Original Paper

Hormone Research

Horm Res 2002;58:287–291
DOI: 10.1159/000066448

Received: November 12, 2001
Accepted after revision: August 11, 2002

Does Growth Hormone Treatment Alter Skeletal Muscle Mitochondrial Respiration in Rats?

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Key Words
Growth hormone · Mitochondria · Substrate oxidation · Lipid

Abstract
Objective: Growth hormone (GH) has been shown to stimulate lipolysis and enhance lipid oxidation. We investigated whether GH could improve mitochondrial oxidative capacity. ***Method:*** Fourteen male Wistar rats received 14-day treatment with biosynthetic human GH (10 IU/kg/24 h) or placebo. Mitochondria were isolated from the total muscle of one hind limb of the rat. Mitochondrial oxygen consumption was measured in vitro using a Clark-type electrode with three substrates: palmitoyl-*L*-carnitine, pyruvate and succinate (+ rotenone). ***Results:*** Muscle mitochondrial yield was not significantly different in the GH-treated group from that in controls. Neither the basal nor ADP-stimulated respiratory state reached a significant difference between the 2 groups with palmitoyl-*L*-carnitine, pyruvate, and succinate. ***Conclusion:*** GH treatment did not improve the oxidative capacity of skeletal muscle mitochondria.

Introduction

Growth hormone (GH) has well demonstrated anabolic properties [1, 2] and also lipolytic action. Indeed, many studies have demonstrated that GH administrations stimulate lipid metabolism in both humans and animals [3–8] and also acutely increase energy expenditure in humans [9, 10]. GH causes lipolysis stimulation in adipocytes by increasing the activity of hormone-sensitive lipase [11, 12]. This results in increased circulating levels of free fatty acids (FFA) and glycerol and increased lipid oxidation rates, as assessed by indirect calorimetry [6]. It is generally accepted that GH stimulates lipid oxidation by increasing the availability of substrates. However, it has been recognized that in other cases as after endurance training, the increased lipid oxidation is due to an increase in mitochondrial activity [13, 14]. Indeed, low-intensity training also promotes lipid utilization, as demonstrated by a lower respiratory gas exchange ratio [15, 16]. In this case, the enhancement in lipid oxidation is explained by a stimulation of lipolysis and also by an increase in mitochondrial capacity. At the level of skeletal muscle, training increases mitochondrial mass [17–19]. This improves the capacity to oxidize fatty acid by

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increasing the specific enzyme rate both in total muscle [14, 17] and isolated mitochondria [20]. Therefore, the greater utilization of fatty acid observed in trained subjects compared with untrained subjects would be explained by a higher lipid mobilization and a higher capacity of muscle to oxidize fat. Then, because GH treatment has been well recognized to also stimulate lipid oxidation, we hypothesized that such treatment could increase skeletal muscle mitochondrial oxidative capacity.

Thus, the aim of this study was to determine if GH treatment would induce mitochondrial adaptations promoting substrate oxidation in the skeletal muscle of rats. In this way we investigated particularly whether GH improves the cellular lipid oxidation pathway.

Material and Methods

Animals

The experimental procedure was in accordance with the recommendations of the American Association for Accreditation of Laboratory Animal Care. Fourteen male Wistar rats (230–320 g) were used. They were provided food and drink ad libitum and maintained in a 12-hour light/12-hour dark cycle. They were paired by weight and randomly separated into 2 groups. The GH-treated group (n = 7) received a dose of 10 IU/kg/24 h of human recombinant GH (genotropin, Pharmacia & Upjohn, Saint-Quentin-en-Yvelines, France) twice daily in subcutaneous injections (9 a.m., 5 p.m.) for 14 days. The control group (n = 7) received twice daily injections of saline for 14 days. The animals were weighed in the morning of every day until experimentation. On day 15, animals did not receive any treatment and were euthanized on day 16 by cervical dislocation.

Blood Samples

Blood samples were collected from the caudal vein with capillary microtubes and immediately centrifuged. Plasma was conserved at –80°C until assay. Samples were drawn 1 day before the beginning of treatment (day 1) and 1 day after the end of the treatment (day 15).

Glycemia was assayed with a Sigma diagnostics kit (glucose (HK) procedure No. 16-UV, Saint Quentin, France), following a method similar to the procedure described by Bondar and Mead [21], which used the coupled enzyme reactions catalyzed by hexokinase and glucose-6-phosphate dehydrogenase.

NADH Dehydrogenase Activity

NADH dehydrogenase activity was assayed using the method described by Holloszy [18]. The reduction of potassium ferricyanide was measured at 420 nm. The reaction mixture contained 40 m*M* of potassium phosphate buffer (pH 7.4), 0.15 m*M* DPNH, and 0.6 m*M* potassium ferricyanide. The reaction was started by the addition of 20 µl of mitochondrial suspension.

Whole Muscle Mitochondrial Isolation

Mitochondria were isolated from the entire musculature of one hind limb of the rat as previously described by Davies et al. [17]. Briefly, all muscles from one of the rat hind limbs were removed and freed of fat and connective tissue. Muscles were immediately placed in ice-cold isolation medium (250 m*M* mannitol, 10 m*M* EDTA, 45 m*M* Tris HCl, 5 m*M* Tris base; pH 7.4), weighed, minced, and incubated with trypsine (bovine pancreas type III; Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 0.35 mg/g of wet muscle weight for 30 min at –4°C. Samples were then homogenized using an Ultra-Turrex tissue homogenizer for 15 s at 40% power. Samples were further homogenized (Wheaton glass homogenizer) using two slow passes of the pestle [22]. Muscle homogenates were centrifuged at 500 *g* for 10 min. Supernatants were centrifuged for 10 min at 900 *g*. Pellets were resuspended in cold isolation medium and centrifuged at 900 *g*. Pellets were then resuspended in 3–4 ml of isolation medium.

Muscle Mitochondrial Protein Yield

The final mitochondrial protein concentration was determined by the Bradford protein assay (Bio-Rad, Marnes-la-Coquette, France). The mitochondrial protein yield was the milligram of mitochondrial protein assayed by gram wet muscle.

Mitochondrial Oxygen Consumption

Rates of oxygen consumption by mitochondria were measured with a Clark-type oxygen electrode (Strathkelvin Instruments, Glasgow, UK). The respiration chamber was kept at a constant temperature (37°C). The chamber was filled with 1.87 ml of respiratory medium (15 m*M* KCl, 15 m*M* K_2HPO, 15 m*M* KH_2PO_4, 25 m*M* Tris base, 45 m*M* sucrose, 12 m*M* mannitol, 5 m*M* $MgCl_2$, 7 m*M* EDTA, 0.2% bovine serum albumin FFA, 20 m*M* glucose, pH 7.4) and one of three substrates: 40 µ*M* palmitoyl-*L*-carnitine and 1 m*M* malate or 10 m*M* pyruvate and 2.5 m*M* malate, or 10 m*M* succinate and 50 µ*M* rotenone. Palmitoyl-*L*-carnitine is used to test β-oxidation pathway and all complexes of the electron transport chain. Pyruvate is used to test the oxidative decarboxylation pathway and all complexes of the electron transport chain. Succinate is used to specifically test the tricarboxylic acid cycle and mitochondrial complexes II–IV of the electron transport chain while complex I was then inhibited by rotenone. Mitochondria (1 mg) were loaded into the chamber. The basal state was measured and the ADP-stimulated state was determined after addition of 500 nmol of ADP. The ratio of the amount of ADP phosphorylated to oxygen consumed (ADP/O) was calculated by dividing the nanomoles of ADP added by nanomoles of oxygen consumed during the ADP-stimulated state respiration. The respiratory control ratio (PCR) was obtained by dividing the ADP-stimulated state respiration by the recovery rate after completion of ATP synthesis (recovery state). All measures of every state were performed at least in duplicate.

Statistical Analyses

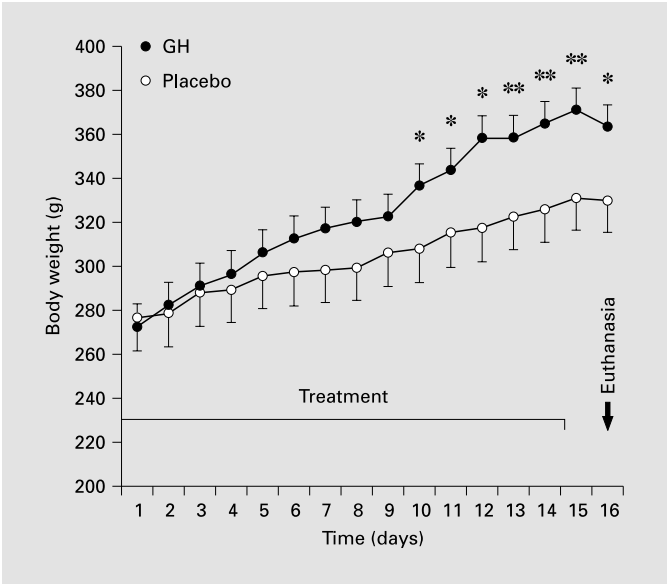
All data are presented as mean ± SEM. To determine the GH effect on weight, animals were paired before treatment and a two-way ANOVA for repeated measures was used. To determine the GH effect on the three respiratory states for each substrate, a two-way ANOVA for repeated measures was used. To compare values of muscle mitochondrial protein yield, NADH dehydrogenase activity, ADP/O, and RCR, Student's t test was used. Verification of a normal distribution for all values was previously done. Statistical significance was assumed at $p < 0.05$.

Results

The body weight of the GH-treated rats was significantly higher than that of the controls above 10 days of treatment and until the end of treatment (364 ± 13 vs. 326 ± 20 g, respectively, $p < 0.01$; fig. 1). The muscle mitochondrial protein yield was not significantly different in the GH-treated group in comparison with the control group: 0.60 ± 0.07 vs. 0.58 ± 0.06 mg/g muscle. NADH activity was not significantly different between the GH and control groups: 2.3 ± 0.6 vs. 2.62 ± 0.5 µmol/min/mg, respectively. Both ADP/O and RCR with the three substrates did not significantly differ between the 2 groups (table 1). Mitochondrial respiratory states did not reach significant differences between the GH-treated group and the control group in the basal state, in the ADP-stimulated state and in the recovery state, with palmitoyl-*L*-carnitine (fig. 2), with pyruvate (fig. 3) and with succinate + rotenone (fig. 4). Moreover, basal glycemia did not differ significantly between the GH-treated group and the control group, before treatment: 1.17 ± 0.05 vs. 1.18 ± 0.05 $g \cdot 1^{-1}$, and after treatment: 1.13 ± 0.07 vs. 1.08 ± 0.04 $g \cdot 1^{-1}$, respectively.

Table 1. ADP/O and RCR (state 3/state 4) ratios (mean ± SEM)

	Control rats (n = 7)	GH-treated rats (n = 7)
Palmitoyl-*L*-carnitine + malate		
ADP/O	2.96 ± 0.08	3.01 ± 0.09
RCR	4.08 ± 0.38	4.14 ± 0.19
Pyruvate + malate		
ADP/O	3.15 ± 0.06	3.13 ± 0.07
RCR	5.81 ± 0.55	6.18 ± 0.35
Succinate + rotenone		
ADP/O	1.58 ± 0.05	1.62 ± 0.06
RCR	1.78 ± 0.07	1.77 ± 0.8



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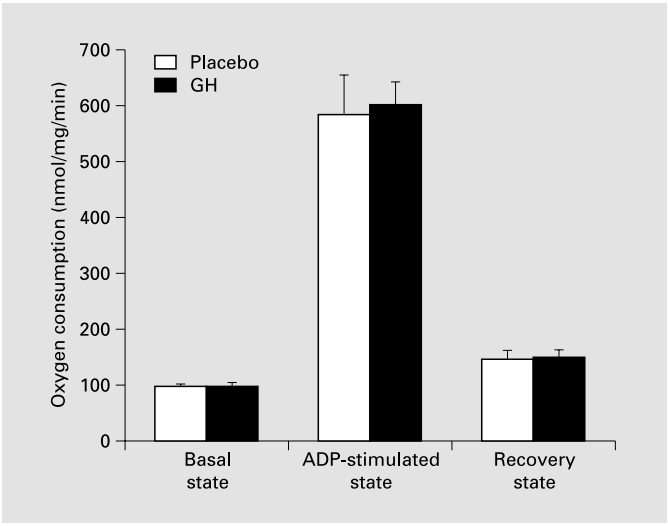

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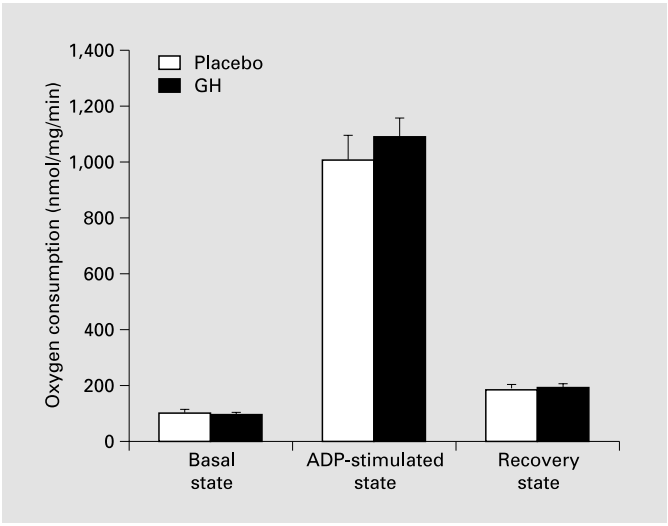

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Fig. 1. Evolution of body weight (mean ± SEM). ○ = Control rats (n = 7); ● = GH-treated rats (n = 7). * $p < 0.05$; ** $p < 0.01$.
Fig. 2. Mitochondrial oxygen consumption measurements with palmitoyl-*L*-carnitine and malate as substrates (mean ± SEM). □ = Control rats (n = 7); ■ = GH-treated rats (n = 7).
Fig. 3. Mitochondrial oxygen consumption measurements with pyruvate and malate as substrates (mean ± SEM). □ = Control rats (n = 7); ■ = GH-treated rats (n = 7).

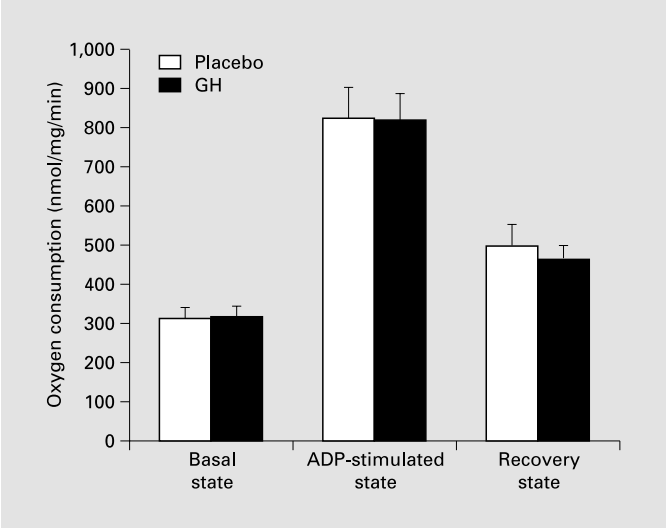

Fig. 4. Mitochondrial oxygen consumption measurements with succinate (+ rotenone) as substrate (mean ± SEM). □ = Control rats (n = 7); ■ = GH-treated rats (n = 7).

Discussion

This study did not show significant effects of GH treatment on oxidative capacity of mitochondria isolated from skeletal muscle.

In a previous study using similar doses for subcutaneous injection of human biosynthetic GH in rats, a significant elevation in plasma IGF1 was noted [23]. In accordance, although we did not assay IGF1, we observed the well-known growth-promoting effect of GH by means of weight measurement. This confirmed the efficacy of using human recombinant GH in rats as in our treatment protocol. Moreover, our treatment did not induce a diabetes state as shown by the glycemia values.

In both the GH-treated and control groups, the values of the ADP/O rate obtained from both pyruvate and palmitoyl-*L*-carnitine were always nearly 3 and for succinate always nearly 2. This demonstrated a good phosphorylation-oxidation coupling of isolated mitochondria. Theoretically, NADH-linked substrates allow three molecules of ATP to be formed per passage of a pair of electrons from NADH along the electron transport chain, while FAD-linked substrates allow two molecules of ATP. The isolation procedure affected neither the chemiosmotic gradient across the inner membrane nor the ATPase complex. The high values of RCR provided further evidence that isolation and incubation resulted in structurally intact mitochondria with good integrity [18]. This indicates, moreover, that GH treatment did not induce alterations in the mitochondrial membrane.

GH has been well recognized to increase lipid oxidation both acutely [6] and chronically [10]. This increased lipid oxidation is usually imputed to an enhancement of lipid mobilization [6, 24]. However, previous studies have shown that cellular adaptations could induce an increased lipid oxidation [13, 14]. These adaptations consist in an increased skeletal muscle mitochondrial content. Indeed, low-intensity training also increases lipolysis and plasma FFA turnover [25], but in skeletal muscle this is associated with an increase in mitochondrial capacity to oxidize lipids due to a higher mitochondrial mass [17–19]. Consequently, the pool of enzymes needed to oxidize fatty acids is increased, i.e., FFA translocation, the tricarboxylic acid cycle, the β-oxidation pathway, and components of the electron transport chain [14]. Indeed, mitochondrial respiration values, with fatty acid as substrate expressed per gram muscle, are higher in trained rats [14]. Recently, other authors demonstrated that similar measurements expressed per milligram mitochondrial protein also show higher values in trained animals [20]. This indicates that the specific activity of mitochondria is also increased. These studies taken together suggest that both the mass of mitochondria and their intrinsic properties to oxidize fatty acid are increased by training. Because GH also promotes lipolysis and plasma FFA turnover, we hypothesized that muscle adaptations similar to those induced by training would be induced by GH treatment.

The muscle mitochondrial protein yield is usually determined by the ratio of mitochondrial protein assayed to muscle wet weight used for mitochondrial isolation. Our values indicate that GH treatment did not improve mitochondrial biogenesis. When the machinery of biogenesis is retarded because of pathological conditions, GH has been shown to increase the rate of mitochondrial protein synthesis [26–28]. This study showed that in normal conditions GH did not improve mitochondrial protein synthesis. This suggests that the mitochondrial pool was not increased by GH. Moreover, we did not observe a significant difference between the 2 groups in terms of isolated mitochondrial respiratory state with palmitoyl-*L*-carnitine as substrate, or with succinate. This indicates that the mitochondrial intrinsic enzymatic system needed to oxidize these substrates, i.e. the β-oxidation pathway and the tricarboxylic acid cycle, was not improved by GH treatment. Moreover, respiration measurements with each substrate were used to determine if changes in the complex of electron transport chain activity occurred. Indeed, if only complex-I activity was modified, changes in respi-

ration measurements could be observed with both pyruvate and palmitate as substrate but not with succinate. If another complex of the electron transport chain was modified, changes in respiration measurements could also be detected with succinate. The results obtained with both palmitoyl-*L*-carnitine and pyruvate indicate that the activity of the whole electron transport chain was not increased by GH. In accordance, the results obtained with succinate confirm that the activity of the electron transport chain above complex I was not increased. Thus, GH did not improve either mitochondrial mass or the oxidative properties of isolated mitochondria. Therefore, the increase in lipid oxidation previously observed with GH treatment could only be the result of greater substrate availability [6, 24] and not associated with muscle mitochondrial adaptations. In spite of the previously demonstrated effect on lipid mobilization, GH treatment did not have the same effect as training on the skeletal muscle oxidative capacity.

In conclusion, this study suggests that the pharmacological use of GH did not improve the muscle mitochondrial oxidative properties investigated on isolated mitochondria in normal rats.

Acknowledgments

We thank Pharmacia & Upjohn for the generous gift of human recombinant GH used in this study.

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