Cortisol Effects on Aerobic and Anaerobic Metabolism, Nitrogen Excretion, and Whole-Body Composition in Juvenile Rainbow Trout

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ABSTRACT

The influence of chronic cortisol elevation on metabolism, body composition, and fuel use patterns was examined in juvenile rainbow trout (*Oncorhynchus mykiss*). Measurements were performed in a control group (day 0) and in two experimental groups at days 3, 10, and 30 after treatment with a cortisol implant or a sham implant. All fish were fed 1% daily ration. Measured plasma cortisol levels were highest at day 3 and returned close to normal values by day 30 in cortisol-implanted fish. No plasma cortisol elevation was observed in the sham group. Growth was depressed in the cortisol-treated fish. Cortisol elevation resulted in increased plasma glucose concentrations during the entire experimental period, elevated CO_2 production at day 3 and 30, and an elevated respiratory quotient (RQ) exceeding 1.0 on these days. Nitrogen excretion, estimated as the sum of ammonia-N plus urea-N excretion, and the nitrogen quotient exhibited small decreases at day 30. Total-N excretion, measured with a nitrogen oxidizer, was approximately twice the sum of ammonia-N plus urea-N excretion but exhibited a similar trend. Aerobic metabolism (routine O_2 consumption) was higher on day 10 compared to sham-implanted fish, although not relative to day 0 control levels. Anaerobic metabolism increased substantially, as evidenced by pronounced plasma lactate elevations at days 3 and 10, a small increase in whole-body lactate on day 10, and the elevated RQ on days 3 and 30. Body composition exhibited an increase in total carbohydrate at days 3 and 10, mainly reflecting increased glycogen levels. Protein concentration was stable, indicating, in accord with the respirometry data, that protein usage did not fuel the increased metabolism or carbohydrate elevation. Redirection of nutrient uptake from food and/or mobilization of lipid stores (which decreased relative to the control group but not relative to shams) are suggested as possible energy sources for these actions of cortisol.

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Introduction

Cortisol is known to have profound effects on intermediary metabolism in fish, but many uncertainties remain (reviewed by Mommsen et al. 1999). Normally, cortisol elevation is followed by hyperglycemia (Barton et al. 1987; Wendelaar Bonga 1993; Vijayan et al. 1997), providing an easily accessible fuel for energy-demanding processes involved in behavioral, physiological, and biochemical acclimation. However, some studies suggest that despite significant increases in plasma cortisol, this increase in plasma glucose is not obligatory (Andersen et al. 1991; De Boeck et al. 2001). Indeed, in the American eel, hypoglycemia has been observed after cortisol stimulation (Foster and Moon 1986). Cortisol elevation causes liver glycogen mobilization through glycogenolysis (Barton et al. 1987; Janssens and Waterman 1988; Vijayan and Moon 1992; Vijayan et al. 1997) as well as gluconeogenic actions (Vijayan and Leatherland 1989; Vijayan et al. 1991, 1997). In short-term responses to stress, hyperglycemia seems to be mediated by the rapid increase of blood catecholamines, while in the long run, the gluconeogenic action of cortisol seems to play a major role (Schreck 1993; Wendelaar Bonga 1997). The energy source used for gluconeogenesis has been suggested to be either proteins (Lidman et al. 1979; Vijayan et al. 1991), lipids (Sheridan 1986; Vijayan et al. 1991), or lactate and glycerol (Leach and Taylor 1982; Vijayan et al. 1991), and the mechanisms involved are unclear. As is stated in the recent review by Mommsen et al. (1999), there is an obvious need for further research to clarify the metabolic effects of cortisol on teleost fish.

Increased aerobic and anaerobic metabolism and decreased growth rates have been observed frequently in stressed fish (Heath 1995). The role of cortisol in these events has been studied to only a small degree. Increased oxygen consumption rates were observed in cutthroat trout after cortisol adminis-

tration (Morgan and Iwama 1996), but in coho salmon, this elevation was only seen after handling stress and not after cortisol administration alone (Davis and Schreck 1997). Gregory and Wood (1999) reported that cortisol administration decreased appetite in rainbow trout, which could explain the reduced growth rates and condition factors often observed. However, the same study indicated that at the same individual food consumption levels, cortisol-treated fish still had lower growth rates, reflecting the "higher cost of living."

One aim of this study was to assess whether we could observe this higher cost of living in cortisol-treated juvenile rainbow trout, and a second was to determine if this increase in energy metabolism was aerobic, anaerobic, or both. As a third goal, we wanted to establish which energy sources were employed under cortisol stimulation. For the latter, we compared two methods to elucidate differences in fuel usage in cortisol-stimulated fish. The instantaneous method (Lauff and Wood 1996) utilizes respirometric determination of O_2 consumption, CO_2 production, and N-waste excretion to stoichiometrically calculate the particular combination of carbohydrate, protein, and lipid actually being oxidized at any point in time. The classical compositional method relies on changes in whole-body fuel content to provide comparable information, averaged over prolonged periods. The compositional approach is less sensitive, revealing only the fuels that have been depleted. These are not necessarily the fuels which have been burned in metabolism. For example, fuels may be interconverted before respiration or they may be secreted or excreted without being burned. A limitation of the instantaneous approach is that it can only be applied when metabolism is aerobic, under steady-state conditions. Measurements for both methods were performed in control fish at day 0 and in experimental fish at days 3, 10, and 30 after receiving either a cortisol implant or a sham implant.

A final goal was to assess whether the sum of ammonia-N and urea-N excretion, traditionally considered to be the end products of nitrogen metabolism in teleost fish, accounted for all the N excretion of the fish in these experiments and whether it was affected by cortisol. The rationale for this comparison came from a recent review (Wood 2001) summarizing a number of articles in the older literature that indicated that total-N excretion (by Kjeldhahl digestion) might significantly exceed the sum of ammonia-N and urea-N excretion. The availability of easier, more accurate modern technology, a total nitrogen oxidizer, facilitated the comparison.

Material and Methods

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and kept at the experimental temperature of 14° ± 1°C in dechlorinated Hamilton tap water for several months before experimentation. They were maintained on a commercial trout feed and were fed 1% of their body weight daily. Water chemistry and food composition were identical to the values reported by Alsop and Wood (1997).

Cortisol Implants and Analysis

In order to provide the fish with either cortisol or sham implants, fish were anaesthetized individually in 0.1 g L^{-1} tricaine methanesulphate (MS-222) set to the normal water pH of ~8.0 with NaOH and then weighed. Experimental fish were then injected intraperitoneally with either melted coconut oil (~27°C) at a ratio of 10 μL g^{-1} of fish (sham groups) or with a mixture of coconut oil plus cortisol (cortisol groups; containing 250 mg kg^{-1} hydrocortisone 21-hemisuccinate; Sigma Diagnostics, St. Louis). This dose of cortisol in the implant was selected on the basis of a preliminary range-finder experiment and is the same as that used by Gregory and Wood (1999). Placing the fish on ice and briefly (10–15 s) keeping them on the ice after the injection avoided leakage of coconut oil out of the body cavity. Following this procedure, success rates in the actual experiment were 98%. Fish were then transferred to a well-aerated recovery tank until they regained consciousness and were placed into one of the experimental tanks.

Treatment Groups

Eight days before actual experiments began, 140 fish weighing 14.6 ± 0.2 g (mean ± SEM, $N = 140$) were divided randomly over seven experimental 25-L tanks. Each tank containing 20 fish was provided with constant aeration and flow of dechlorinated Hamilton tap water at ~0.5 L min^{-1}. Tanks were covered with perforated lids to minimize visual disturbance, and photoperiod was set at 12.5L : 11.5D. Feeding regime remained at 1% of their body weight, and food was administered every morning through a hole in the lid. Food was given in small consecutive portions to ensure it was all eaten, a fact which was confirmed visually. Following this procedure, trout could remain undisturbed until the day the implant was injected and, thereafter, until the day of sampling.

The seven experimental groups consisted of one uninjected control group that was sampled at day 0; a series of three groups that were injected with sham implants containing coconut oil alone and sampled 3, 10, and 30 d after implantation, respectively (hereafter referred to as sham groups); and a series of three groups that were injected with cortisol implants containing coconut oil with 250 mg kg^{-1} cortisol and sampled 3, 10, and 30 d after implantation, respectively (hereafter referred to as cortisol groups).

Body Composition

At the time of sampling (midafternoon, approximately 6 h postfeeding), 10 fish were quickly killed in 3.0 g L^{-1} MS-222

(pH ~8.0), a concentration which is sufficiently high to prevent the induction of a cortisol response (Beitinger and McCauley 1990). The fish were put on ice and a blood sample was withdrawn from all with a heparinized syringe by caudal puncture. Blood samples were stored in heparinized microcentrifuge (1.5 mL) tubes on ice until all samples were taken (max. 6–7 min) and were subsequently centrifuged for 5 min at 5,000 *g*. Plasma was removed and stored at −70°C for further analysis. Fish were blotted dry and weighed, and the coconut-oil implant was carefully removed and weighed for comparison to the initial weight. Fish were frozen in liquid nitrogen and stored at −70°C for analysis of the body composition.

Plasma cortisol levels were determined by ^{125}RIA using an ImmuChem Cortisol CT kit (ICN Biomedicals, Costa Mesa, Calif.). Plasma glucose and lactate were measured using Sigma Diagnostic kits Glucose (HK) 16-UV and Lactate 735, respectively (Sigma Diagnostics, St. Louis). Whole bodies of the fish were ground in liquid nitrogen, weighed, and lyophilized. The lyophilized tissue was reweighed to calculate the percentage dry weight and percentage water content of the body tissues. All assays were done in duplicate on these freeze-dried samples. Protein was determined via the Lowry method (Miller 1959) using bovine serum albumin (Sigma) as standard. Glucose, glycogen, and lactate were determined as in Bergmeyer (1985). The sum of these three carbohydrates is referred to as total carbohydrates. Lipids were determined gravimetrically after extraction in chloroform-methanol (2 : 1), exactly as described in Lauff and Wood (1996).

Respirometry

Immediately following sampling, eight of the remaining fish from each group were placed in individual separate Blazka-style swimming respirometers of known volume (≈3.2 L), similar to those described by Beamish et al. (1989). Velocity was set to one body length per second, the speed used in the subsequent respirometric measurements; at this speed, the fish would orient to the current and swim gently. They were allowed to recover from handling stress overnight, and measurements were performed the next morning. An opaque sheet protected the fish from visual disturbance. For temperature control, respirometers were submerged on a wet table, and a head tank provided a continuous flow of air-saturated water through each respirometer at a rate of 150 mL min^{-1} (total content of the recirculating system approximately 500 L). About half of the water content of this system was replaced on a daily basis.

An experiment was performed to assess whether the handling and confinement involved in the experimental procedure disturbed plasma cortisol levels. Six control fish were transferred to individual respirometers exactly as described above and allowed to recover overnight at one body length per second. After an identical period in the respirometers as in the actual respirometry experiments, these fish were rapidly killed with 3.0 g L^{-1} MS-222 (pH ~8.0) and blood was sampled for plasma cortisol measurement.

Respirometry measurements were made by closing the respirometers until Po_2 had dropped to approximately 120 Torr (approximately a 2-h period). During measurements, fish were swimming at a speed of one body length per second to ensure equal activity levels for all fish and good mixing of the water within the respirometer. At the start of the experiment, a 20-mL water sample of each respirometer was immediately frozen at −20°C for later analysis of ammonia and urea content and total N. An additional 8-mL water sample for CO_2 analysis was collected in a precooled glass vial that (after overflowing) was quickly capped to prevent loss of CO_2 by diffusion and was kept at the water temperature until analysis immediately after the experimental run had ended. Respirometers were closed, and every 20 min, a sample for Po_2 determination was taken. The samples were immediately analysed with a water-jacketed O_2 electrode (Radiometer E-5046) thermostated to the temperature of the test system. When oxygen levels had dropped to 120 Torr, samples for ammonia, urea, total CO_2, and total N were taken as described above. Fish were removed from respirometers, blotted dry, and weighed. Po_2 was converted to oxygen concentrations using tabulated solubility coefficients for freshwater from Boutilier et al. (1984).

Water samples for CO_2 analysis were measured in triplicate against a series of $NaHCO_3$ standards freshly made in the test medium. A Shimadzu GC-8A gas chromatograph equipped with a Poropack Q column and a Shimadzu-CR3A integrator were employed. Samples for ammonia determination were analysed using the salicylate-hypochlorite assay (Verdouw et al. 1978). Samples for urea determination were concentrated fivefold by freeze-drying before analysis by the diacetyl monoxime assay (Rahmatullah and Boyde 1980). Samples for total-N analysis by oxidation were analysed in triplicate on an Antek 7000V N analyser, employing the same standards as used for ammonia and urea determination.

Rates of O_2 consumption, CO_2 production, ammonia, urea, and total-N excretion were calculated in the traditional manner from the change in molar concentration of the substance in the water, factored by individual fish mass, time, and respirometer volume. For calculation of total-N excretion, urea excretion was multiplied by 2 to yield urea N and was added to ammonia excretion. This value was compared to the total-N excretion measured independently by oxidation in the N analyser. Respiratory quotient (RQ) and nitrogen quotient (NQ) were calculated as the ratios of CO_2 production rate to O_2 consumption rate and total-N production rate to O_2 consumption rate, respectively.

Statistics

All values are mean values ± SEM. The influence of treatment and time was evaluated using two-way ANOVA, followed by

the least significant difference test (LSD test) when significant differences occurred. Data were checked for normality of distribution and, where necessary (cortisol data), were appropriately transformed (to logarithms) before analysis. For the comparison between ammonia-N plus urea-N excretion and total-N excretion, a paired Student's *t*-test was used to evaluate differences between the two measurements. A significance level of $P < 0.05$ was employed throughout.

Graphs show significant differences with the control group at day 0 (indicated by a dagger) and differences between the sham and cortisol group at the same moment in time (indicated by an asterisk; one asterisk and one dagger: $P < 0.05$; two asterisks and two daggers: $P < 0.01$; three asterisks and three daggers: $P < 0.001$). Significant differences between moments in time within the same treatment group are mentioned in the "Results."

Results

Implanting the fish with either sham- or cortisol-containing coconut-oil implants did not cause any mortality. However, despite identical food consumption (1% daily ration, all consumed), cortisol-implanted fish showed a significantly lower growth rate over the whole experimental period compared to sham-implanted fish. At day 3, cortisol fish had actually lost weight at a rate of 0.25 ± 0.02 g d^{-1}, whereas sham fish had maintained a stable weight (rate $= 0.04 \pm 0.04$ g d^{-1}), which was a significant difference ($P < 0.001$). This weight loss was not due to water loss because the percentage dry weight of the body tissues was stable in the cortisol-implanted fish ($P > 0.05$, results not shown). Later in the experiment, fish of both groups were growing at a steady rate, but for the cortisol fish, this growth rate remained lower: 0.06 ± 0.01 g d^{-1} compared to 0.14 ± 0.01 g d^{-1} for the sham-implanted fish at day 10 ($P < 0.001$) and, at day 30, 0.09 ± 0.01 g d^{-1} for the cortisol fish compared to 0.14 ± 0.01 g d^{-1} for the sham-implanted fish ($P < 0.01$).

Figure 1*A* clearly shows that cortisol implants induced a significant rise in plasma cortisol levels, while sham implants did not. Cortisol was significantly higher at day 3 relative to both the simultaneous sham and the day 0 control groups and remained so at day 10. Nevertheless, it had significantly declined by day 10 ($P < 0.001$ compared to day 3). Cortisol did not change significantly thereafter but was no longer significantly elevated at day 30 relative to either control or sham-implanted trout. In the test performed to assess the effects on plasma cortisol of the handling and confinement involved in the respirometry procedure, plasma cortisol levels were 35.7 ± 6.7 ng mL^{-1} ($N = 6$). While these values were elevated relative to control values, they were not significantly different from values in the sham treatment and were significantly lower ($P < 0.001$) than those in cortisol-implanted fish (Fig. 1*A*).

Plasma glucose concentrations (Fig. 1*B*) increased significantly in the cortisol trout relative to the sham group for the whole experimental period. As plasma cortisol concentrations leveled off toward day 30, so did plasma glucose concentrations ($P < 0.05$ compared to day 10), but a significant difference from both the sham-implanted fish and the day 0 control value remained prominent during the entire experimental period. A transient increase in plasma glucose levels of sham fish (relative to day 0 control) was observed at day 10. Plasma lactate (Fig. 1*C*) followed the same trend as plasma glucose (Fig. 1*B*), with a clear elevation at days 3 and 10 in cortisol-implanted fish relative to shams, after which lactate levels returned close to

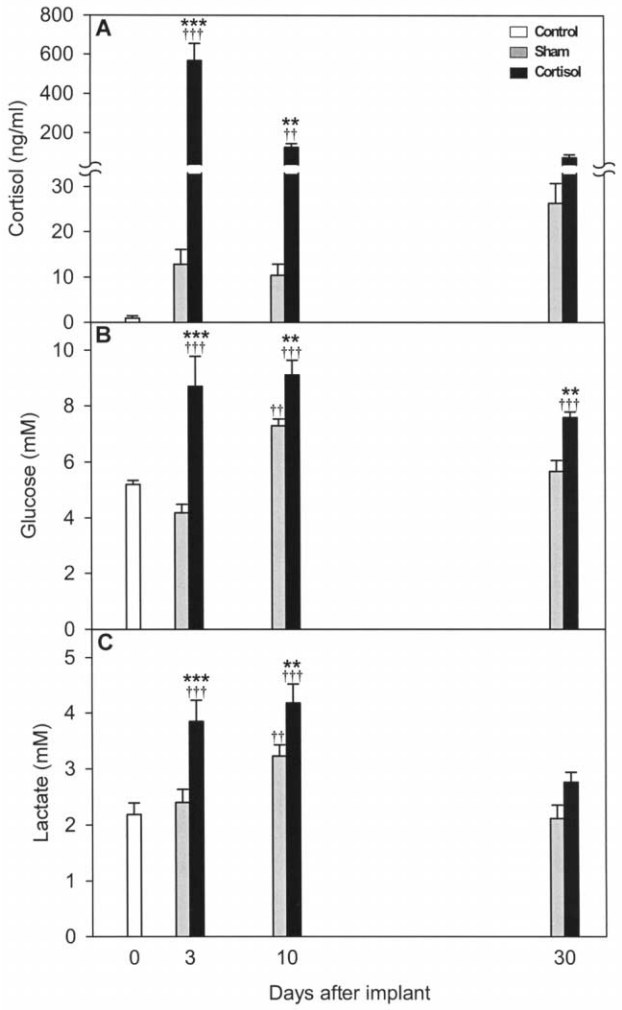

Figure 1. *A*, Plasma cortisol levels, *B*, plasma glucose, and *C*, plasma lactate of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 10$). An asterisk indicates significant difference between sham and cortisol treatment at the same moment in time, and a dagger indicates significant difference relative to control group on day 0 (two asterisks or two daggers: $P < 0.01$; three asterisks or three daggers: $P < 0.001$).

normal ($P < 0.01$ compared to day 10). Although a transient increase in plasma lactate was also observed at day 10 for the sham trout relative to the day 0 controls, lactate levels remained significantly lower than in the cortisol-implanted group.

At first, oxygen consumption remained unchanged for both groups (Fig. 2*A*) but, in the long run, declined in the sham treatment compared to the control situation (day 10: $P < 0.01$; day 30: $P < 0.05$). Cortisol fish did not show this reduction in aerobic energy metabolism and therefore exhibited a significantly higher oxygen consumption compared to the sham fish at day 10. Carbon dioxide excretion, on the other hand, clearly increased relative to both shams and day 0 controls in the cortisol-treated fish and remained unchanged in the shams (Fig. 2*B*).

Nitrogen excretion, estimated as the sum of ammonia-N plus urea-N excretion, showed little change (Fig. 3*A*), with a small decrease at day 30 for the cortisol group compared to the control group ($P < 0.05$) but not relative to the simultaneous sham treatment. Breaking nitrogen excretion down into its components, the ammonia-N component was dominant (>80%) and therefore exhibited the same trend as the sum of ammonia-N plus urea-N excretion (Fig. 4*A*). Urea-N excretion was rather variable and exhibited no differences related to either treatment or time (Fig. 4*B*).

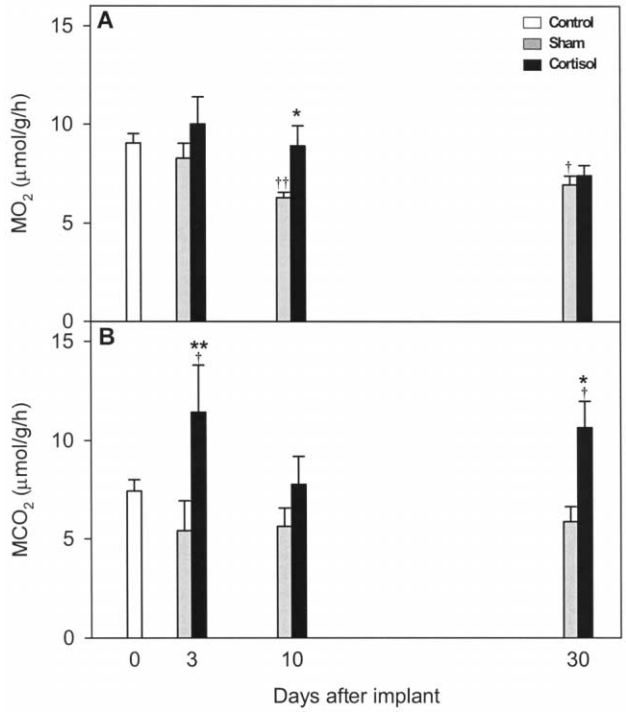

Figure 2. *A*, Oxygen consumption and *B*, carbon dioxide excretion of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 8$). An asterisk indicates significant difference between sham and cortisol treatment at the same moment in time, and a dagger indicates significant difference relative to control group on day 0 (one asterisk or one dagger: $P < 0.05$; two asterisks or two daggers: $P < 0.01$).

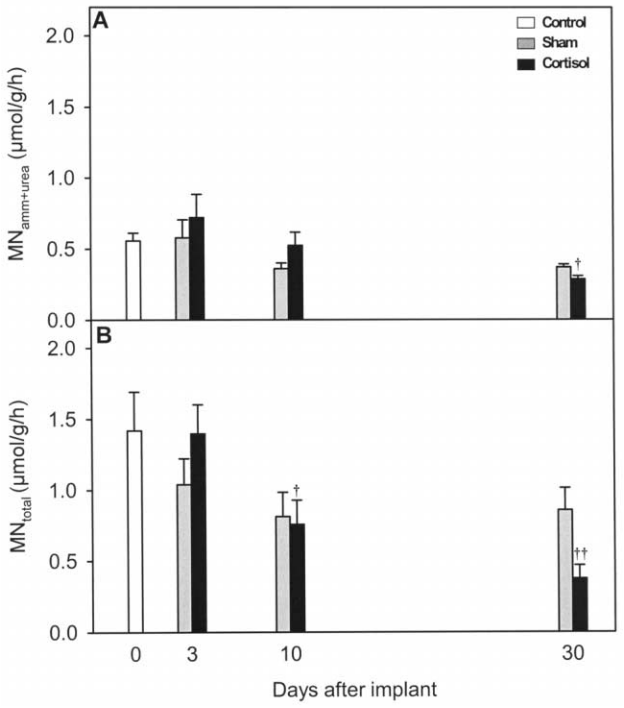

Figure 3. *A*, Nitrogen excretion rates (calculated as the sum of ammonia N and urea N) and *B*, total-N excretion rates (measured by oxidation) of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 8$). A dagger indicates significant difference relative to control group on day 0 (one dagger: $P < 0.05$; two daggers: $P < 0.01$).

Total-N excretion (Fig. 3*B*) measured by oxidation with the nitrogen analyser was approximately twice as large the sum of ammonia-N plus urea-N excretion (Fig. 3*A*). Total-N excretion was decreased in the cortisol group both at day 10 ($P < 0.05$ compared to controls) and day 30 ($P < 0.01$ compared to controls; $P < 0.01$ compared to day 3). The difference between total N measured by oxidation and the sum of ammonia N plus urea N (Fig. 3*A* vs. Fig. 3*B*) was significant in the control group ($P < 0.01$) throughout the sham treatment (days 3, 10: $P < 0.05$; day 30: $P < 0.01$) and at day 3 in the cortisol group ($P < 0.05$).

Figure 5 shows the RQs and NQs calculated from the data of Figure 3*A*. For reasons laid out in the discussion, NQ was calculated from the sum of ammonia-N plus urea-N excretion rather than from total-N excretion measured by oxidation in the N analyser. There was an increase in RQ (Fig. 5*A*) for the cortisol trout relative to shams at day 3 and an even more distinct increase at day 30. At both these times, the RQ exceeded 1.0 in the cortisol fish. The NQ (Fig. 5*B*) decreased moderately

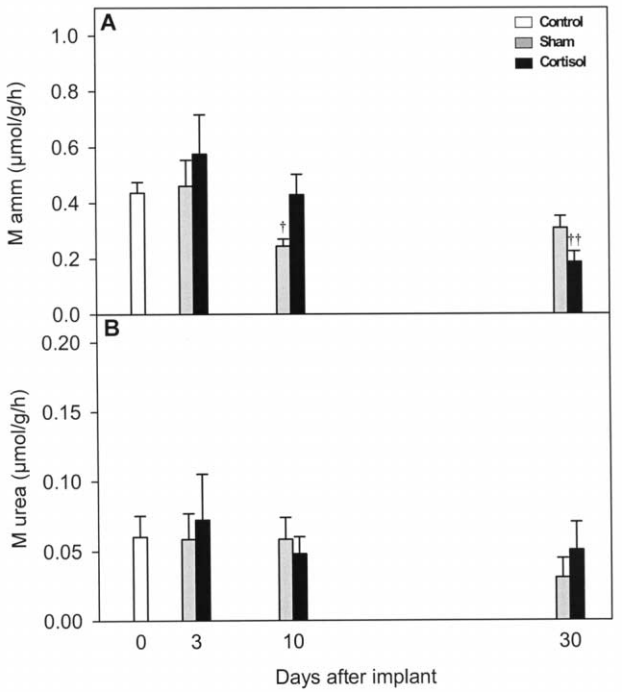

Figure 4. *A*, Ammonia-N and *B*, urea-N excretion rates of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 8$). A dagger indicates significant difference relative to control group on day 0 (one dagger: $P < 0.05$; two daggers: $P < 0.01$).

after 30 d of treatment for the cortisol group compared to the day 0 control situation.

While the respirometric data were valuable in demonstrating increases in both aerobic and anaerobic metabolism caused by cortisol, they could not be used to calculate instantaneous fuel utilization. Due to the obvious presence of anaerobic metabolism (i.e., RQ > 1.0, plus lactate data below) and the dramatically changing proximate body composition of the experimental fish (data below), neither of the basic criteria (aerobic metabolism only, steady-state conditions) needed for the application of the instantaneous fuel utilization equations were met (Lauff and Wood 1996). Nevertheless, the fall in NQ clearly indicated that there was no increase in the metabolic utilization of protein.

Body composition data (Fig. 6) showed the usual pattern in control fish, with protein being the largest component on a simple dry weight basis (57%), lipid slightly lower (41%), and carbohydrate accounting for only about 2%. When these data were converted to total calories using standard caloric equivalents for fish (Lauff and Wood 1996), the percentages of the total fuel caloric content were 45% for protein, 54% for lipids, and 1% for carbohydrates. Sham implants did not affect the body composition at all compared to the control situation (Fig. 6*A*–6*C*). In fact, the coconut-oil implants did not exhibit any significant weight changes over the 30 d of implantation in either group and thus did not seem to participate as an energy source for metabolism.

There was a considerable increase in carbohydrate content (Fig. 6*A*) during the first weeks of increased cortisol levels compared to the sham group and the control situation. After 30 d of treatment, the carbohydrate content dropped again ($P < 0.001$ compared to day 10) and returned to control levels. Lipid content was significantly depressed at day 30 in cortisol-treated fish relative to day 0 controls, although not relative to simultaneous shams (Fig. 6*B*). There were no significant changes in protein content in the cortisol or sham treatments (Fig. 6*C*).

The increase in carbohydrate content at days 3 and 10 in the cortisol trout was explained primarily by the increase in glycogen content (Fig. 7*A*), with an additional contribution of increased lactate levels at day 10 (Fig. 7*C*). Changes in glucose content were negligible, but at the end of the treatment, the small increase in glycogen content that was still present was

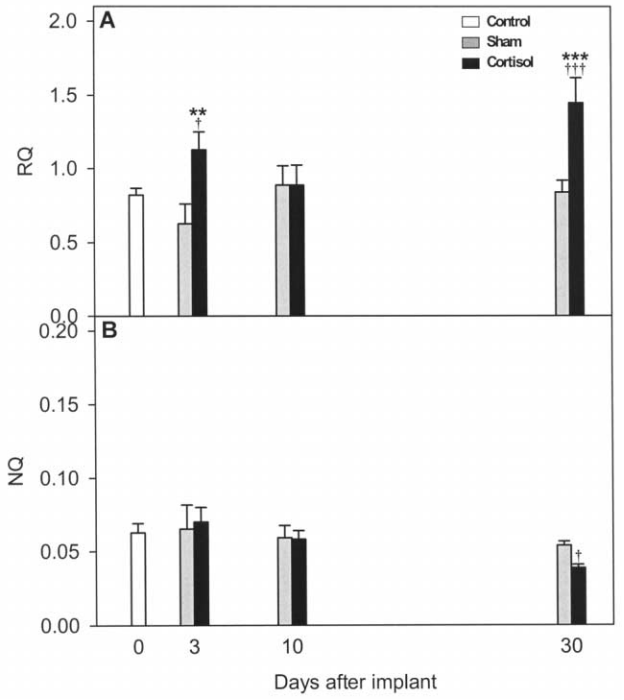

Figure 5. *A*, Respiratory quotients (RQ) and *B*, nitrogen quotients (NQ) of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 8$). An asterisk indicates significant difference between sham and cortisol treatment at the same moment in time, and a dagger indicates significant difference relative to control group on day 0 (one dagger: $P < 0.05$; two asterisks: $P < 0.01$; three asterisks or three daggers: $P < 0.001$).

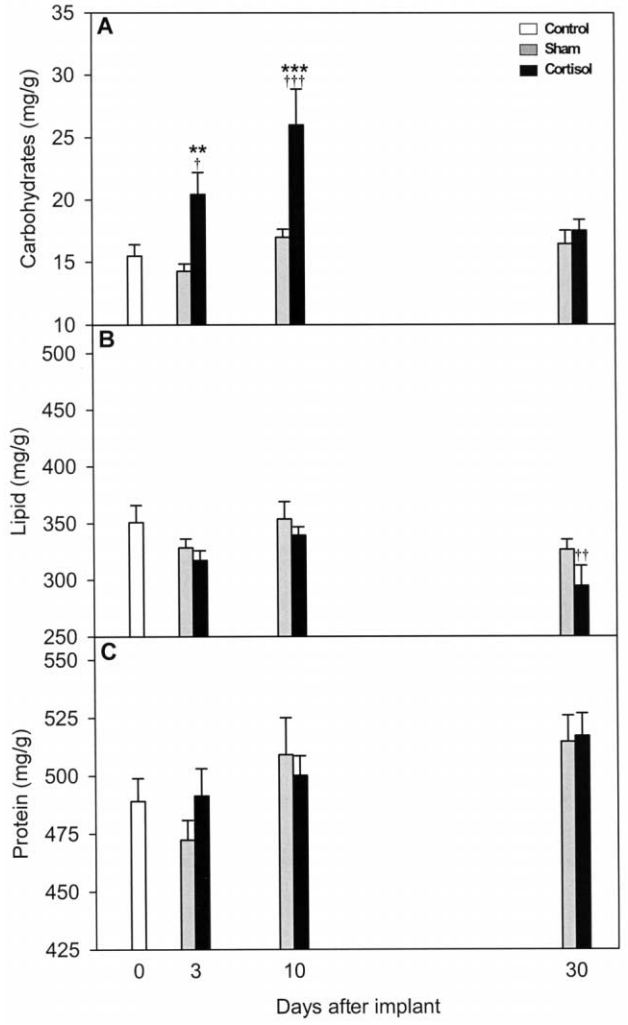

Figure 6. Total *A*, carbohydrate, *B*, lipid, and *C*, protein content of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 10$). An asterisk indicates significant difference between sham and cortisol treatment at the same moment in time, and a dagger indicates significant difference relative to control group on day 0 (one dagger: $P < 0.05$; two asterisks or two daggers: $P < 0.01$; three asterisks or three daggers: $P < 0.001$).

counteracted by the small decrease in glucose concentration (Fig. 7*B*). At this time, body lactate levels had returned to normal ($P < 0.05$ compared to day 10).

Discussion

The effects observed in this study are attributed to the observed elevation of plasma cortisol caused by cortisol implants. The handling and confinement involved in the respirometry procedure in itself marginally elevated plasma cortisol levels, which were significantly higher relative to controls but not relative to shams. However, they remained much lower than in cortisol-implanted fish. In our experience, the use of a small current to induce slow-speed swimming helps prevent disturbance, standardizes activity levels, and minimizes plasma cortisol elevation. Because respirometric changes in the shams were minimal, while respirometric changes in the cortisol-implanted fish were significant relative to shams, we feel that the marginal elevation in plasma cortisol associated with the respirometric procedure had minimal influence on the results and their interpretation.

Plasma glucose concentrations remained elevated over the

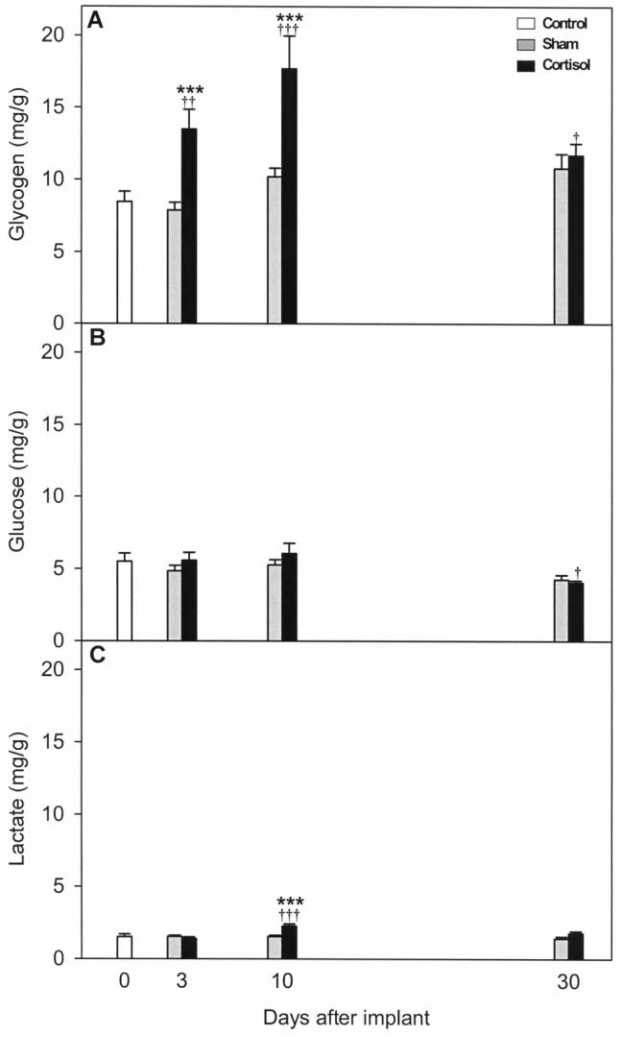

Figure 7. *A*, Glycogen, *B*, glucose, and *C*, lactate content of juvenile rainbow trout without implant (controls, day 0) or with sham- or cortisol-containing coconut-oil implants ($N = 10$). An asterisk indicates significant difference between sham and cortisol treatment at the same moment in time, and a dagger indicates significant difference relative to control group on day 0 (one dagger: $P < 0.05$; two daggers: $P < 0.01$; three asterisks or three daggers: $P < 0.001$).

entire experimental period, and growth rates remained reduced compared to sham-treated trout, indicating that the effects of the cortisol elevation worked over an extended period of time. The reduction in growth rate consisted of two phases: an initial strong drop in body weight during the cortisol peak followed by a phase of reduced growth. The initial drop in weight was not caused by water loss, nor could it be attributed to reduced appetite since all groups consumed an equal amount of food, as was confirmed visually at all times. Aerobic metabolism was not elevated at this time, but anaerobic metabolism was significantly elevated, as shown by elevated RQ and plasma lactate; therefore, some depletion of fuels may have contributed to the initial weight loss. Compromised food absorption may also have played a role under these circumstances. Cortisol is known to modulate amino acid transport in the intestine (Mommsen et al. 1999) and to depress the secretion of triiodothyronine (T3) secretion into the plasma (Redding et al. 1986; Vijayan and Leatherland 1989; Brown et al. 1991), which is a key regulator of glucose and amino acid transport (Collie and Ferraris 1995). Furthermore, chronic cortisol elevation causes intestinal regression and degradation of the intestinal lining in fish (Barton et al. 1987). This regression in itself, independent of any direct effects on nutrient transport, may lead to reduced absorption of food. In the second phase of the experiment, when cortisol levels declined and both aerobic and anaerobic metabolism were higher relative to shams, increased metabolism was more likely to have been the cause of depressed growth rates.

Stressful situations often lead to elevated cortisol levels and increased energy expenditure in fish (Chan and Woo 1978; Barton and Schreck 1987; Morgan and Iwama 1996). Recently, Gregory and Wood (1999) found that cortisol-implanted rainbow trout showed reduced growth rate, condition factor, appetite, and food conversion efficiency, all indicating a higher cost of living. Following the same methodology of cortisol administration, we showed that elevation of cortisol indeed accelerated energy metabolism and that the increased energy usage was powered not only by aerobic metabolism but also by anaerobic metabolism.

In this study, higher aerobic metabolism was only significant on day 10 and occurred against a background of declining oxygen consumption in the sham treatment. Morgan and Iwama (1996) observed a more pronounced increase in oxygen consumption in cutthroat trout and did not observe the reduced oxygen consumption in sham-implanted fish. Davis and Schreck (1997), using an implant in coho salmon, detected increased aerobic metabolism only after handling stress and not after cortisol administration alone, but they measured respiration rates only during the first few hours following the implantation. While the differences in oxygen consumption in this study were limited, elevation in plasma cortisol clearly increased CO_2 excretion. As a result, RQ was elevated above 1.0 at days 3 and 30. This obvious presence of anaerobic metabolism, as confirmed by elevated plasma and whole-body lactate in cortisol-treated fish, violates one of the key conditions necessary for instantaneous fuel use calculations from respirometry (Lauff and Wood 1996). Nevertheless, the available respirometry data do allow us to exclude protein from being this energy source. If protein had been the source, nitrogenous waste excretion and NQ would have undoubtedly increased.

Nitrogen excretion as well as NQ were generally stable, and in fact, both decreased slightly after 30 d of cortisol administration. These indices are far more sensitive than total protein measurements (i.e., the compositional approach); indeed, it would be difficult to detect the source of a 10 mg g^{-1} dry weight (Fig. 6*A*) increase in carbohydrate against background protein levels of 490 mg g^{-1} dry weight (Fig. 6*C*). Nevertheless, the fact that there were no differences in protein content between the two groups supports the conclusion. This result is quite surprising since a general agreement seems to have developed, based on mammalian literature, that cortisol exerts a proteolytic action, especially on fish white muscle and possibly in the liver (Barton et al. 1987). However, this agreement is based on very little piscine literature (Mommsen et al. 1999). While cortisol-induced increases in plasma amino acids have been observed in some previous studies (Andersen et al. 1991; Vijayan et al. 1997), Mommsen et al. (1999) have recently cautioned against making conclusions about protein turnover or metabolism from such measurements alone. Whatever their cause, the key finding of this study based on direct measurements of N excretion is that cortisol does not promote the use of protein as an energy source, at least under the conditions of our experiment.

Total-N excretion measured with a nitrogen oxidizer was approximately twice as large as the sum of measured ammonia-N plus urea-N excretion (Fig. 3). This finding is in accord with a considerable body of early literature that mainly employed Kjeldhahl digestion (e.g., Smith 1929; Wood 1958; Olson and Fromm 1971; McCarthy and Whitledge 1972; Beamish and Thomas 1984), although the discrepancy appears to be somewhat larger in this study, perhaps because the fish in this study were fed daily. In reviewing this literature, Wood (2001) concluded that the most likely explanation is the excretion of nonmetabolized proteins and amino acids, especially in soluble mucus. Assuming this explanation is correct, it would be inappropriate to use total-N excretion measured in this manner to calculate NQ or protein oxidation because the excreted protein and amino acids would not have been metabolized. Nevertheless, the impact on the total fuel budget would be substantial. This discrepancy has been generally ignored in modern studies; clearly, it is an important area for renewed investigation.

Cortisol increased plasma glucose levels over the entire experimental period, indicating either increased glucose synthesis or glycogen breakdown by the liver or decreased glucose utilization by the tissues. But cortisol also proved to be glyconeogenic; the greater part of the increased body carbohydrate levels

seen after 3 and 10 d of elevated cortisol levels was explained by increased levels of glycogen, with small additional effects of body lactate on day 10. Increased glucose levels were limited to the plasma and did not occur in body tissues. In this study, we measured only whole-body glycogen. Increases as well as decreases in liver glycogen and increases or no change in skeletal muscle glycogen have been reported in cortisol-stimulated fish (for review, see Mommsen et al. 1999). Recent work of Vijayan and coworkers on tilapia and rainbow trout hepatocytes confirms the gluconeogenic, but not the glyconeogenic, action of cortisol (Mommsen et al. 1999).

Increased circulating lactate levels have been observed as a result of cortisol treatment for the European eel and New Zealand snapper (Lidman et al. 1979; Bollard et al. 1993) but not for brook char, rainbow trout, and tilapia (Andersen et al. 1991; Vijayan et al. 1991, 1997). Lactate has been shown to be an important substrate for gluconeogenesis in fish (Suarez and Mommsen 1987), and lactate gluconeogenesis is stimulated by cortisol (Janssens and Waterman 1988; Mommsen et al. 1992); thus, the lactate production seen in this study probably contributed to the increased levels of circulating glucose.

The coconut-oil implants did not seem to participate in metabolism, a notion that is confirmed by the fact that sham implants had little or no effects on whole-body composition, N excretion, or aerobic or anaerobic metabolism. Since whole-body protein levels did not decrease, only two other possibilities remain for the extra energy spent to fuel increased metabolism and to produce glucose, lactate, and glycogen in cortisol-treated fish: food and mobilization of lipid stores. Although food consumption was equal in both experimental groups, cortisol-implanted trout did not use their food supply for increased growth; therefore, it is possible that it was used specifically for these purposes. Vijayan et al. (1991) reported that cortisol administration caused increased lipolysis in brook charr, resulting in increased glycerol levels that would be available for gluconeogenesis. In this study, in cortisol-treated fish, endogenous lipid stores decreased by day 30 compared to control but not compared to sham-implanted trout. Against the high background levels of total lipid (around 350 mg g^{-1} dry weight; Fig. 6*B*), the absolute difference (about 25 mg g^{-1} dry weight) was certainly large enough to explain the observed 10 mg g^{-1} dry weight elevation in total carbohydrate (Fig. 6*A*). Clearly, more work is needed to differentiate between these two possibilities.

We conclude that rather than investing energy in growing, fish with elevated plasma cortisol levels invest their energy in easily accessible energy sources such as carbohydrates, which can be very important for escape responses and burst swim activities needed to flee from the stressor. Cortisol-implanted fish exhibit no impairment of aerobic swimming performance (Gregory and Wood 1999); it would be interesting to test whether "burst" anaerobic swimming performance is actually improved.

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