THE EFFECTS OF SUSTAINED EXERCISE AND ENVIRONMENTAL STRESS ON PLASMA GROWTH HORMONE LEVELS IN JUVENILE SALMONIDS

by

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The effects of sustained exercise and environmental stress on plasma growth hormone levels in juvenile salmonids

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ABSTRACT

Plasma growth hormone (GH) levels of juvenile salmonids were determined, in response to various conditions simulating different phases of migration, using an homologous radioimmunoassay (RIA). The conditions of study were: 1) Physical exercise; a) sustained exercise, b) variations of exercise intensity and duration, and c) exercise training. 2) Environmental stress; a) long term starvation, b) seawater transfer, and c) temperature acclimation. Environmental stresses were tested both individually and in combination with sustained exercise. Fish were exercised in an open top swim tunnel apparatus.

The standard exercise bout of 1.5 body lengths per second (bl/s) for 24 h resulted in an eight-fold increase in plasma growth hormone concentration ten minutes after exercise as compared to non-exercised control fish. Peak growth hormone levels appeared to be more related to the intensity rather than the duration of exercise, although a minimum duration of 4 h was essential for any GH response.

Daily exercise training (2.0 bl/s for 4 h/day) modified peak growth hormone levels and recovery time compared to non-trained counterparts following the standard exercise bout. Peak GH levels doubled in trained fish and returned to normal levels four times faster than non-trained fish.

Long term starvation increased plasma growth hormone levels six-fold. Transfer of freshwater fish to seawater

increased plasma GH seven-fold after 24 h compared with fish transferred to freshwater. Sustained exercise in combination with starvation or transfer to seawater significantly increased levels of growth hormone compared with non-exercised fish that were starved or transferred to seawater.

Temperature acclimation from 17° C to 9° C had no significant effect on plasma GH levels in resting fish. However, growth hormone levels increased in both groups when exercised at their respective temperatures.

Preliminary analysis shows an increase of free fatty acids in plasma after exercise, starvation, seawater transfer, and temperature acclimation.

Increases in plasma growth hormone in response to physical exercise and environmental stress appear to mobilize energy reserves to satisfy increased fuel demands under stress.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

Growth hormone - Its nature, function, regulation and method of detection in the blood

Growth hormone (GH) or somatotropin (STH) is a pituitary hormone which, in vertebrates, is a simple straight chain protein of approximately 23,000 daltons molecular weight. Growth hormone is secreted from somatotroph cells located in the anterior pituitary (adenohypophysis; pars distalis) in all vertebrates (Gorbman et al. 1983, Martin 1985). The primary function of GH is the stimulation of somatic growth, both skeletal and soft tissues, and at least some of the influences of GH on the skeletal system require the intermediary production of peptides that are collectively termed somatomedins. GH acts to stimulate amino acid transport into cells, incorporation of these amino acids into protein, and a general inhibition of gluconeogenesis (Martin 1985). All of these factors are consistent with its role in growth. As might be expected, a hormone which affects the complex nature of somatic growth might necessarily play a role(s) in the many other aspects of body function associated with bone and tissue growth. Indeed GH possesses a variety of other functions associated with somatic growth such as; 1) increasing appetite, such that GH may act directly on centres in the hypothalamus that influence food intake, 2) elevating metabolic rate, such that GH's growth promoting actions increase the need for oxygen, 3) maintenance of the immune system, as GH is needed

for the normal development of the immune system and plays a role in primary antibody response, and 4) interaction with other hormones to regulate protein, lipid and carbohydrate metabolism (Gorbman et al. 1983, Martin 1985, Hall et al. 1986). The metabolic effects of GH may be affected by GH directly or by GH influencing the secretion of other proteins. GH particularily influences lipid metabolism by increasing lipolysis, decreasing lipogenesis, and increasing uptake of glucose by adipose tissue (Rudas and Scanes 1983). GH also exerts limited effects on the secretion (and to some extent metabolism) of insulin, glucocorticoids, and thyroid hormones (Scanes and Lauterio 1984).

The secretion of GH is regulated by at least three other peptide hormones of hypothalamic origin. Somatotropin release-inhibiting factor (SRIF) or somatostatin is inhibitory in nature and GH secretion, in vertebrates, is depressed with infusion of SRIF (Scanes and Lauterio 1984, Hall et al. 1986). Growth hormone releasing factor (GRF) or somatocrinin and thyrotropin releasing factor (TRH) are both potent stimulators of GH secretion in vertebrates (Hall et al. 1986). Growth hormone secretion in humans is more sensitive to the effects of GRF than TRH (Sonntag et al. 1982), however these potencies appear to be reversed in other vertebrate groups such as the birds (Class - Aves) (Scanes and Lauterio 1984). A variety of non-hypothalamic factors also modulate GH release. Among these steroids, amino acids, glucose, fatty acids, and various inorganic ions exert effects on GH secretion at both

hypothalamic and pituitary sites of action (Hall et al. 1986).

There are several different techniques used in the study of endocrine factors. The most powerful and advanced endocrine tool for measuring minute levels of a hormone in the serum or plasma is the radioimmunoassay (RIA), first developed for insulin by Berson and Yalow in the late 1950's and early 1960's. The fundamental prerequisite of the RIA technique is a sufficient quantity of the purified hormone to be studied. The purified hormone is used to 1) generate antisera, 2) provide a source of hormone to be radiolabelled, and 3) provide a source of unlabelled hormone to generate a standard curve. The basic principle of the RIA technique is that the labelled (usually ^{125}I, which adds to tyrosine residues of protein) and unlabelled hormone compete equally well for the antibody binding sites. Following an incubation period, which allows the system to reach an equilibrium (24 h in the GH-RIA), the antibody-bound hormone is separated from the free unbound hormone by a variety of techniques (in the GH-RIA by the double antibody technique). The antibody-bound hormone precipitates out of solution, the supernatant is poured off, and the radioactivity of the remaining pellet is counted. In such a competitive assay the amounts of antisera and the labelled hormone are fixed in all tubes, and known amounts of unlabelled hormone are used to generate a standard curve. It follows that unknown samples with relatively large amounts of hormone will have corresponding lower counts as compared to unknown samples which contain small quantities of the hormone. Growth hormone

levels in the present study were determined using such an RIA developed in salmonids and validated in the genera Salmo (Wagner and McKeown 1986).

External factors effecting GH secretion in higher vertebrates

A variety of external stimuli cause the release of GH from the mammalian and avian pituitary (Muller 1974, Scanes and Lauterio 1984). Among these are various stresses, including restraint and exercise, temperature fluctuations, dehydration, anaesthetics, and food shortage (Shephard and Sidney 1975). For the purposes of this investigation the author will confine the present discussion to the influences of exercise, food shortage, and temperature effects on GH concentration.

Roth et al. (1963) and Hunter and Greenwood (1964) first reported an increase in GH concentrations of human subjects in response to physical exercise. Since that time a variety of exercise types and regimes (ie. weightlifting, running, cycling and climbing) have been shown to result in elevated GH levels (Galbo 1983, 1986). So predictable is the growth hormone response to exercise that stationary cycling and treadmill running are used as provocative tests for detecting growth hormone deficiencies in young children (Buckler 1972, Parkin 1986). In exercising humans, the typical GH profile is one of a gradual increase above resting values (1-5 ng/ml) during the exercise bout, with peak levels (15-40 ng/ml) generally reached between exercise termination and fifteen minutes post-exercise (Sutton and Lazarus 1974, Hartley 1975, Van Helder et al. 1984,

Chang et al. 1986). The minimum time for this GH response to exercise appears to be related to the work intensity, such that the lag phase in plasma GH concentration may only be a few minutes in heavy exercise (Buckler 1973). In contrast, a 30-60 min lag phase exists for exercise at work loads of only 10-15% of maximum oxygen uptake (Hansen 1973). As well, this exercise-induced GH peak appears to be most related to exercise intensity rather than total work output (Galbo 1983, Van Helder et al. 1984). However, some peculiar features to the GH response to exercise have been described. Some studies have demonstrated lower GH concentrations during maximal than submaximal exercise (see Galbo 1986). This may be related to findings that GH concentration decreases if exercise is of long duration (Shephard and Sidney 1975). Indeed, GH concentration may be at or below basal levels following a marathon race (Sutton et al. 1969). The growth hormone response to exercise is also affected by the physical condition of the human subjects such that athletic or trained individuals attain higher peak levels of GH post-exercise than their non-trained counterparts (Hartley 1975, LeBlanc et al. 1982, Bunt et al. 1986). Sutton et al. (1969) has also demonstrated that trained individuals recover more rapidly (within 20 min) from elevated GH levels following exercise termination than non-trained individuals which exhibited above basal GH levels up to 2 h post-exercise. Exercise also increases GH concentration in birds. McKeown et al. (1974) reported elevated levels of GH following 10 min of exercise in the pigeon, Columbia livia (by

electrically stimulating the pectoral muscle). GH levels were decreased following 2 and 5 h exercise bouts, however plasma free fatty acid levels were increased. McKeown et al. (1974) suggest that a possible feedback inhibition of GH release is exerted by increasing levels of plasma free fatty acids (FFA).

It appears that GH plays some regulatory role in the mobilization of lipid reserves during bouts of increased physical activity as evidenced by elevated levels of plasma free fatty acids (Sutton et al. 1969, Lasarre et al. 1974, Shephard and Sidney 1975, Galbo 1986). The mechanism by which growth hormone exerts this effect remain unclear. Several studies have reported the lipolytic actions of pituitary derived GH (Hunter et al. 1965, Goodman and Grichting 1983, Bulow and Madsen 1986), however, conflicting evidence has been reported using biosynthetic GH. Kuhn et al. (1983) found no lipolytic properties associated with a synthetic GH molecule and suggested that the lipolytic action of the pituitary derived GH molecule was a function of some extraction contaminant. More recently however, work by Van Vliet et al. (1987) has shown that GH's produced by pituitary extractions and molecular techniques possess lipolytic potency, and that this effect in humans is an intrinsic property of the GH molecule.

As mentioned previously, food or protein deprivation also results in the release of growth hormone from the pituitary in mammals (Hall et al. 1986). During starvation in humans GH levels are elevated and appear to play a role(s) in the

maintenance of blood glucose levels, increased use of body lipids, and the sparing of body protein (Sandek and Felig 1976). Similarily, in some birds GH levels rise in response to food deprivation and Scanes et al. (1981) have shown that in chickens fed two equal caloric diets, containing 5 and 20% protein, GH levels were higher in the chicks fed the low protein diet. In humans, the additional stress of physical exercise in fasted individuals augments this starvation induced GH response (Galbo et al. 1981).

There also appears to be a temperature-dependent relationship with resting GH levels in humans, such that an increase in core temperature results in an elevation of plasma GH levels (Shephard and Sidney 1975). Buckler (1973) reported a smaller elevation of GH following 20 min of cycling when the environmental temperature was reduced from 21° C to 4° C. Frewin et al. (1976) and Christensen et al. (1984) have also reported a diminution in GH response following cold temperature exercise. Pethes et al. (1979, as cited in Scanes and Lauterio 1984) found a similar reduction in plasma GH concentration in cold (10° C)-adapted ducks. John et al. (1975) also reported such increases plasma GH and free fatty acid levels in the pigeon following 3 days of heat stress and dehydration.

Involvement of GH in salmonid fish

Our knowledge of the physiology of growth hormone in fishes is limited. However valuable information regarding the metabolic effects of GH has been gained through investigations

using mammalian GH's (ie. human GH, bovine GH, ovine GH, porcine GH) (Donaldson et al. 1979). Replacement therapy and administration experiments using purified mammalian GH in hypophysectomized fish support a role in promoting growth in fish (see Donaldson et al. 1979). Markert et al. (1977) have also reported increased food conversion rates in yearling coho salmon, Oncorhynchus kisutch, following the administration of bovine growth hormone. Mammalian GH has also been shown to possess lipolytic properties in fish. Clarke (1976) reported a diminution in total body lipid of sockeye salmon, O. nerka, in response to GH treatment and McKeown et al. (1976) demonstrated elevated levels of plasma free fatty acids in coho salmon fry at times of increased GH concentration. Sweeting (pers. comm.) has subsequently shown a lag phase in FFA elevation in relation to increased plasma GH. More recently Sheridan (1986) provided evidence for the lipolytic role of GH in fish and reported an increase in lipolytic enzyme activity following injection of bovine GH into coho salmon parr. As has previously been discussed however, discrepencies exist with respect to the lipolytic actions of mammalian GH's and there is a real need to assess the cause and effect relationship of lipolysis using salmonid GH.

Some data also exists regarding the effects of physical exercise on circulating GH levels in salmonids. GH levels in migratory sockeye salmon were measured at sea, upon entering the Fraser River (British Columbia, Canada), during the upstream migration, and on the spawning grounds (McKeown and

van Overbeeke 1972). Pituitary GH levels were decreased during the upstream migration, but serum levels were unchanged. McKeown and van Overbeeke (1972) suggest that the hormone was being utilized from the blood, possibly as a result of metabolic demands. Although these findings did not provide evidence that exercise may effect GH levels in adult fish, plasma GH was elevated 25% in juvenile kokanee salmon, O. nerka, forced to exercise in groups at 6.0+ bl/s for 24 h in a swim tunnel apparatus (McKeown et al. 1976). Thus there is preliminary evidence that exercise in juvenile salmonids stimulates GH in a similar manner as in humans and birds. However, such exercise in group fashion (N=9) allows for streamlining, greater water turbulence, and behavioural changes, all of which may cause sufficient stress to explain such increases in GH.

Prior to the mid 1980's, studies reporting GH levels in salmonids employed the use of heterologous RIA's, using anti-ovine GH as the antisera to bind salmon GH and radiolabelled ovine GH as the tracer antigen. Purified salmon GH was not available at this time and therefore a standard curve could not be generated. Hence such studies were qualitative rather than quantitative in nature. More recently, however, the purification of salmon GH has allowed the development of homologous RIA's using anti-salmon GH as the antisera and labelled salmon GH as the tracer antigens (Bolton et al. 1986a, Wagner and McKeown 1986). Since that time valuable information on the physiology of salmon GH has been gained, primarily with

respect to the processes of smoltification and to a lesser extent nutritional deficiencies. It is important that these new studies, and confirmation of previous findings using this technique allow investigators to assign real values to plasma GH levels thus enabling comparisons with existing mammalian data.

The parr-smolt transformation (smoltification) of juvenile salmonids involves many physiological and biochemical adaptations which prepare the salmonid for the transition from freshwater to the hyperosmotic medium of the marine environment (McKeown 1984, Barron 1986). Among the most obvious and well documented of these adaptations are 1) the loss of parr marks and the subsequent acquistion of the typical silvery colouration of a salmon smolt, 2) increased growth rate, 3) a preference for increased salinity, and 4) an increase in gill microsomal Na^+/K^+-ATPase activity (Johnston and Eales 1968, Hoar 1976, Folmar and Dickoff 1980, Loretz et al. 1982, Wickes et al. 1983, Barron 1986). As might be expected, entry into seawater (SW) typically results in elevated levels of plasma sodium, which usually peak within 12 h post-exposure, and decrease to pre-transfer levels between 24-48 h following SW transfer (Clarke and Blackburn 1977, Miwa and Inui 1985, Sweeting and McKeown 1987). Sweeting et al. (1985) were the first to report increased levels of plasma GH during smoltification and Bolton et al. (1986b) and Sweeting and McKeown (1987) have subsequently reported several fold increases in plasma GH levels following direct SW-transfer of

pre-smolt salmonids. Peak levels of GH generally occur between 24-48 h post-transfer and recover to pre-transfer levels within one week. Recent work has demonstrated that GH may be working in concert with cortisol to decrease plasma sodium concentration through an increase in gill Na^+/K^+-ATPase activity (Richman and Zaugg 1987). Furthermore, administration of mammalian GH induces smoltification related changes in pre-smolting salmonids (ie. changes in skin pigment, condition factor, and seawater survival) (Komourdjan et al. 1976, Wedemeyer et al. 1980). SW-transfer also elevates levels of plasma free fatty acids and increases lipolytic enzyme activity (Sweeting pers. comm., Sheridan pers. comm.). Another stressor which appears to effect GH levels is reduced food intake. McKeown et al. (1975) reported no change in plasma GH levels of kokanee salmon following a 30 day starvation period. However, Wagner and McKeown (1986) reported an 890% increase in plasma GH levels of rainbow trout fasted for a three week period. Thus the effect(s) of food deprivaton on plasma GH concentration remains equivocal.

Experimental objectives

The purpose of this study then was to attempt to assess the effects of physical exercise and environmental stress on salmonids through a laboratory simulation of the various factors associated with migration. The study was composed of six individual experiments, three of which directly related to exercise, and three related to changes in specific stress

conditions (reduced food intake, seawater transfer, and temperature change). The experiments and corresponding hypotheses to be tested were 1) fish were exercised individually in a swim tunnel apparatus (see Fig. 1) at 1.5 bl/s for 24 h to test the hypothesis that submaximal, sustained exercise increases plasma GH concentration, 2) variations in exercise regimes were adopted to test the hypothesis that swimming intensity, rather than total work, effects GH response to a greater extent, 3) a group of fish were submitted to a daily training routine to test the hypothesis that trained individuals recover from exercise-induced increases in GH more rapidly than non-trained individuals, 4) a group of fish were deprived food for a 30 day period to test the hypothesis that long term starvation elevates GH concentration, 5) individuals were transferred to seawater to test the hypothesis that steelhead trout, like other salmonids, respond with an elevation in circulating levels of GH, and 6) a group of fish were cold-acclimated to test the hypothesis that there exists a temperature dependent relationship for seasonal GH levels.

A subset of individuals from each of the experimental stress groups were also submitted to the standard exercise bout (see Methods, p. 14) to test the hypothesis that stress-induced increases in GH can be exaggerated or augmented when the additional stress of physical exercise impinges further on the physiology of a given individual.

In addition, plasma free fatty acid levels were determined for a small (n=3) subsample of experimental animals (from

experiments IV-VI; starvation, SW-transfer, and temperature acclimation) in order to provide preliminary evidence to support the hypothesis that GH possesses lipolytic properties.

The juvenile salmonid (primarily steelhead trout, Salmo gairdneri) was chosen as the animal of study for this project. The reason for this selection is two-fold; first, the physical limitations of the swim tunnel apparatus dictated the use of smaller individuals. Secondly however, the very nature of this study has obvious aquacultural implications and therefore the use of juvenile fish also has economic justification.

MATERIALS AND METHODS

Experimental Animals

Juvenile presmolt steelhead trout, Salmo gairdneri, were obtained from the Capilano and Alouette River hatcheries. The juvenile presmolt coho salmon, Oncorhynchus kisutch, used in experiment I were obtained from the Capilano hatchery, and the juvenile domestic rainbow trout, S. gairdneri, used in experiment VI were obtained from West Creek Trout Farms (Aldergrove, B.C.) (see individual experimental methods for physical characteristics). All fish were maintained in 120-L flow through tanks supplied with fresh, dechlorinated water under natural photoperiod (according to the light:dark regime outside Simon Fraser University) and temperature (refer to individual experimental methods for the time of year and temperature). Fish were fed once daily to satiation with M & H Trout and Salmon Feed (3/16" pellets, Surrey, B.C.).

Exercise Protocol

Following a typical three week holding period fish were individually placed in a recirculating swim tunnel (adapted after Brett, 1964) (see Fig. 1) and exercised at 1.5 body lengths per second (bl/s) for a 24 h period. This shall be referred to as the 'standard exercise bout' (see individual experimental methods for exercise modifications). Control fish were placed in an identical chamber to control for the environment but were not exercised. All fish were deprived of food at least 24 h prior to experimentation.

Analytical Techniques

Following the desired period of exercise fish were sacrificed and blood was collected via the caudal vessels in heparinized capillary tubes. In experiments I(series A) and III fish were sub-sampled at 0, 10, 30, 60, 120, and 240 min post-exercise. In experiments I(series B), II, IV, V, and VI fish were bled 10 min post-exercise, according to the peak levels of experiment I (see Results section). Following centrifugation, the plasma was immediately frozen on dry ice and stored at -20° C until assayed. Plasma GH levels were determined by the method of Wagner and McKeown (1986) using a radioimmunoassay (RIA) employing anti-chum salmon GH and iodinated coho salmon GH as the tracer antigen. Values are expressed as means ± SEM with statistical differences compared using the Student's t-test at 95% confidence intervals. Plasma free fatty acids were determined by high performance liquid chromatography (HPLC) (Borch 1975).

Experiment I

Juvenile steelhead trout (35.6 ± 3.1 g; 16.3 ± 0.7 cm; N=102) and coho salmon (29.1 ± 4.3 g; 15.7 ± 0.4 cm; N=98) were held as described (p.14) from February to April (T=4-7° C).

Series A. Individual fish were exercised at 1.5 bl/s for 24 h (Standard exercise bout) and sampled in time course fashion.

Series B. Individuals were exercised at 1.5 bl/s for 0, 1, 2, 4, 6, 12, 24, and 48 h. Fish were bled 10 min post-exercise in accordance with peak GH levels as determined in Series A.

Experiment II

Juvenile steelhead trout (42.6 ± 6.1 g; 16.7 ± 0.4 cm, N=35) were maintained from April to June (T=7-9° C) and submitted to one of three exercise regimes of varying intensity and duration.

Series C. Individuals were exercised at 2.0 bl/s for 24 h and sampled 10 min post-exercise.

Series D. Individuals were exercised at 3.0 bl/s for 12 h and sampled 10 min post-exercise.

Series E. Individuals were exercised stepwise to exhaustion and sampled 10 min post-exhaustion. Fish began swimming at 1.0 bl/s with 0.5 bl/s increment increases at 30 min intervals. Fish were defined as exhausted when they made contact with the downstream electric gate 3 times in succession. All fish were exhausted within 4 h (average U_{crit} = 3.85 bl/s). Critical swimming speed was calculated from:

$$U_{crit} = u_i + (t_i / t_{ii} \times u_{ii})$$

where, u_i = the highest velocity maintained for the prescibed time (bl/s)
u_{ii} = velocity increment (bl/s)
t_i = time fish swam at the fatigue velocity (min)
t_{ii} = prescribed period of swimming (min)

Experiment III

Juvenile steelhead trout were divided into two groups of similar weights and lengths (A= 34.9 ± 4.2 g, 15.8 ± 0.3 cm, N=28; B= 36.1 ± 3.5 g, 16.2 ± 0.5 cm, N=30) and maintained from February to April (T= 4-7° C). Fish from tank A were exercised in an open top flume at 2.0 bl/s for 4 h/day for a five week

period (these fish were designated 'trained'). Tank B fish were netted daily during this period in order to simulate handling stress, but were not exercised (these fish were designated 'non-trained'). Following this training period both experimental and control fish were submitted to the standard exercise bout and blood was collected in time course fashion (refer to p.15).

Experiment IV

Juvenile steelhead trout were divided into two groups of similar weights and lengths (A= 36.5 ± 3.1 g, 16.4 ± 0.7 cm; B= 34.8 ± 2.8 g, 15.8 ± 0.4 cm) and maintained from March to May (T= 5-8° C) and fed as described previously for a three week period. Food was then withheld from tank B (N= 25) for a 30 day period while tank A (N= 32) remained on the same daily feeding regime. Following this starvation period a subgroup of tanks A and B were exercised by the standard method, final weights and lengths were determined, and blood was collected 10 min post-exercise. Control subgroups were not exercised.

Experiment V

Juvenile steelhead trout (N= 60; 41.1 ±2.3 g; 17.1 ± 0.4 cm) were maintained as previously described (p. 14) from March to May (T=5-8° C).

Series F. Following a three week holding period a subgroup of 16 individuals was transferred to a 120-L recirculating tank containing 34 o/oo seawater. Control fish were transferred to an identical tank containing fresh, dechlorinated water.

Fish were sampled and sacrificed simultaneously 24 h post-transfer.

Series G. Following the same three week holding period a similar subgroup of 12 fish were individually submitted to the standard exercise bout in 34 o/oo seawater. Control fish were placed in an identical swim chamber containing fresh, dechlorinated water and were subjected to the same standard exercise bout (1.5 bl/s for 24 h) on alternating days.

Experiment VI

Domestic rainbow trout were divided into two groups of similar weights and lengths (A= 31.5 ± 2.8 g, 15.1 ± 0.4 cm; B= 32.8 ± 3.1 g, 15.2 ± 0.6 cm) and held for two weeks in July.

Series H. The temperature of tank A (N= 21) was then lowered to 8.5-9.5 C using a min-o-cool refrigeration unit (#BHL-1089-3, Frigid Units Inc., Blissfield, Mich.). Tank B (N= 20) was maintained under natural seasonal temperature (16-18 C). The temperature regimes were maintained for a four week period. Following this period a subgroup of fish from tanks A and B were submitted to the standard exercise bout at their respective temperatures on alternating days. Control fish were not exercised and blood was collected from both groups 10 min post-exercise.

Series J. Juvenile steelhead trout used in each of the decribed experiments and held at various times of year and corresponding seasonal temperatures (Oct., 1986 - Aug., 1987) were sampled. These samples were assayed to assess changes in plasma GH levels in response to seasonal temperature change.

RESULTS

Experiment I

Sustained exercise significantly increased plasma GH levels (Fig. 2). Plasma GH levels peaked at 10 min post-exercise (41.3 ± 2.6 ng/ml) and exceeded control levels (4.96 ± 1.1 ng/ml) by 800%. There was no significant difference in peak levels of GH in steelhead trout and coho salmon and plasma GH concentrations returned to basal levels by 4 h post-exercise. Hematocrit levels of exercised fish (31.8 ± 1.6 %) were not significantly different from those of non-exercised control fish (31.0 ± 2.1 %) following the standard exercise bout. Therefore, the observed increase in plasma GH is not simply an artefact of haemoconcentration. Swimming fish (at 1.5 bl/s) exhibited GH levels similar to control animals until the fourth hour of exercise (Fig. 3). Plasma GH concentrations rose to peak levels at 24 h exercise duration (36.1 ± 3.4 ng/ml) and individuals exercised for 48 h did not achieve GH levels in excess of the 24 h peak.

Experiment II

Fish exercised at 3.0 bl/s for 12 h achieved significantly higher GH levels (87.0 ng/ml) than fish exercised at 2.0 bl/s for 24 h (49.0 ng/ml) (Fig. 4). Fish which were exercised stepwise to exhaustion exhibited plasma GH levels (4.3 ng/ml) not significantly different than non-exercised control fish (8.4 ng/ml) (Fig. 4).

Experiment III

Exercise-acclimated fish had significantly higher levels of GH (80.0 ng/ml) post-exercise as compared to tank maintained fish (37.0 ng/ml) when required to undergo the standard exercise bout (Fig. 5). Trained fish also restored control levels faster (30 min) in comparison to non-trained fish (60-120 min). There was no significant difference in control GH levels between trained and non-trained fish.

Experiment IV

Table I indicates that starved individuals weighed significantly less (35%) than fed individuals following the 30 day starvation period. Long term starvation resulted in approximately a 600% increase in plasma GH levels (38.6 ng/ml) as compared with animals which were fed daily (6.4 ng/ml) and similar to fed fish that were swam (Fig. 6). However, starved fish which were exercised exhibited exaggerated GH levels (146 ng/ml) approximately 1400% greater than that of fed controls and 400% greater than starved controls.

Experiment V

Direct FW-SW transfer resulted in a significant 700% elevation of plasma GH levels as compared to sham transfer (FW-FW) controls, and this level was similar to that of FW-exercised fish (Fig. 7). Individuals transferred from FW to SW and concurrently exercised at 1.5 bl/s for 24 h exhibited plasma GH levels significantly higher (79.8 ± 9.6 ng/ml) than control individuals required to undergo the standard exercise

bout concurrent with FW-FW transfer (34.6 ± 3.8 ng/ml).

Experiment VI

Cold-acclimated rainbow trout (8.5 - 9.5° C) exhibited lower mean levels of plasma GH (21.8 ± 6.1 ng/ml) than fish held at seasonal ambient temperatures (16-18° C; [GH] = 37.9 ± 9.8 ng/ml). Plasma GH levels were elevated in both groups when exercised, with high temperature fish attaining peak levels 70% greater than low temperature fish post-exercise (Fig. 8). Table 2 illustrates seasonal variation in steelhead trout held at various times throughout the course of the project. Fish held during the winter months exhibited the lowest levels of plasma GH (T= 4-6° C; [GH] = 6.1 ± 3.8 ng/ml), while fish maintained during the summer months contained the highest levels (T= 16-18° C; [GH] = 38.3 ± 8.4 ng/ml). Fish held during the spring months (May - June) exhibited intermediate levels (19.0 ± 5.6 ng/ml).

FIG. 1. Diagram of the swim tunnel apparatus used for all experiments (total volume 69.1 L; chamber dimensions 41x6x6 cm; baffle grids were the only attempt made to straighten the flow profile).

A. propeller
B. motor
C. shock gate
D. chamber entry door
E. baffle grids
F. fish chamber

o air stone locations

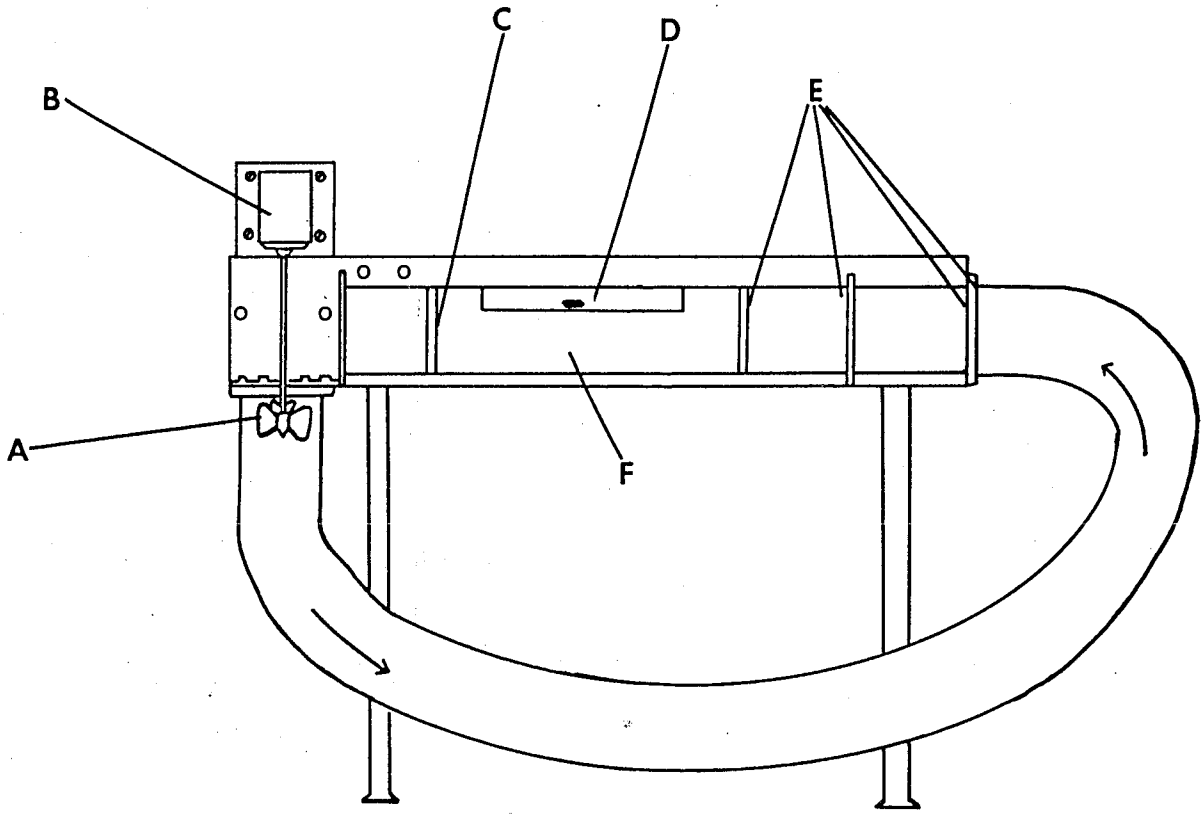

A
B
C
D
E
F

FIG. 2. Levels of plasma GH of juvenile steelhead trout and coho salmon at varying intervals post-exercise (Expt I, Series A). Each point represents the mean of eight individuals. Vertical bars indicate SEM.

* significant difference ($P<0.05$)

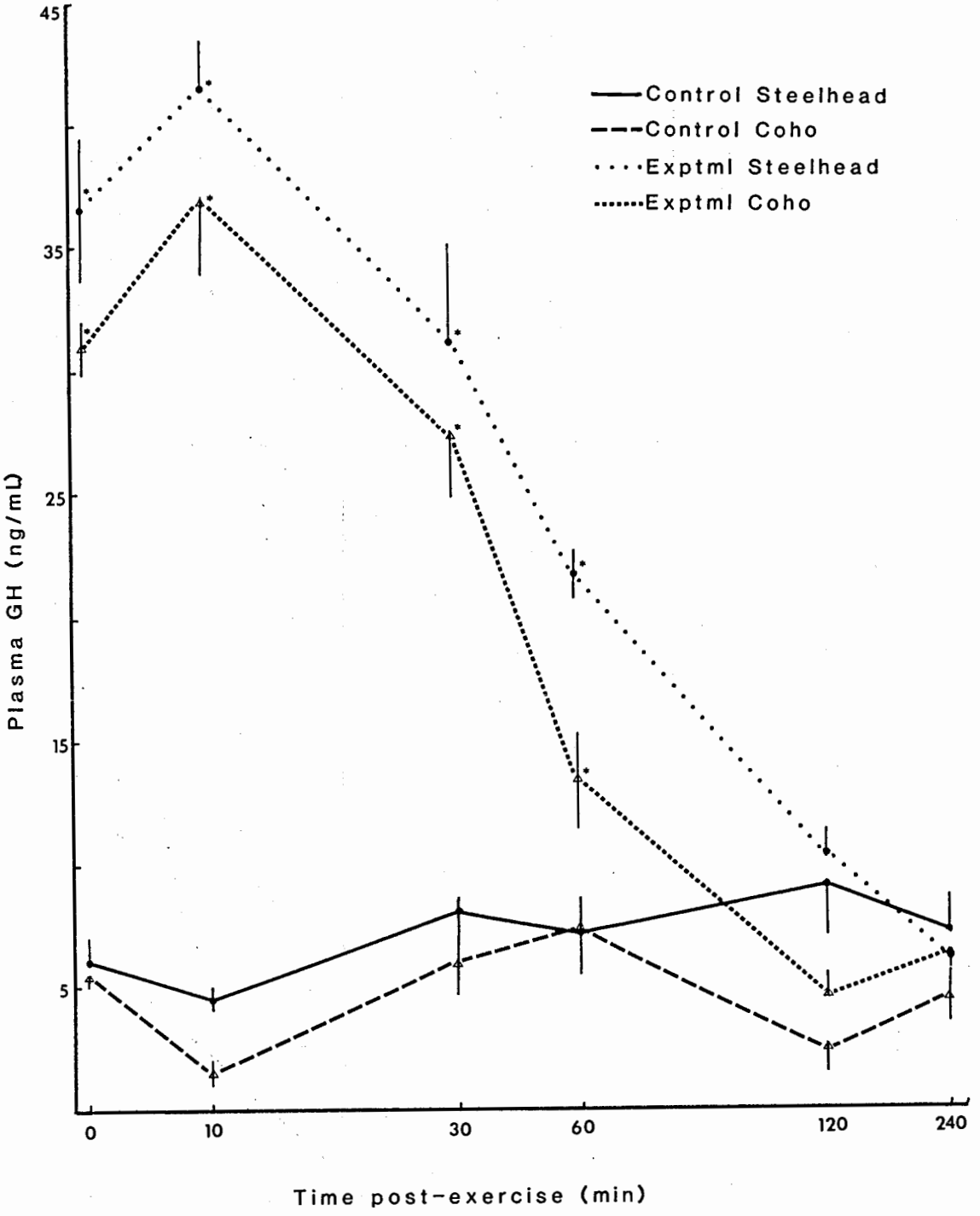

45
35
25
15
5
Plasma GH (ng/mL)
Control Steelhead
Control Coho
Exptml Steelhead
Exptml Coho
0
10
30
60
120
240
Time post-exercise (min)

FIG. 3. Plasma GH levels of juvenile steelhead trout 10 min after varying bouts of exercise at 1.5 bl/s (Expt I, Series B). Each point is the mean of at least eight fish. Vertical bars indicate SEM.

* significant difference ($P<0.05$)

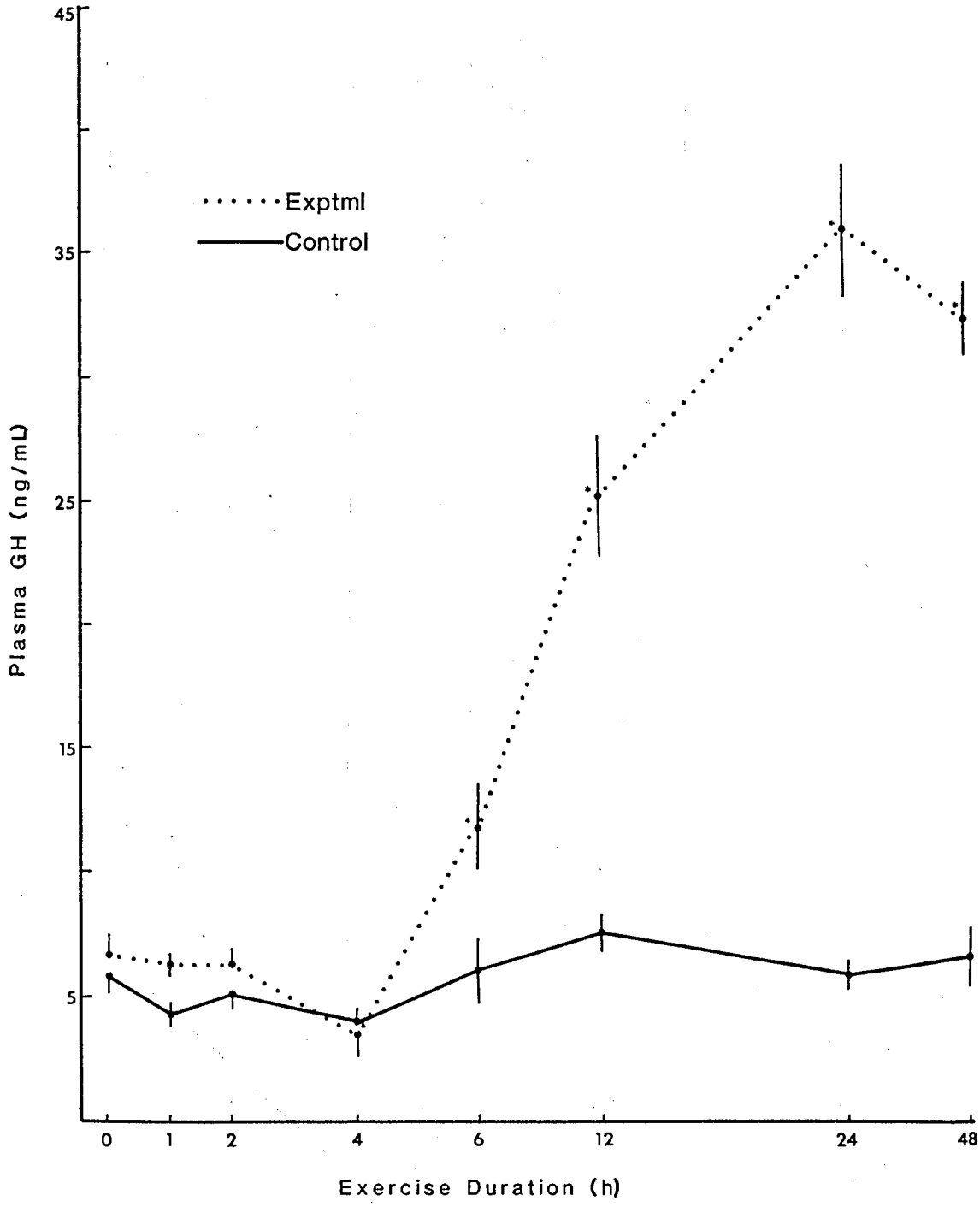

45
35
25
15
5
Exptml
Control
Plasma GH (ng/mL)
0
1
2
4
6
12
24
48
Exercise Duration (h)

FIG. 4. Plasma GH levels of juvenile steelhead trout subjected to one of three exercise regimes (Expt II; Series C,D,E). Each point is the mean of testing at least eight fish. Vertical bars indicate SEM. Control fish were bled simultaneously with experimental fish but were not exercised.

a,b ($P<0.05$)
b,c ($P<0.05$)

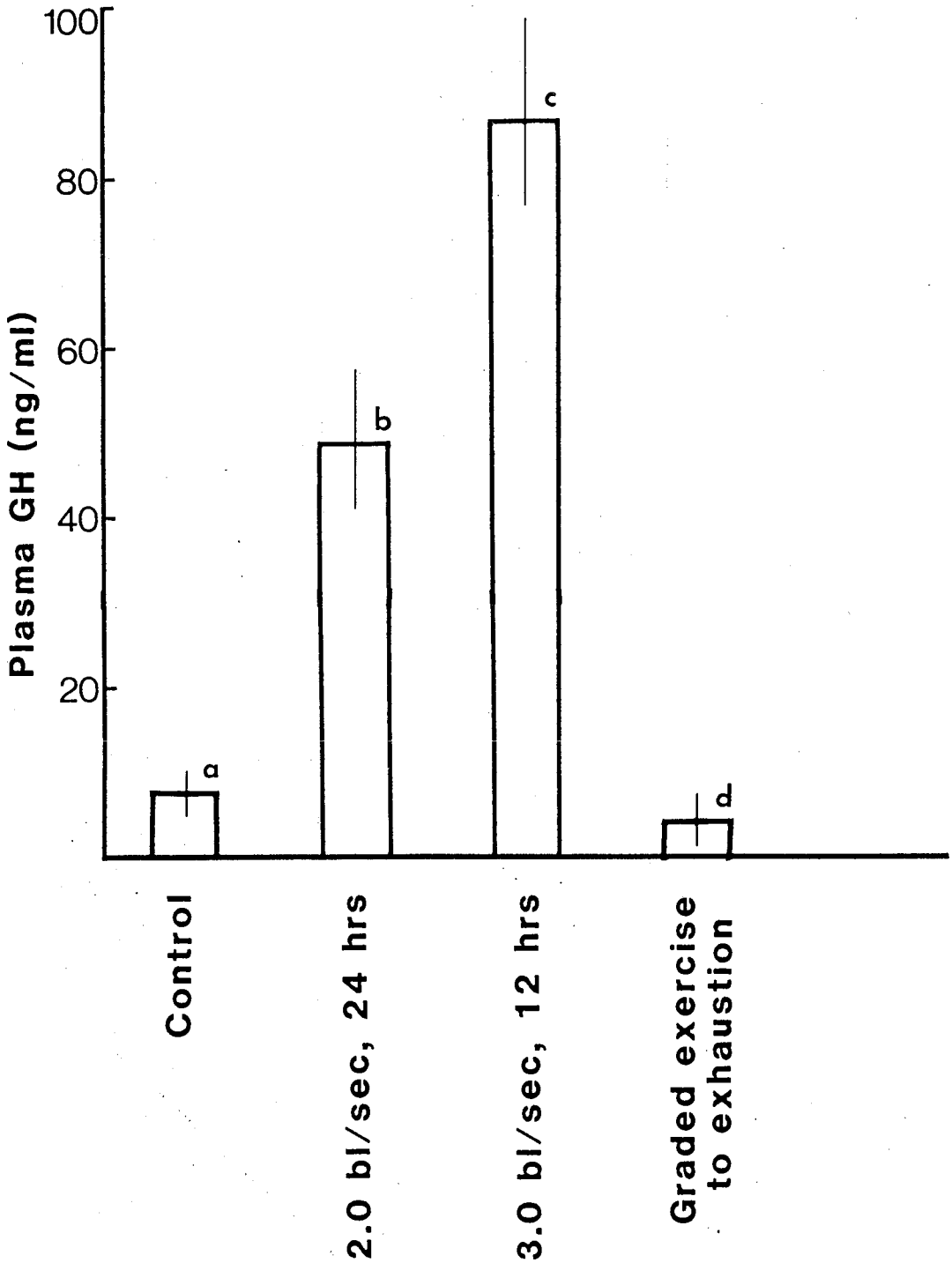

Plasma GH (ng/ml)
100
80
60
40
20
a
b
c
d
Control
2.0 bl/sec, 24 hrs
3.0 bl/sec, 12 hrs
Graded exercise to exhaustion

FIG. 5. Plasma GH levels in trained and non-trained fish at varying time intervals following 24 h of exercise at 1.5 bl/s (Expt III). Each point is the mean of testing at least five fish. Vertical bars indicate SEM. Control fish were bled simultaneously with experimental fish but were not exercised.

* significant difference ($P<0.05$)

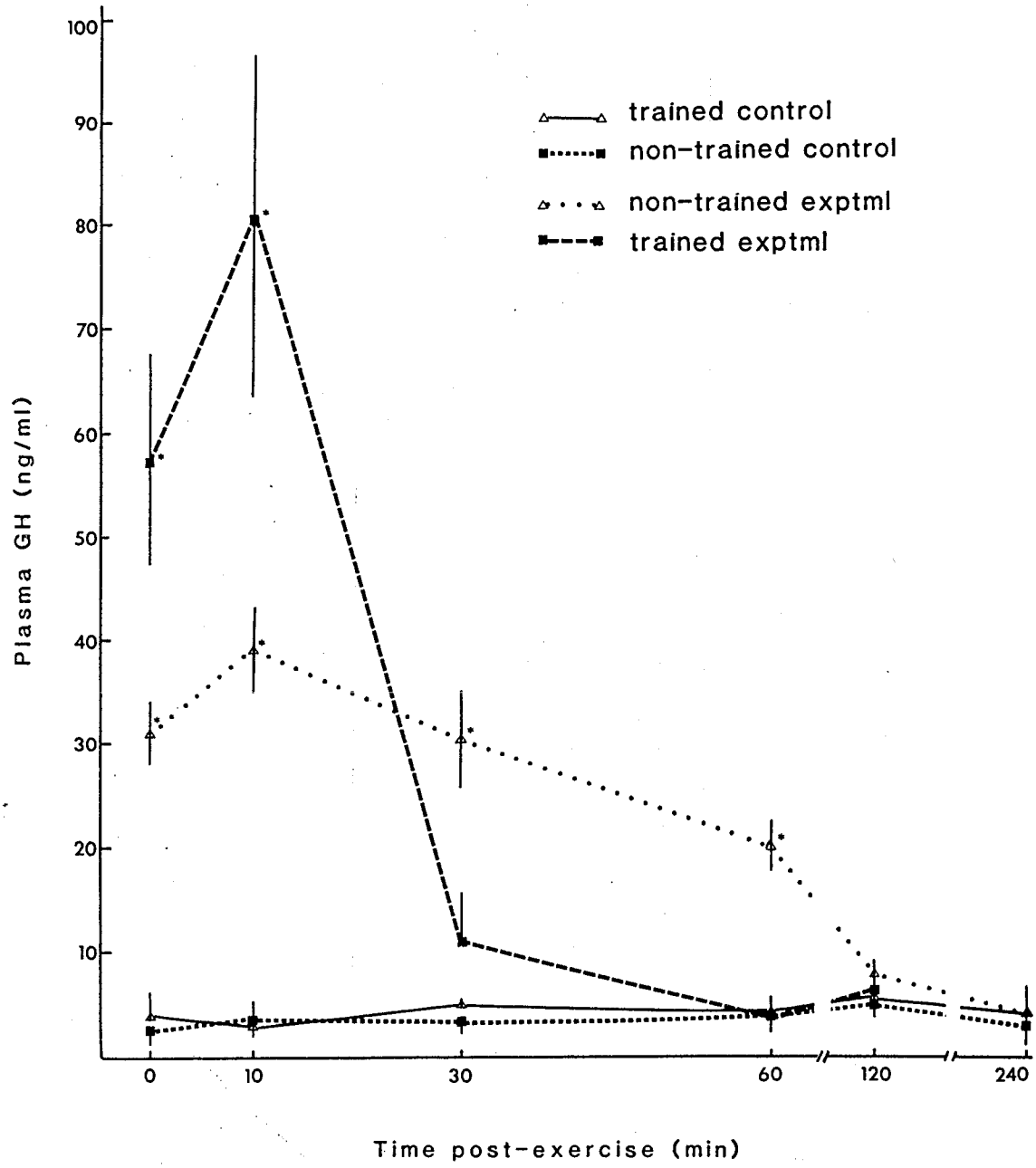

trained control
non-trained control
non-trained exptml
trained exptml
Plasma GH (ng/ml)
100
90
80
70
60
50
40
30
20
10
0
10
30
60
120
240
Time post-exercise (min)

TABLE 1. Mean weights prior to and following fed and starved treatments in juvenile steelhead trout.

	Initial mean weight (gms)	Final mean weight (gms)
Group A (fed)	36.5 ± 3.1	43.1 ± 2.6*
Group B (starved)	34.8 ± 2.8	27.3 ± 1.9*,**

* Significantly different ($P<0.05$) within treatments

** Significantly different ($P<0.05$) between treatments

FIG. 6. Mean levels of plasma GH of juvenile steelhead trout after starvation, exercise, or both. Exercised individuals were bled 10 min post-exercise (Expt IV). Control fish (fed and starved) were placed in an identical swim chamber but were not exercised. Vertical bars indicate SEM. There were at least twelve individuals per group.

a,b ($P<0.05$)
c,d ($P<0.05$)
b,d ($P<0.05$)

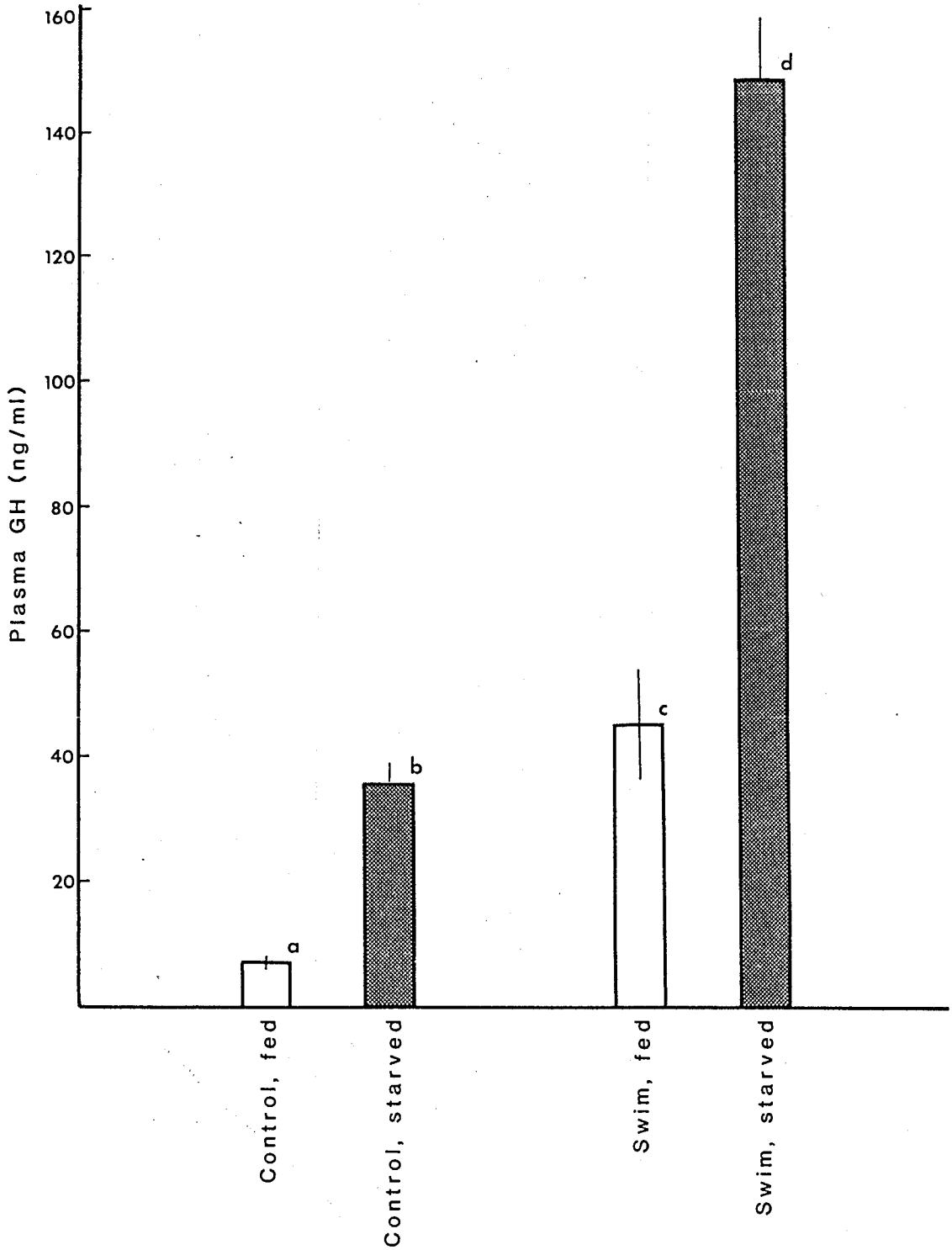

Plasma GH (ng/ml)
160
140
120
100
80
60
40
20
a
b
c
d
Control, fed
Control, starved
Swim, fed
Swim, starved

FIG. 7. Mean plasma GH levels of juvenile steelhead trout following; 1) FW-FW transfer, 2) FW-SW transfer (Expt V, Series F), 3) FW-FW transfer exercised for 24 h at 1.5 bl/s, and 4) FW-SW transfer exercised for 24 h at 1.5 bl/s (Expt V, Series G). Individuals were sampled 24 h after treatment initiation. Vertical bars indicate SEM. Each treatment consists of at least twelve fish per group.

a,b ($P<0.05$)
c,d ($P<0.05$)
b,d ($P<0.05$)

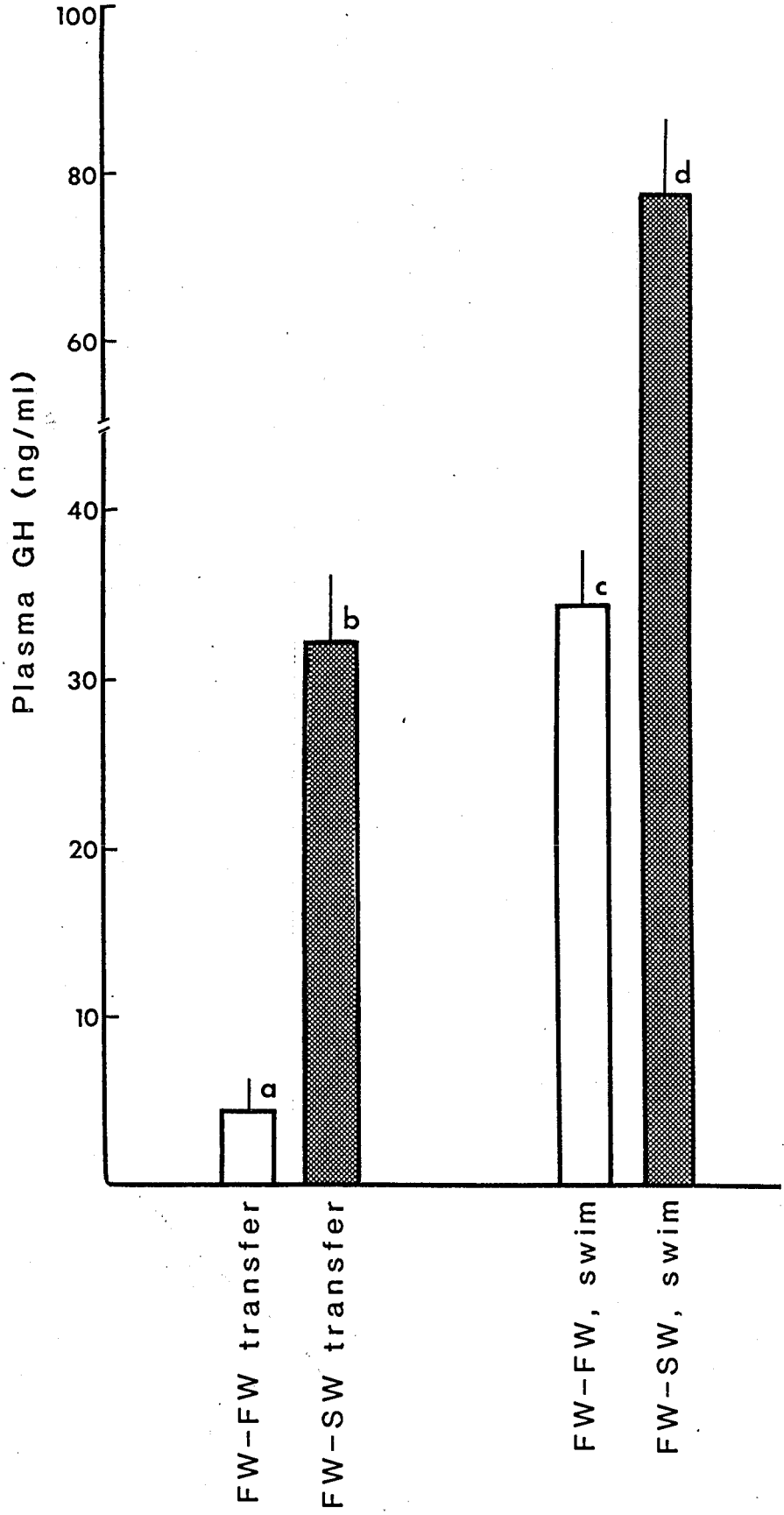
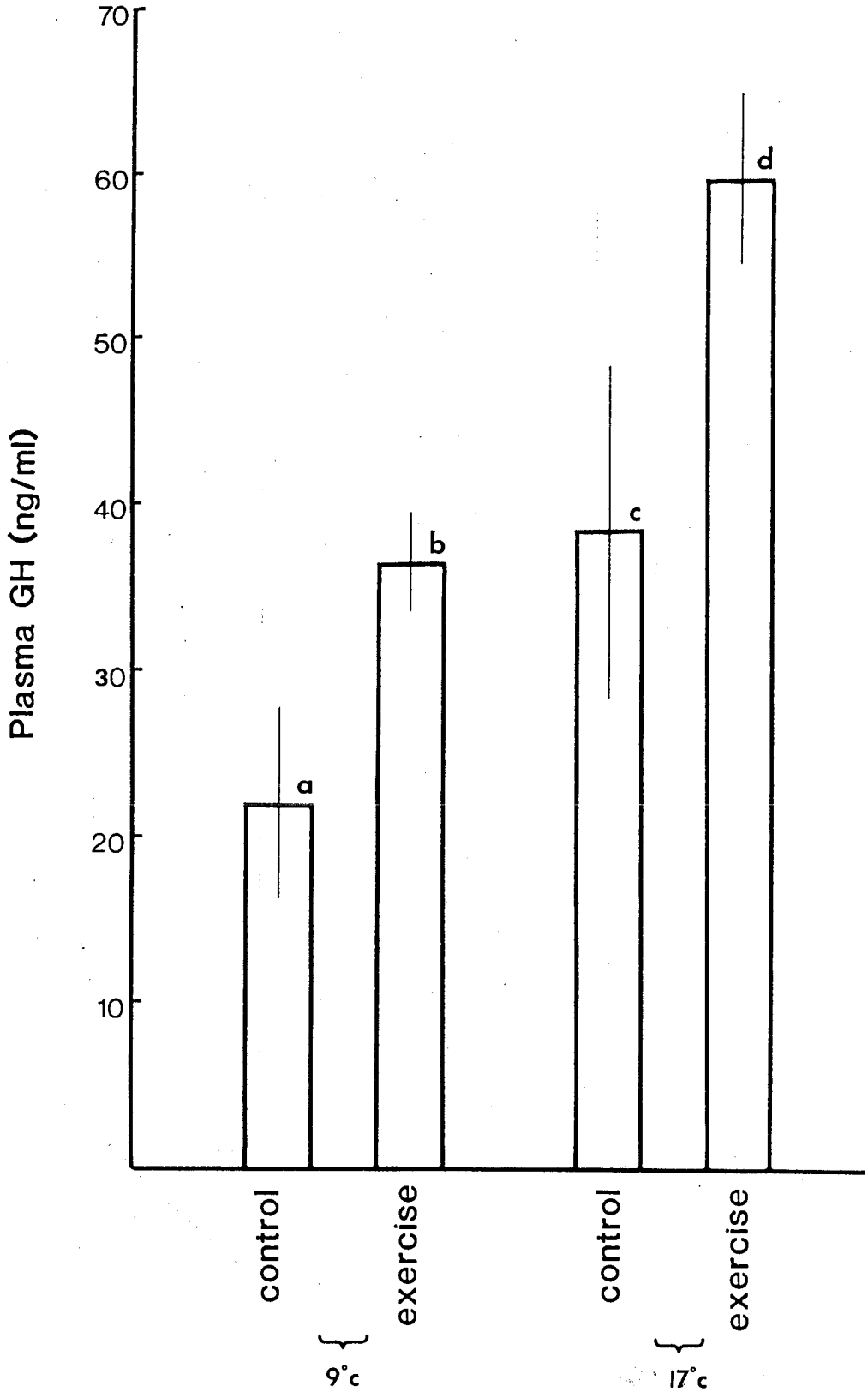

Plasma GH (ng/ml)
100
80
60
40
30
20
10
a
b
c
d
FW-FW transfer
FW-SW transfer
FW-FW, swim
FW-SW, swim

Table 2. Plasma GH levels of steelhead trout held at ambient temperatures during different months of the year.

	JAN – MAR	APRIL – MAY	JULY
	4 - 6 C	8 - 11 C	15 - 16 C
STEELHEAD TROUT (Salmo gairdneri)	6.1 ± 3.8	19.0 ± 5.6	38.3 ± 8.4

FIG. 8. Plasma GH levels in response to sustained exercise in temperature acclimated rainbow trout (Expt VI, Series H). Each group represents the mean of testing at least nine individuals. Vertical bars indicate SEM.

a,b ($P<0.05$)
c,d ($P<0.05$)


Plasma GH (ng/ml)
70
60
50
40
30
20
10
a
b
c
d
control
exercise
control
exercise
9°c
17°c

DISCUSSION

The results reported here clearly indicate that plasma GH levels of steelhead trout and coho salmon respond to sustained submaximal exercise as has previously been described in humans (Fig. 2). The profile of the GH response curve following exercise (Fig. 2) is similar to that reported in humans (Sutton and Lazarus 1974, Karagiorgos et al. 1979, VanHelder et al. 1984, 1986, 1987) with mean peak levels occurring 10 min after exercise and a return to basal levels within 2-4 h after exercise. Indeed, both the relative differences and absolute values observed in fish (1-10 ng/ml to 30-60 ng/ml) are within that range reported for human subjects (1-5 ng/ml to 15-40 ng/ml). Under such conditions of increased physical activity fat and glucose are the primary sources of metabolic fuel (Galbo 1983) and infusion experiments have demonstrated that low levels of circulating glucose or an increase in plasma FFA increase and decrease GH response to exercise respectively (Hansen 1971, Casanueva et al. 1981). In humans, sustained exercise results in the mobilization of lipid reserves (Bulow and Madsen 1986) and increasing GH levels are known to stimulate this mobilization of lipid (Lassarre et al. 1974, Goodman and Grichting 1983). Lassarre et al. (1974) reported increases in specific plasma free fatty acids 16:0, 18:0, 18:1, and 18:2 in human subjects following 1 h of submaximal stationary cycling. Similarily, actively swimming fish derive energy from fat and glucose (Driedzic and Hochachka 1978). Krueger et al. (1968) reported an elevation in the levels of

plasma free fatty acids 16:0, 16:1, 18:1, 18:2, 20:4, and 22:6 of coho salmon following periods of extended exercise (> 24 h, covering distances of 20.3 and 41.6 km). In agreement with the findings of Lasarre et al. (1974) in humans, the increased fatty acid levels in coho salmon were in response to sustained, submaximal exercise (Krueger et al. 1968). Johnston and Moon (1980) have reported that slow cruising speeds (< 1.8 bl/s) are maintained almost exclusively by the red muscle mass (located along the lateral surface and at the base of the fins). This is in contrast to more explosive burst swimming which utilizes the more predominant, and largely anaerobic white muscle fibres (Beamish 1978). The red muscle fibres also contain twice the level of stored fat as compared to the white muscle fibres (Johnston and Moon 1980) and lipids are also stored extensively in liver tissue, in adipose-like tissue along the mesenteries and around the pyloric caecae (Bilinski 1969). The present study supports the role of lipids as a fuel source during sustained, submaximal exercise as evidenced by increases in the specific free fatty acids 16:0, 16:1, 18:2, 20:4, and 22:6 in the plasma samples of exercised steelhead trout.

As has been discussed previously, GH response to exercise in humans typically exhibits a lag phase dependent upon exercise intensity and duration (1-5 min for heavy exercise to 30-60 min for mild exercise of longer duration) (Hansen 1973). The four hour lag phase described in this study (Fig. 3) supports earlier findings in humans that the GH response to submaximal exercise is delayed. This delay in GH response may

be a reflection of changes in various GH pools (ie. pituitary, blood, tissue) such that secretion rates may be elevated at the onset of exercise, however target tissues may readily clear GH from the circulation, therefore its increase would not be reflected in the plasma. The continued liberation of GH as the exercise bout continues may then flood target tissue uptake mechanism(s), possibly causing receptor desensitization (ie. internalization of the hormone-receptor complex), thus resulting in the elevated GH levels from 6-48 h of exercise (Fig. 3). Alternatively, available carbohydrates may be exhausted early during the exercise bout prior to the utilization of lipid reserves (Driedzic and Hochachka 1978).

From previous data regarding swimming speed and oxygen consumption rates, it is known that rainbow trout consume slightly more than twice as much oxygen while swimming at 3.0 bl/s as compared to 2.0 bl/s (Beamish 1978). Exercise regimes (see Methods section p. 16, Series C and D) were designed to yield swimming bouts of approximately equal work loads but of varying intensity and duration. The data clearly indicate that fish required to swim at 3.0 bl/s for 12 h exhibit markedly higher GH levels than fish exercised at 2.0 bl/s for 24 h (Fig.4). Thus, GH response appears to be more related to the peak intensity of exercise rather than the total work output. Complicating this hypothesis, however, is the lack of GH reponse elicited by fish exercised stepwise to exhaustion (< 4 h total duration, terminated by approximately 2-5 min of burst activity) (Fig. 4). It appears that there is some relationship

between exercise intensity and GH response, however, it is possible that a minimum duration requirement must first be satisfied as was previously illustrated in figure 3. It should also be noted that the non-rectilinear flow, in the swim tunnel used, would require fish to expend more energy to maintain their position in such turbulent flow (Beamish 1978). Thus definitive statements regarding GH response to swimming intensity using the described apparatus (see Fig. 1) remain equivocal. McKeown et al. (1975) reported a 25% increase in plasma GH following 24 h of exercise at 6.0+ bl/s in juvenile kokanee salmon. However, such speeds could not be maintained by either steelhead trout or coho salmon in this investigation. Perhaps the much lower percent increase reported by McKeown et al. (1975) (as compared to the 800% reported here) is a function of this more intense, sustained swimming regime.

Figure 5 clearly indicates that fish required to undergo an exercise training program exhibit a markedly different GH response following bouts of sustained submaximal exercise as compared to their tank maintained counterparts. In support of these findings Hartley (1975) and Bunt et al. (1986) demonstrated a higher GH peak in trained than non-trained human subjects. These higher levels are reached early during exercise and maintained throughout exercise duration. There is some evidence to suggest that circulating levels of insulin, catecholamines, and possibly β-endorphins play a role in the increased GH response (Galbo 1986). It is possible that the exercise-acclimated trout in the present study may exhibit a

similar response during the swimming bout itself, however this requires sequential sampling and the use of much larger, cannulated fish would be essential for such a study. It is also evident from this investigation that non-trained control fish, which were periodically netted to simulate handling stress, did not achieve the high levels of GH exhibited by the trained individuals (Fig. 5). This suggests that the high peak GH levels of trained fish following exercise is not an exaggerated stress-induced response elicited by the repeated handling of the experimental group. It should also be noted that the area under the curves are virtually identical and thus the total GH pulse is approximately equal in both trained and non-trained fish (Fig. 5). The faster recovery to basal GH levels exhibited by trained fish is typical of the physiological adjustments (ie. metabolic rate) in response to training in mammals (Galbo 1986) and presumably enables them to undergo subsequent exercise bouts while, at the same time, non-trained fish remained physiologically stressed. However, the significance of this result is equivocal since GH profiles during the actual exercise bout itself may be quite different. Unfortunately, the small size of the fish in the present study precluded sequential sampling using cannulation techniques.

Much of the data presented herein provides preliminary endocrine support for recent studies documenting the benefits of low speed, sustained swimming as an effective rearing method for increasing growth, stamina, and food conversion efficiency among a variety of salmonids (coho salmon, Oncorhynchus

kisutch, Besner 1980; Atlantic salmon, Salmo salar, Kuipers 1982, Totland et al. 1987; rainbow trout, S. gairdneri, Davie et al. 1986; Brook trout, Salvelinus fontinalis, Leon 1986). These studies have demonstrated that swimming speeds ranging from 0.5 bl/s - 1.5 bl/s over long periods of time (generally > 8 weeks) provide optimum conditions for muscle growth and feed conversion efficiency.

As has been discussed previously, under conditions of physical exertion fat and glucose are the primary fuel stores utilized. During periods of reduced food intake however, fat and (or) protein become the major source of fuel for body maintenance. Moreover, the combination starvation and exercise would further encroach on fuel requirements. Food deprivation for 30 days resulted in a 603% increase in plasma GH concentration of steelhead trout (Fig. 6). This is consistent with the previous work of Wagner and McKeown (1986) who reported an 890% increase in the plasma GH concentration of domestic rainbow trout following a three week starvation period. Starved fish also weighed significantly less than fed individuals, although this difference was not as drastic as those reported by Wagner and McKeown (1986) (35% versus 151%). Indeed, reductions in body weight have been reported in response to food deprivation in several salmonids (Jezierska et al. 1982, Dannevig and Norum 1983, Black and Skinner 1986). During the early stages of starvation (< 2 weeks) nonessential proteins are catabolized as a fuel source in rainbow trout (Moon 1983, Loughna and Goldspink 1984). However, beyond the

early stages of starvation body protein is conserved and lipid reserves are mobilized (Love 1980). It is known that long term starvation (> 3 weeks) generally results in the depletion of lipid reserves (Greene and Selivonchick 1987) and Jezierska et al.(1982) have shown that visceral lipid makes a substantial contribution to energy utilization during starvation in the trout. Furthermore, increases in lipolytic enzyme activity have been reported as a result of starvation in a number of fish species (Zammit and Newsholme 1979, Black and Skinner 1986). Jezierska et al. (1982) have demonstrated that saturated fatty acids are conserved or their utilization is delayed in comparison to unsaturated fatty acids over the course of reduced food intake. It is suspected that this is due to their relative costs of production (Jezierska et al. 1982) with long chain saturated fatty acids requiring more energy for elongation. Similarily, Dave et al. (1976) demonstrated that long term starvation in the European eel, Anguilla anguilla, had a pronounced effect on the fatty acid composition of the liver while the muscle, the main fat depot in eels, showed much smaller alterations. Decreases in the unsaturated liver fatty acids 14:total, 16:1, and 18:total were reported in response to starvation in the eel (Dave et al. 1976). Although the precise mechanism(s) is not well understood it is suspected that GH levels increase lipid mobilization during periods of starvation through the stimulation of RNA dependent synthesis of an adipolytic lipase (Martin 1985).

It should also be noted that starved individuals required

to undergo the standard exercise bout exhibited exaggerated GH levels beyond those of non-exercised, starved fish (Fig. 6). Such augmented GH levels under the conditions of exercise and food deprivation have similarily been reported in humans (Galbo et al. 1981). Presumably the additional stress of exercise impinges further on metabolic demands and this increased demand for fuel is reflected in the exaggerated GH level. In light of previous findings regarding fuel utilization preferences under conditions of both starvation and (or) exercise, and by the process of elimination, GH is likely playing some role in lipid mobilization. Indeed, plasma samples from starved individuals in this study show increases in the specific free fatty acids 14:1, 16:1, 18:2, 21:3, and 22:6 and although no direct cause and effect association can be ascribed to such correlative data it nonetheless lends support to this hypothesis. It is also known that during fasting, growth is attenuated and inhibitors of somatomedin-mediated growth are produced (Martin 1985). It follows that GH secretion may be accelerated during such conditions, in part, due to the loss of the negative feedback influences of somatomedins on the somatotropes of the pituitary. Galbo et al. (1981) have also suggested that, in humans, insulin availibility, before exercise, is an important determinant of the overall hormonal response (catcholamines, GH, prolactin, cortisol and glucagon) to exercise. However this remains to be investigated in salmonids.

Figure 7 illustrates that SW-transfer of presmolt steelhead trout results in a marked increase in plasma GH

levels confirming previous data reported for chum salmon, O. keta, (Bolton et al. 1986b), coho salmon (Sweeting and McKeown 1987), and domestic rainbow trout (Bolton et al 1986b). Following 24 h of SW-transfer there was a 600% increase in plasma GH concentration in presmolt steelhead trout (Fig. 7). This is consistent with the previous work of Sweeting and McKeown (1987) who reported a 700% increase in GH levels of presmolt coho salmon (presmolts, ca. 20 g), while Bolton et al. (1986b) reported a 300% increase in GH concentration of chum salmon and an 89% increase in rainbow trout (100 g). However, these differences can likely be attributed to differences in the size of experimental animals as it is known that there exists an inverse relationship between fish size and plasma GH concentration in seawater (Bates, pers. comm.). That is, juvenile salmonids under 13 cm in length typically exhibit higher levels (180-220 ng/ml) of plasma GH in seawater than do larger fish (16-18 cm, 35-60 ng/ml) under the same conditions. Similarily, there exists an inverse relationship between size and plasma Na^+ levels in rainbow trout (Bolton et al. 1987). That these levels represent differences in relative difficulties to seawater adaptation between small and large presmolts appears likely.

Seawater transfer also results in the mobilization of lipid (Sheridan, pers. comm.) and this is reflected by changes in free fatty acid levels in the plasma (Sweeting, pers. comm.). In the present study seawater transfer resulted in increases in plasma free fatty acids 16:1, 18:0, 18:1, 18:2,

20:0, 20:4, and 22:6. These levels increased a further 50-100% in fish which were exercised concurrent with seawater transfer.

The enhanced levels of plasma GH exhibited by fish which were exercised concomitant with direct SW-transfer (Fig. 7) may reflect the compounded effects of increases in osmoregulatory and metabolic rates. This is supported by the work of Rao (1968) who demonstrated that fish transferred to 30 o/oo seawater and then forced to exercise exhibit a 3-fold increase in metabolic rate as compared to fish transferred to the same seawater but not exercised. However, Febry and Lutz (1987) reported that although the cost of osmoregulation for resting cichlids is higher in SW (35 o/oo) than FW, when these fish are exercised at 1.8 bl/s the cost of osmoregulation is slightly less in SW than FW. It is important to note however, that Febry and Lutz (1987) allowed their fish to acclimate at the desired salinities for a one week period prior to forcing them to exercise. It is well documented that both plasma Na^+ and GH levels return to pre-transfer concentrations well within one week (Clarke and Blackburn 1977, Bolton et al. 1987, Sweeting and McKeown 1987). This is in sharp contrast to the present study in which fish were exercised concurrent with seawater transfer for a 24 h period, during which time GH levels are still rising (Sweeting and McKeown 1987). It has previously been discussed that SW-transfer on its own results in lipid mobilization, and it thus seems likely that this exaggerated GH response to the combined effects of SW-transfer and exercise may be two-fold. GH is possibly functioning in osmoregulation

to directly or indirectly enhance gill Na^+/K^+-ATPase activity as well as a lipid mobilizing agent to satisfy energy requirements under conditions of increased stress.

It is evident from the results presented in Table 2 that there exist seasonal differences in plasma GH concentration. Steelhead trout used throughout the project duration showed a significant difference between the cold, winter months (6.1 ± 3.8 ng/ml) and the relatively warmer temperatures of summer (38.3 ± 8.4 ng/ml). This diminution of GH concentration in response to low temperature is not surprising in light of the absolute decrease in both respiration rate and aerobic enzyme activity found at cold temperature (Johnston and Dunn 1987), and may be reflected in corresponding decreases in both appetite and food conversion efficiency (Kuipers 1982, Markert et al. 1977). However, it should be noted that these levels were recorded at different times of the year and the author acknowledges that other variables such as photoperiod may also play some causal role in the reported changes. Thus the temperature-acclimation experiment was designed to control for seasonal changes other than temperature which might also affect plasma GH concentration (see Methods p. 17). Unfortunately, Figure 8 shows no significant difference between resting levels of GH in fish held at 9° C and 17° C respectively. Perhaps this is due, in part, to inadequate lowering of the temperature such that 9° C only corresponds to the seasonal temperatures of spring and fall (8-11° C) for the data in Table 2. Upon comparison of these groups it becomes evident that the two

groups are not significantly different (seasonal steelhead 19.0 ± 5.6 ng/ml; acclimated rainbow 21.8 ± 6.1 ng/ml). It is suspected that a temperature acclimation of < 7°C would have been more appropriate, however limitations of the cooling system precluded such desired temperatures. Alternatively, this may indicate that basal GH levels are more conserved over a wider range of temperatures typically encountered by fish. It should also be noted however, that even at lower ambient temperatures (7°C) domestic (ie. farmed) rainbow trout exhibit resting levels of plasma GH greater than those of the wild hatchery steelhead trout and coho salmon used throughout the majority of this project (unpubl. obs.). Perhaps this is a reflection of artificial selection for faster growing fish in the farming industry.

Previous investigations on humans have reported that heat stress produces an increase in GH levels and that these levels are comparable to those of non-heat stressed individuals required to undergo bouts of exercise (Francesconi et al. 1984). Furthermore, Frewin et al. (1976) and Christensen et al. (1984) have reported that individuals exercised at cold temperature showed no increase in plasma GH. It was concluded that exercise 'per se' was not the stimulus for elevated levels of GH, but rather the increase in core body temperature resulting from exercise. My data indicates however, that sustained swimming stimulates plasma GH levels regardless of temperature (Fig. 8). This is not surprising, given that low temperature is not as stressful among poikilotherms as is the

case in both humans and birds. Thus one might not expect the typical stress response observed in hypothermic humans and birds and it appears obvious that such a response is under much different control in fish.

Heat stress and dehydration resulted in a two-fold increase in plasm FFA levels in the pigeon (John et al. 1975) and preliminary results from this study indicate an increasing trend in plasma FFA's in conjunction with temperature acclimation. Specific free fatty acids 14:0, 16:0, 18:1, 20:0, and 22:6 are all elevated in individuals at high temperature (16-18°C).

In conclusion, it is evident that a pronounced GH response can be elicited under a variety of exercise regimes, as well as under certain environmentally limiting conditions. A central theme throughout this study has been that GH is acting in some way to stimulate fat mobilization to satisfy fuel requirements under such conditions. Indeed, a correlative relationship between elevated GH levels and increased FFA in the plasma lend support to this hypothesis. However, a direct stimulation hypothesis possesses numerous shortcomings; 1) during mild exercise lipolysis is increased long before a rise in GH is observed, and 2) FFA and glycerol levels increase in response to exercise in subjects incapable of increased GH concentrations (ie. hypopituitarism or administration of SRIF). Certainly a time course of FFA elevation with respect to increasing GH levels in response to the submaximal exercise regime of this investigation would have provided useful

information as to the role of GH in fish. However, given the nature of the findings reported here, and in light of existing data it seems more likely that GH may, by the stimulation of synthesis of lipolytic and gluconeogenic enzymes and by the stimulation of growth, serve to prepare the organism for future exercise bouts or for increased access to stored reserves under environmentally imposed stresses rather than to directly stimulate lipid mobilization.

SUMMARY

By the use of an homologous radioimmunoassay changes in plasma growth hormone levels in response to sustained exercise and environmental stress were evaluated in juvenile salmonids using a recirculating, open top swim chamber. It is clear from the findings presented in this study that circulating levels of growth hormone are highly responsive to periods of sustained submaximal swimming.

1. Juvenile steelhead trout, Salmo gairdneri, coho salmon, Oncorhynchus kisutch, and domestic rainbow trout, S. gairdneri, all exhibit a several fold increase in growth hormone levels when subjected to bouts of sustained swimming of 1.5 bl/s for a 24 h period.

2. This exercise-induced growth hormone response appears to be more closely associated with swimming intensity than duration as evidenced by the fact that fish required to swim at 3.0 bl/s for 12 h exhibit twice the plasma growth hormone levels of fish exercised at 2.0 bl/s for 24 h. Furthermore, it should be noted that maximal exercise to exhaustion resulted in lower mean growth hormone levels than those of the non-exercised, control fish.

3. Fish which were acclimated to exercise over a one month period exhibited elevated levels of growth hormone as compared to non-trained fish when both were subjected to the sustained

exercise protocol. Furthermore, the trained individuals recovered from the exercise induced increase in growth hormone four times earlier than non-trained fish following exercise cessation.

4. Long term starvation also results in drastic increases in growth hormone levels and this response is compounded when starved individuals are required to undergo sustained exercise.

5. Direct seawater transfer results in a 700% increase in plasma growth hormone and these levels are further augmented in fish subjected to bouts of sustained exercise. However, from analysis of existing studies regarding growth hormone and seawater entry it appears that growth hormone function during FW-SW transfer concomitant with sustained swimming is two fold; with a percentage of the augmented increase likely being associated with osmoregulation and the remaining amount a result of increased fuel requirements. The present experimental design however does not lend the investigator to properly assess what degree of the growth hormone response is associated with osmoregulation and what degree is in response to energy acquisition. It seems unlikely that a simple additive effect would explain such a relationship since osmoregulation in actively swimming fish is known to be more taxing than osmoregulation in inactive fish (Rao 1968).

6. Temperature change (9 and 17 C) effects the relative mean differences of plasma growth hormone in both resting and exercised individuals such that fish maintained at higher

temperatures (17°C) exhibit elevated mean basal growth hormone levels when compared to fish held at lower constant temperatures (9° C). Similarly, higher peak growth hormone values were attained by warm water fish following exercise.

It is suspected that the increase in plasma growth hormone documented in the present study is, in part, a reflection of the increased demand for energy to satisfy 1) periods of greater physical activity, 2) diminished fuel intake, 3) temperature dependent increases in metabolism and, 4) combinations of some or all of the above. Furthermore, it is presumed that the reported increases in plasma growth hormone reflect an increase in secretion from existing growth hormone pools. However decreased uptake and receptor desensitization cannot be ruled out given the nature of this investigation. It should also be reiterated that the present experimental design only assesses plasma GH levels after exercise and this may be in no way a reflection of what such levels are during the exercise bout itself.

It should similarly be noted that the effects of exercise training on growth hormone response requires a more detailed analysis of the involvment of lactate, insulin, catecholamines, and the acquisition of oxygen debt to more fully assess the role of growth hormone during exercise in such individuals. Furthermore, due to the nature of the exercise itself and its duration much of this discussion has centred on lipid metabolism. However, it must also be emphasized that GH is likely involved in the metabolism of other organic compounds

such as proteins and carbohydrates under different conditions of exercise, or within the same conditions but at different times.

Many questions of interest resulting from this investigation remain to be addressed. Future studies are needed to properly assess; 1) the role of GH on fuel reserves in actively swimming fish, 2) GH response during the actual exercise bout itself, and 3) possible sites of action of GH in response to exercise. The present investigation remains to be validated in adult salmonids.

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