140]. It is interesting that omnivorous species such as carp (*C. carpio*) appear to have more numerous insulin receptors, with higher tyrosine kinase activity than carnivorous species (trout (*Salmo trutta*), sea bream (*Sparus aurata*)). The most striking difference was observed between carp and trout. In fed fish, the number of insulin receptors was significantly higher in carp than in trout (Fig. 2). Fifteen days of fasting resulted in a decrease to 50% of the respective control values in insulin binding to semipurified receptors of carp and trout muscle. This observation is supported by the concomitant decreased rate of anabolic processes and utilization of glucose by this tissue during fasting. After 30 days of fasting this tendency is a special feature in carp[17,18].

In summary, a number of strategies are available to fish tissues to respond to changes in insulin secretion rate and changes in plasma insulin titers. At least in liver and in white skeletal muscle, the number of hormone binding sites or their affinity can be adjusted, at times concurrently, with different nutritional states, thus resulting in the observed metabolic alterations.

2. *Glucagon and glucagon-like peptides*

It was not until quite recently that attempts have been made to elucidate the role of glucagon in fish under fasting conditions. Unlike insulin, glucagon levels in fish

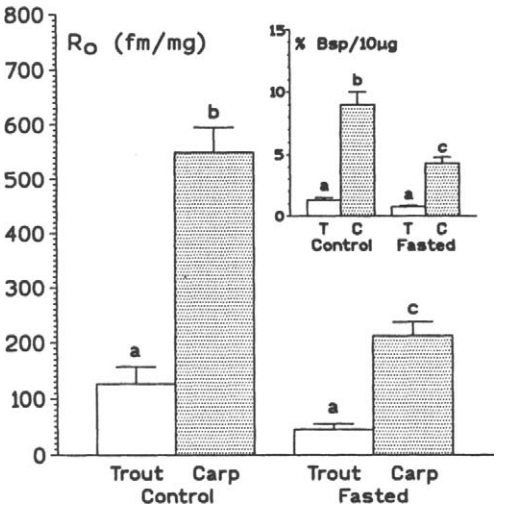

Fig. 2. Specific binding of insulin (inset) and binding capacity of muscle insulin receptors (main panel) of starved fed trout (*Salmo trutta fario*) and carp (*Cyprinus carpio*). Bars with identical letters are not significantly different at the 0.05 level. From ref. 140, with permission.

plasma can be measured successfully with a mammalian RIA[64], in spite of the fact that an appreciable variability exists in fish glucagons[42]. What helps matters is that key regions of the peptide are highly conserved[26] and show many structural similarities to the mammalian hormone. Nevertheless, some authors have used homologous RIA systems to assay glucagon titers in teleostean fishes[152].

In mammals, pancreatic insulin and glucagon act together in response to fasting. After a short-term fasting, insulin titers decrease while glucagon levels increase substantially resulting in a high molar ratio of glucagon/insulin. After a more prolonged fast, glucagon levels remain high and plasma insulin concentrations continue to decrease. Thus, the glucagon/insulin (G/I) molar ratio appears to be a key regulatory parameter providing a link to the nutritional state of the organism[179]. In most mammals, a normal G/I molar ratio after an overnight fast would be approximately 0.30. Undernutrition or a low carbohydrate meal will drive this molar ratio up towards unity and in total starvation the G/I value will likely approach 1.5–2.0. When the animal is in a catabolic situation, glucagon is instrumental in mediating the retrieval of stored nutrients at a time of need. On the other hand, in an anabolic situation, such as after a meal, the excess of nutrients must be stored, and in mammals G/I ratios may decrease to 0.1–0.05 or even lower if the diet carbohydrate content is high.

In fasting experiments in fish, plasma hormone levels follow a somewhat different pattern. In fasted juvenile sea bass (*D. labrax*) a steep increase in plasma glucagon was found after 4 days of fasting (Table 6) (similar to that occurring in higher vertebrates). This was accompanied by a stabilization of glucose levels. In contrast to mammals, this increased hormone level is not maintained and glucagon

TABLE 6

Plasma glucagon levels in fed or fasted teleosts

	State		Glucagon (ng/ml ± SE)
Dicentrarchus labrax[65]	Fasted	1 d	0.72 ± 0.14
	Fasted	3 d	1.31 ± 0.30
	Fasted	8 d	0.54 ± 0.11
	Fasted	15 d	0.37 ± 0.05
	Fasted	22 d	0.32 ± 0.05
Salmo trutta (summer)[135]	Fasted	1 d	0.65 ± 0.09
	Fasted	3 d	0.91 ± 0.03
	Fasted	8 d	0.51 ± 0.03
	Fasted	15 d	0.46 ± 0.04
	Fasted	30 d	0.25 ± 0.03
	Fasted	50 d	0.25 ± 0.03
	Refed	8 d	0.52 ± 0.06
Salmo trutta (winter)[135]	Fasted	1 d	0.44 ± 0.04
	Fasted	3 d	0.46 ± 0.05
	Fasted	5 d	0.55 ± 0.09
	Fasted	8 d	0.45 ± 0.03
	Fasted	30 d	0.32 ± 0.03
	Fasted	50 d	0.23 ± 0.03
	Refed	8 d	0.43 ± 0.04
Cyprinus carpio[17]	Fasted	1 d	0.42 ± 0.05
	Fasted	5 d	0.28 ± 0.04
	Fasted	50 d	0.14 ± 0.01
	Refed	12 d	0.54 ± 0.05
Cyprinus carpio[18]	Fasted	1 d	0.37 ± 0.04
	Fasted	50 d	0.64 ± 0.04
	Fasted	65 d	0.75 ± 0.12
Gadus morhua[73]	Fed		1.10 ± 0.32
	Fasted	7 d	0.30 ± 0.09
	Fasted	21 d	0.30 ± 0.03
Gadus morhua[73]	Fed		2.00 ± 0.73
	Fasted	7 d	0.30 ± 0.06
	Fasted	21 d	0.40 ± 0.13

concentrations declined again after 8 days. An increase of glucagon levels has also been observed in brown trout after 3 days of fasting[135], although this response was dampened compared to sea bass. It is possible that species differences can account for this difference in response, but surprisingly, no such increase was detected following a two- to four-day fast in the rainbow trout, another salmonid with a very similar lifestyle and diet as the brown trout[152,173].

The changes in glucagon and insulin in this short-term fasting lead to a rise in G/I molar ratio. Contrary to mammals, this ratio remains usually below 0.5. Increases of G/I values from 0.1 to 0.2 after a four-day fast in sea bass or from 0.2 to 0.4 in three-day fasted brown trout have been observed[65,135]. Immediately following a meal in fish, G/I molar ratios do not decrease as clearly as in mammals.

High levels of insulin a few hours after ingestion in salmonids[76,134] are compensated by subsequent elevation of glucagon levels which may be related to the high protein content of the diet. Sea bass (*Dicentrarchus labrax*) fed a natural diet shows a similar pattern of post-feeding hormonal changes[133].

In fasted sea bass and trout a correlation exists between high glucagon levels and decreased liver glycogen reserves. The glycogenolytic action of glucagon has been demonstrated *in vivo* and *in vitro* in numerous species of teleosts, although the degree of glycogenolytic action can vary depending on the season. Glucagon injections induced a decrease in liver glycogen in eel (*A. japonica*)[22], coho salmon (*O. kisutch*) or chinook salmon (*O. tshawytscha*)[152]. Similar potent glycogenolytic actions of glucagon have been seen in isolated hepatocytes from sea raven (*Hemitripterus americanus*)[52], coho salmon (*O. kisutch*)[156] or catfish (*Ictalurus melas*)[139]. Thus it can be envisaged that increasing plasma levels of glucagon at the beginning of fasting may induce the mobilization of glycogen reserves.

In contrast to mammals, long-term fasting in fish is accompanied by a drop in plasma glucagon (Table 6) and in glucagon-like peptide levels (Table 7)[126]. The relative changes in insulin and glucagon family peptides increased the G/I ratio from 0.11 in fed fish to 0.16 in fasted fish and GLP/I molar ratio from 0.08 to 0.25 after 6 weeks of fasting in coho salmon. Moon and coworkers[126] concluded that the relative hormonal changes are responsible for the observed activation of the gluconeogenic pathway during the later stages of food deprivation. Fasting-dependent increases in liver gluconeogenic capacity – as assessed by maximum enzyme activities of key enzymes – have been described in different species of fish (reviewed in ref. 125). *In vitro* experiments demonstrated that glucagon and especially GLP appear to be key peptides for regulating hepatic gluconeogenesis in fish[17,120,121]. It is interesting that this potent gluconeogenic effect of GLP has been found only in fish and not in mammals, where only insulinotropic and intestinal-related functions of GLP have been described[126]. In fishes, a high GLP/I ratio appears to enhance gluconeogenic processes and mobilization of carbohydrate reserves.

TABLE 7

Plasma glucagon-like peptide (GLP) levels in fed or fasted teleosts

	State		GLP (ng/ml ± SE)
Oncorhynchus mykiss[126]	Fed		0.6 ± 0.10
	Fasted	42 d	0.3 ± 0.04
Oncorhynchus mykiss[150]	Fed		1.9 ± 0.40
	Fasted	42 d	0.4 ± 0.02
Gadus morhua[73]	Fed		0.2 ± 0.06
	Fasted	7 d	0.2 ± 0.03
	Fasted	21 d	0.2 ± 0.03
Gadus morhua[73]	Fed		0.3 ± 0.06
	Fasted	7 d	0.2 ± 0.06
	Fasted	21 d	0.2 ± 0.06

A high G/I ratio associated with long-term fasting has also been described in 50-day starved brown trout (increase from 0.20 to 0.48)[135] but only in the summer season and not in the winter. Immature carp food-deprived for 50 or 65 days demonstrated an unusual increase of plasma glucagon levels[18]. Nevertheless, these increases in G/I ratios are not always clearly seen in fish during long-term fasting experiments[17,65]. It is quite apparent that generalizations are difficult at this point and that many other factors, such as the physiological state or previous diet, can influence hormone responses during fasting in fish. It is interesting that in anorexic individuals of brown trout (during the period of reproduction) maintained in captivity, a G/I ratio higher than unity was found in peripheral blood and portal vein[21]. Such a high molar ratio was never described before in fish. It is possible that during natural processes of anorexia, the hormonal response is enhanced in comparison to experimental fish.

Although it is clear that lipid reserves are mobilized during fasting in fish, the role of glucagon (a strongly lipolytic hormone in mammals) and other members of the glucagon family of hormones in this process needs to be elucidated. An indication of lipolytic action of glucagon has been found in *Esox lucius*[85] or *D. labrax*[142] where injections of glucagon induced an increase in plasma free fatty acids. However, administration of glucagon remained without effect in *Opsanus tau*[176] or eel (*A. anguilla*)[95]. It appears that hepatic lipolysis in fish is mediated by triacylglycerol lipase which hydrolyses stored triacylglycerol to glycerol and fatty acids. Glucagon family peptides have been found to influence lipolysis in salmonids. *In vivo* administration of glucagon or glucagon-like peptide induces an elevation of plasma fatty acids accompanied by enhanced hepatic triacylglycerol lipase activity, although the effectiveness of GLP in lipid metabolism in salmon varies depending on the time of the year[152]. *In vitro* experiments support the role of glucagon in lipid metabolism during fasting. It has been also demonstrated that glucagon acts directly on the liver since glucagon stimulates triacylglycerol lipase activity in liver slices as well as fatty acid and glycerol release into the culture medium. More recently, Harmon and coworkers[70] concluded that the increased lipolytic activity by glucagon in trout hepatocytes is mediated by phosphorylation of the enzyme.

The previous nutritional state of fish can modulate hormonal mediated lipolysis: trout basal hepatic lipolysis is enhanced in liver slices from four weeks fasted fish, and glucagon-stimulated lipolysis was more pronounced in liver slices from these food-deprived animals than in liver from fed fish. Surprisingly, glucagon failed to affect hepatic lipolysis in the liver of six-week fasted animals[69], maybe as a result of already advanced lipid store depletion, or alternatively, as a consequence of alterations at the receptor level. Thus, all the above studies support the idea of glucagon and GLP being key hormones in regulating lipid mobilization during fasting in fish.

Although plasma glucagon titers give us important information about the role of this hormone during fasting, receptor studies help in understanding the significance of the hormonal regulation. Navarro and Moon[136] have recently characterized, for the first time in fish, specific binding of glucagon in hepatocytes isolated from two teleostean species, the American eel (*A. rostrata*) and the brown bullhead

(*I. nebulosus*). Two classes of binding sites have been described with apparent dissociation constants (K_d) of 1.97 nM (high affinity) and 17.3 nM (low affinity) for bullhead and 2.68 and 22.9 nM, respectively, for eel cells. These values are approximately ten times higher than those generally reported for mammalian hepatocytes[11,80]. This reflects the relatively slow association of glucagon binding in these fish which may be attributed to intrinsic characteristics of ectothermic animals. It is interesting that, a higher number of binding sites was found in the hepatocytes from the eel (10,413/cell) in comparison to the bullhead (3811/cell). Mommsen and Moon[121] reported a higher increase in intracellular cAMP in response to the same glucagon concentrations in eel compared to bullhead hepatocytes. This suggests the existence of a correlation between the responsiveness of liver for glucagon binding and adenylyl cyclase in these fish species. However, it should also be noted that the eel is a species which fasts in captivity, while bullheads adapt to captivity quickly and feed actively. Nevertheless, more studies are needed to elucidate if such differences reflect species characteristics, or nutritional state. Liver cells of both eel and bullhead were able to regulate the abundance of their own receptors, since receptor number decreased by about 55% in both species with incubation of 100 nM glucagon. This 'down regulation' has been described in mammalian species[79,160]. These findings raise the question as to whether plasma glucagon levels could regulate hepatic glucagon receptors *in vivo* and especially in catabolic situations such as fasting.

Variations in the number of receptors could help explain discrepancies described in glucagon sensitivity for fasted chinook salmon (*O. tshawytscha*)[93], and for sea raven (*Hemitripterus americanus*)[56]. Glucagon-stimulated glycogenolysis in liver pieces isolated from fed and from short-term (one-week) fasted salmon, but failed to stimulate glycogenolysis in liver slices from long-term (three-week) fasted fish. In contrast, epinephrine maintains its glycogenolytic action regardless of nutritional state. In this fish species, a short fast induces an initial liver glycogen degradation while in longer term fasted salmon, mobilization ceases in the face of high glucagon/insulin molar rates. Thus it appears likely that glucagon is modulating fasting-associated adjustments in the metabolism of salmon by decreasing hormone sensitivity. Thus these salmon are able to maintain some minimum pool of glycogen.

Distinctive hormonal features of the sea raven (*Hemitripterus americanus*) fasting strategies distinguish it from those species in which glycogen is partially conserved as in salmon. In six-week fasted sea raven, endogenous carbohydrate stores are used preferentially for the production of glucose instead of gluconeogenic precursors. Hepatocytes isolated from six-week fasted sea raven had an increased apparent sensitivity of rates of total glucose production (presumably by glycogenolysis) to glucagon. This phenomenon would retain the effectiveness of glucagon in the liver and allow for increased glucose production in this species under fasting conditions. All the findings mentioned above show that nutritional state modifies hormone effects and may explain some of the seasonal differences in hormone action previously reported for this and other fish species[52,53].

In conclusion, fasted fish have proven to be a good model to study glucagon-like hormone actions. It seems that glucagon family peptides in fish are key hormones

in regulating energy reserves in fish under fasting conditions. Recent and detailed studies on the glucagon-related hormones reveal fine adjustments to the nutritional state of fish involving tissue receptors and responsiveness of target cells. Clearly such studies are to be favored since they may help to understand the metabollic mechanisms of fish adapting to adverse conditions.

3. Glucocorticoids

Administration of adrenocortical hormones, such as cortisol, to higher vertebrates normally stimulates gluconeogenesis, provokes a rise in liver glycogen and leads to an inhibition of glucose uptake in several peripheral tissues. Concomitantly, protein catabolism is stimulated. However, the immediate importance of such hormones under fasting conditions is under debate since generally, a decrease in cortisol secretion in early fasting is observed[46]. Nevertheless, these hormones may have a supportive function in the control of normoglycemia, even in fasting conditions.

It appears that corticosteroid hormones could have similar actions in teleost fish, but the importance of these hormones during fasting remains controversial. With respect to carbohydrate metabolism, the studies performed in whole animals concluded that cortisol administration resulted in enhanced liver gluconeogenesis based mainly on tissue carbohydrate changes[19,97,132]. Since activation of gluconeogenic processes during fasting has been demonstrated also in fish as well as in higher vertebrates, cortisol may contribute to its regulation. In mammals, it has been reported that cortisol may exert, in addition to the direct metabolic effects, an indirect action on other hormones such as thyroid hormones, glucagon or catecholamines. Also, some authors have suggested that the insulin/cortisol ratio could regulate the direction of metabolic fluxes during fasting in fish[132]. However, to date none of the *in vivo* studies analyzing the role of corticosteroids in fish systems have attempted to separate direct from permissive effects. Arguably the best example of such permissive effects in mammals is the potentiating effect of corticosteroids on glucagon's multiple actions, an area that remains to be analyzed with piscine systems. Vijayan and colleagues[182–184] have looked at the interactive effects of cortisol and other glucoregulatory hormones. These authors demonstrated that cortisol implantation for 7 days in fed sea raven (*H. americanus*) enhances the responsiveness of hepatocytes to the actions of epinephrine and insulin, but not glucagon, on carbohydrate metabolism. Although hepatocytes from food-deprived (eight weeks) animals showed enhanced responsiveness to pancreatic hormones, these effects were not modified by cortisol implantation. These slow-release implants of steroid hormones have been used successfully to evaluate the chronic effects of cortisol on the physiology of teleosts[181,182,194].

The effects on carbohydrate metabolism in isolated fish hepatocytes are not definitive. Recent studies show that cortisol increases hepatic activities of glycerol kinase (GK, EC 2.7.1.30) and fructose 1,6-bisphosphatase (FBPase, EC 3.1.3.11) in brook charr (*Salvelinus fontinalis*) indicating enhanced gluconeogenic potential from glycerol[181]. Metabolic flux in eel hepatocytes was altered by cortisol administration, shifting the preferred gluconeogenic substrate from lactate to amino acids[51]

as previously suggested by other authors[29,87]. Following administration of cortisol, activities of phospho*enol*pyruvate carboxykinase and glutamate-oxaloacetate transaminase (AspAT, EC 2.6.1.1) increased, suggesting an enhanced amino acid gluconeogenesis. Surprisingly, the absolute rate of gluconeogenic substrates incorporation into glucose actually declined in hormone-injected fish[51]. However, repeated injection of cortisol (which is considered a stress hormone itself), requires repeated handling of specimens and may lead to superimposing metabolic stress reactions mediated by catecholamines or permissive effects of cortisol plus catecholamines (or others) skewing the experimental results. In addition, repeated injection of cortisol may actually lead to increased turnover, i.e. speed-up removal of the injected hormone and thus the separation of cortisol-induced effects from those induced by fluctuating hormone titers, or drastically altered endogenous turnover, is difficult.

Cortisol is known to stimulate glycogen synthesis in mammals, but in fish the data are not clear-cut. At this point, it is not clear whether species differences hormone application or other parameters are responsible for the observed inconsistent results. In fed goldfish (*C. auratus*), for instance, cortisol failed to increase liver glycogen concentration, but the hormone seemed to be effective in maintaining liver glycogen content in fasting fish[170]. Administration of cortisol in the Japanese eel, however, resulted in increased liver glycogen levels[23].

Cortisol has been implicated in the regulation of protein metabolism. Administration of cortisol in fed goldfish resulted in a loss of body weight and the hormone accelerated the rate of loss of body weight in fasted animals[170]. Associated with this observation, a rise in ammonia excretion and a two-fold increase in the concentration of alanine aminotransferase (AlaAT) in the liver were noted for treated fish. This suggests that cortisol is important in controlling the utilization of tissue protein during fasting at least in the goldfish. Similarly, Chan and Woo[23] demonstrated that injections of cortisol induce a rise in liver transaminase activities in the eel, together with an elevation of ammonia excretion, and hyperaminoacidemia; the latter is likely a reflection of increased peripheral proteolysis. These observations coalesce into the suggestion that in teleostean fishes, cortisol shifts the preference towards amino acids as gluconeogenic substrates[29,51], conceivably together with increases in proteolysis in extrahepatic tissues.

Studies on the lipid metabolism of the eel indicate that cortisol induces lipolysis, increasing production and utilization of free fatty acids from triglycerides for energy production[36,103]. Implantation of juvenile coho salmon with cortisol resulted in lipid depletion, mainly in triacylglyerols accompanied by elevated lipase activity in the liver, red muscle and mesenteric fat[163]. Similarly, one step removed from the immediate effects of cortisol, administration of mammalian adrenocorticotropic hormone (ACTH) increased plasma FFA in goldfish within 6–24 h[116]. However, the same treatment failed to affect the concentration of plasma FFA in carp[45] and rainbow trout[174]. Unfortunately, no information is available on the interplay of ACTH with lipid metabolism during fasting.

In natural fasting situations, such as during the non-feeding migration periods in some fish, glucocorticoids are believed to play an important role, especially in

enhancing the gluconeogenic processes[132]. Plasma glucocorticoid levels increase in sturgeons (*Acipenser güldenstädti*) during the upstream migration period, particularly in males and this increase in hormone titer is accompanied by a depletion of the interrenal tissue.

During the anorexia period of migration of the sockeye salmon (*O. nerka*), a six-fold increase in the concentration of corticosteroids has been observed. This fact was accompanied by a massive (up to 60%) breakdown of body, primarily muscle, protein[82,84]. Other authors have also found high corticoid levels associated with migration in salmon. McBride *et al.*[111] found that plasma cortisol levels increase in pink salmon (*Oncorhynchus gorbusha*) during migration, reaching levels similar to those normally associated with stressed fish[7]. It appears that this hyperadrenocorticism is not directly associated with food deprivation, although it is clear that the catabolic effect of the increased levels of corticosteroids would facilitate the mobilization of energy from body reserves. On the other hand, it has been described that teleost interrenals are able to metabolize androgenic steroid precursors to testosterone and to synthesize progesterone[25,83]. The enhanced activity of the adrenal may contribute to the process of sexual maturation.

Experimental fasting studies made mainly in salmonids have shown an opposite trend. In *O. mykiss* (*Salmo gairdneri*) plasma cortisol levels were not affected by prolonged fasting (65 days), suggesting that this hormone is not involved in the processes of energy mobilization in this species[115]. In an attempt to simulate natural migration conditions, continuous swimming of experimentally food-deprived coho salmon (*O. kisutch*) changed neither plasma cortisol levels nor cortisol binding sites in liver[184]. However, plasma cortisol titers decrease with fasting in sea raven[183]. Although cortisol levels seem to be representative indicators of the fish responses to stress, these are not greatly affected by fasting. Barton *et al.*[7], found that plasma cortisol elevations in response to handling stress in juvenile chinook salmon were not appreciably modified in 20-day fasted fish in comparison to controls. Nevertheless, the hyperglycemia response to stress was lower in fasted than in fed fish.

In summary, experimental fasting situations have not been conclusive in determining the precise role of cortisol in fish, although some general trends such as the activation of gluconeogenic pathway from amino acids, are apparent. Data on glucocorticoid actions under fasting conditions in fish are scarce and plasma variations are not always clearly correlated with the physiological state of the fish. The interpretation of the data is complicated by the possible permissive effects of cortisol on other hormone actions.

4. Growth hormone

Few studies have been performed on the role of growth hormone (GH) during fasting in fish. In mammals, the plasma GH concentration increases with a short-term fast. Similarly, very large increases in plasma GH levels have been reported for trout after fasting. Periods of fasting of 20–30 days resulted in a 7-fold increase in plasma GH, compared to controls in steelhead trout (Fig. 3)[6]. The same species

fasted for six weeks also maintain higher levels of growth hormone than comparable, fed animals[44]. It has been postulated that the increase in lipid mobilization is needed to sustain energy production during periods of continuous activity and food deprivation such during migration time[112]. In concordance with this hypothesis, Sheridan[163] has reported that GH stimulates an increase in lipolytic enzyme activity in coho salmon parr.

Barret and McKeown[6] also observed an exaggerated GH response when starving fish were exercised for 24 h in comparison with exercised fish maintained on a normal feeding regime (Fig. 3).

Obviously fasting leads to a paradoxical situation in which starved animals although having high levels of GH do not grow. GH acts indirectly through insulin-like growth factors (IGFs) in most tissues. Thus, Sumpter and coworkers[172] working on rainbow trout suggest that the most probable mechanism to explain this

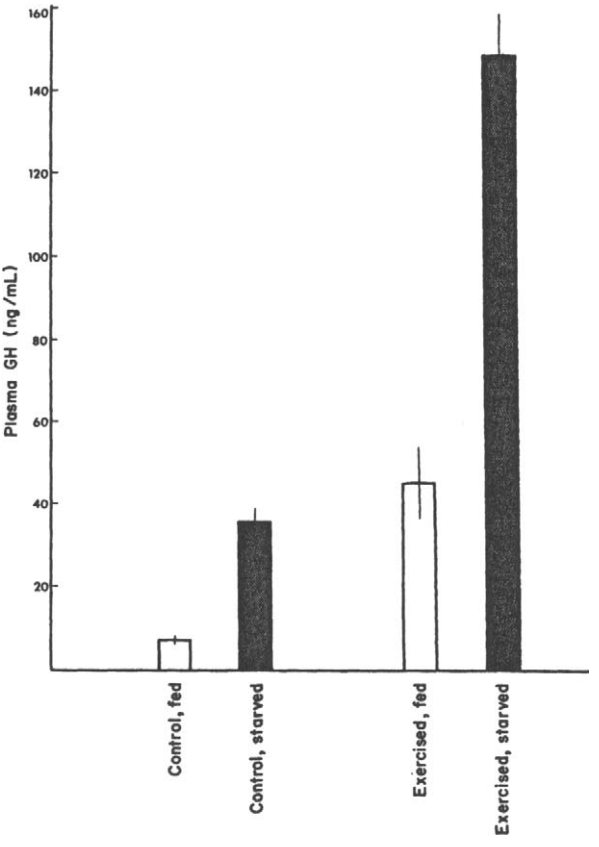

Fig. 3. Mean levels of plasma growth hormone (GH) of juvenile steelhead trout (*Salmo gairdneri*; now: *Oncorhynchus mykiss*). Some treatment groups were starved for 30 days, others were forced to swim at 1.5 body length per s for 24 h. Growth hormone levels were measured with a radioimmunoassay. Reprinted from ref. 6, with permission.

paradoxical situation would be a suppression of insulin-like growth factors similar to the situation described for mammals[24].

In vitro studies with tilapia (*Oreochromis mossambicus*), have shown that fasting results in a substantial increase in GH release from pituitary cell incubations[158]. An interesting outcome from these studies on the regulation of fish GH secretion during fasting is that the decline in glucose levels would be the signal to induce hormone secretion as it does in mammals.

In conclusion, there is a clear response of growth hormone to fasting in fish, although the specific functions of this hormone in the metabolic adjustments demand further investigations.

5. *Thyroid hormones*

It is generally agreed that thyroid hormones, tri-iodothyronine (T_3) and thyroxine (T_4), play an important role in vertebrates affecting a variety of processes such as metabolism, growth, differentiation and reproduction. Studies on the action of thyroid hormones are complicated because even in higher vertebrates thyroid hormones mostly affect metabolism indirectly being required for the permissive actions of other hormones. It seems that, as in mammals, T_4 in fish acts mainly after conversion to T_3 which represents the active thyroid hormone at the level of target cells[33]. The production of T_3 is increased during short-term overfeeding which is correlated with an increased diet-induced thermogenesis in mammals. On the contrary, plasma levels of T_3 decrease during fasting. Some of the known metabolic effects of thyroid hormones on mammals are the potentiation of the mobilization of fat reserves, induction of hyperglycemia and activation of protein synthesis. The general picture that emerges from studies on administration of thyroid hormones in fish is that these hormones appear to regulate fish intermediary metabolism in a similar way as in other vertebrates. However contradictory findings have been described depending on the hormonal dose, fish species or season (reviewed in refs. 98 and 153). The thyroid hormone-induced increase in the metabolic rate, consistently reported in the mammalian literature[11], has not been unequivocally proved in fish[145,193].

Fasting effects on fish thyroid have been studied primarily in salmonids. Short-term fasting for several days or more prolonged food deprivation for several weeks induced a decrease of plasma levels of both T_3 and T_4 in *Oncorhynchus mykiss*[50,99,115]. A similar lowering of plasma thyroid hormone levels was found in starved *Platichthys* sp.[138]. In contrast, no significant changes or even increases in plasma T_4 have been described in *S. fontinalis*[37,74]. Decreased thyroid activity in response to fasting in teleost is indicated by histological changes observed in the thyroid of starved *Oncorhynchus nerka*[110] and *Anguilla anguilla*[78].

Kinetic studies on thyroid hormones are not clearly correlated with changes in plasma hormones although they demonstrate a reduction in thyroid activity in fasted fish as well as in mammals. Since thyroid activity is generally related to a well fed and optimal metabolic state of the animal, this reduction may represent a homeostatic protective mechanism to prolong survival of the organism under conditions of food deprivation. Surprisingly, fasting induces a reduction in T_4 and

T_3 metabolic clearance similarly in fish or mammals[39,74]. Thus it appears that fast-induced low levels of plasma T_3 could be due to a reduction in peripheral conversion of T_4 to T_3 and its release to the plasma. The changes in blood hormone levels may not depend solely on the balance between production and degradation. A major complication is that, at least in mammals, food intake also influences the plasma protein binding of thyroid hormones with attendant changes in total and free hormone levels[40]. Some *in vivo* studies support the hypothesis of decreased mono-deiodination from T_4 to T_3. Injection of ^{125}I-T_4 in trout demonstrated that the transformation of T_4 to T_3 is already reduced after 3 days of fasting[38,50,74].

The number of putative T_3 receptors in the liver nuclei is also reduced by 3 days of fasting[180]. At least in salmonids, fasting may produce a peripheral hypothyroidism by decreasing both T_3 production and the density of hepatic T_3 binding sites.

This general decrease in thyroid activity associated with fasting observed in fish and also in higher vertebrates, may reflect the need to limit an exaggerated metabolite mobilization of energy reserves[115].

VI. General conclusions

In conclusion, there is no doubt that fish tissue metabolism is finely regulated under fasting conditions by the actions of many hormones. Interpretation of experimental data have to be done carefully, taking into consideration the previous nutritional and physiological state of the fish. Especially in the case of starvation, laboratory experiments, generally done on heavily inbred fish previously fed on a diet maximizing meat production, give quite different results to those done on naturally starving fish. Thus, understanding how fish respond to fasting requires research at multiple levels from molecular to ecological. Studies involving plasma hormone levels and energy tissue reserves gives us general information on which metabolic processes are favored during a catabolic fasting situation. Bearing in mind that fish metabolism is the result of a complicated integration of multiple processes in the whole animal, and numerous environmental factors, *in vitro* studies are preferred in order to identify which metabolic pathways are activated during fasting and how they are regulated by the specific actions of the hormones. Piscine cell systems are a relatively new approach for studies at the cellular and metabolic level and could be particularly valuable for integrating molecular and physiological studies. Recent research at the molecular level, the principal ones being, for instance, the characterization of fish tissue hormone receptors and the identification of new regulating peptides, open new questions on the particular strategies of metabolic regulation to survive food deprivation situations, an interesting field of research that demands further investigation in fish models.

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