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Effects of resistance exercise and growth hormone administration at low doses on lipid metabolism in middle-aged female rats

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Abstract

hGH(human growth hormone) is a key factor for metabolism as well as for growth. Lack of hGH usually increases LDL(low-density lipoprotein) while impairing exercise capacity and cardiac function [Amato G., Carella C., Fazio S., La Montagna G., Cittadini A., Sabatini D., Marciano-Mone C., Sacca L., and Bellastella A., 1993. Body composition, bone metabolism, and heart structure and function in growth hormone (GH)-deficient adults before and after GH replacement therapy at low doses. J. Clin. Endocrinol. Metab. 77, 1671-1676.]. It is still unclear, however, whether the administration of hGH to humans or animals has a synergic effects on body composition and the desired metabolic parameters with endurance resistance exercise. Therefore, the present study seeks to establish whether or not lipid metabolism is altered by both recombinant GH(growth hormone) and X(resistance exercise) in middle-aged female rats. GH administration resulted in greater weight gain compared with control and exercised animals, but the effect was reduced when combined with exercise. The increased body weight was largely due to increased muscle mass. Exercise did not result in any additional effect of GH on muscle mass. In the exercise group, hepatic HDL(high density lipoprotein) increased compared to the C(control group). The GX(growth hormone+exercise) group's serum and X group's kidney IGF-I(Insulin-like growth factor-I) concentration increased compared to the C group. In G and GX groups, serum insulin and leptin concentrations were higher than in the control, suggesting that GH may induce an insulin resistant state. Any gains in muscle mass were minimal and were not synergistic with exercise. These results suggest that hGH may not be useful for increasing performance in athletes and may induce and acquired insulin resistance.
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Keywords: Human growth hormone; Resistance exercise; Lipid metabolism; (Aged female rat)

1. Introduction

GH(Growth hormone) secretion from the anterior pituitary gland has profound anabolic effects, increasing lypolysis and partitioning food energy toward protein synthesis (Sonksen, 2001). GH secretion declines dramatically with age (Benbassat et al., 1997). Aging is also associated with alterations in body composition such as decreased muscle protein quantity and quality (Yarasheski et al., 1995) and increased body fat (Dionne et al., 2004). Age-related decline in bone density (Schuit et al., 2004) and cellular enzymes or receptor activities (Feuers, 1998) have also been documented. In addition to providing biological markers for aging, alterations in plasma hormone levels including GH (Bartke, 2003), estrogen (Danilovich et al., 2003), progesterone (Schumacher et al., 2003) and testosterone (Lunenfeld, 2003) are associated with age-related physical decline in both humans and experimental animals. Gradual declines in GH during the adult life have been reported to be involved with aging-associated alterations in body composition (Lamberts et al., 1997). GH administration in older rats (Andersen et al., 2000) and GH-deficient adults or elderly subjects has improved body composition (Lange et al., 2001), plasma lipid profiles (Viidas et al., 2003), increased insulin levels and reduced serum glucose concentrations (Micic et al., 2004). GH has been used illicitly by young athletes as a performance enhancer to decrease body fat and increase muscle mass (Ambrose, 2004). Conversely, elderly people were given GH to reverse various age-related decreases in muscle mass, sarcopenia (Taaffe et al., 1994). Many of the effects of GH are similar to the benefits of exercise in young athletes. However,

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the beneficial effects of using GH are not sufficiently documented in either humans or animals. A contradictory report also exists, which suggests that chronic exercise training may reduce the effects of exogenous GH (Chwalbinska-Moneta et al., 1996; Godfrey et al., 2003). Furthermore, there seems to be an interaction between GH controlled lipid metabolism, IGF-I and exercise (Seguin and Nelson, 2003). CPT-I(Carnitine palmitoyltransferase-I) is the rate-limiting enzyme for fatty acid oxidation and is the first step specific to fatty acid oxidation (McGarry and Brown, 1997). In contrast, ACS(acyl-CoA synthetase) catalyzes the synthesis of acyl-CoA (Coleman et al., 2002), and ACC(acetyl-CoA carboxylase) plays a pivotal role in both fatty acid oxidation and biosynthesis (Barber et al., 2003). Therefore, the focus of this study concentrated on the following. First, to investigate interactive metabolic effects of GH and exercise on the transcriptional regulation of lipid metabolism-related enzymes (ACS, ACC and CPT-I), in mature female rats, who's age corresponded to late middle aged humans. Second, to investigate changes in body composition that might correlate with GH induced lipid metabolism and the effects of exercise.

2. Materials and methods

2.1. Animals and diets

Thirty-two female Sprague–Dawley rats, aged 50 weeks, were purchased from Daehan Biolink Inc. (Eumsung, Chungbuk, Korea). All rats were randomly divided into four groups. Each day for 8 weeks, all animals were injected in the nape of the neck with either GH or a sham treatment (5 times per week for 8 weeks). GH was injected 1 h prior to exercise. Group C was injected with saline using the same method used for the GH group. Group G was injected with 2 mg/kg (bw) per day with GH (Somatotropin, Italy). Group X was injected with saline and subjected to exercise. Group GX was injected with GH and also subjected to exercise. All rats were weighed weekly, and the hormone dose was adjusted in relation to the actual body weight. All rats were caged separately and had unlimited access to water and pellet food (Sam Yang, Seoul, Korea). The animal room temperature was maintained at 23 ± 1 °C, a humidity of 53 ± 2%, and a 12 h light/dark cycle. Experimental protocols were approved by the Institutional Animal Ethics Committee of Chonbuk National University and were conducted according to the guidelines of the Korean Science Academy for the use and care of experimental animals.

2.2. Training

After the first week of adaptation, exercised rats were trained three days per week on alternating days for the next 8 weeks. Resistance training was accomplished by using a 1 m high ladder with 2 cm grid steps and an 85° grade. In their first week, rats were familiarized with climbing up to the top cage with and without weight on their tails. Training sessions, from the second week, were started with the intensity at 50% of each rat's body weight; the weight in an acryl tube was attached to the tail with a plastic belt and tapes. Rats started their climbing from the bottom of the ladder and they were forced to climb up to the top by touching and shouting. When they reached to the top, 2 min of rest was given and the next trial was followed. Subsequent trials were performed from the bottom, and 30 g of weight was added to the prior weight at every trial. If a rat was able to climb with the increasing weights for 10 times, a session of training was considered as completed. In case of the failure of increasing weight as planned, the rat was forced to complete 10 times of trials with the last succeeded weight without increasing the weight any more.

2.3. Sample preparation

Food was withheld for 12 h, and GH or exercise was withdrawn for 48 h before the rats were decapitated. Serum was collected after centrifugation at 1100 ×*g* for 15 min at 4 °C and stored at −20 °C until analysis commenced. Tissue from the liver, kidney, heart, brain, skeletal muscle and spinal cord were collected and weighed. Next, the specimens were immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.4. Analysis of lipid profiles

Lipid profiles were analyzed in both the serum and liver. TG (Triglycerides) were enzymatically analyzed using a commercially available kit (Asan pharm. Co., Seoul, Korea). Total- and HDL-cholesterols were measured using kits (Asan pharm. Co.) that were based on the cholesterol oxidase method (Allian et al., 1974). Then, LDL-cholesterol was calculated (LDL-cholesterol = Total-cholesterol − HDL-cholesterol − TG/5) using the Friedewald equation (Friedewald et al., 1972).

2.5. Analysis of insulin, glucose, leptin and IGF-I concentration

Insulin, glucose and leptin concentrations were analyzed in serum. Insulin and glucose were analyzed using a radioimmunoassay kit (ICN Pharmaceuticals, Inc., USA) and a commercially available kit (Asan pharm. Co. Seoul, Korea) respectively. Leptin was measured with a rat leptin radioimmunoassay kit (LINCO Research Inc., USA). IGF-I concentrations were measured in both the serum and the tissues samples (of muscle, heart, liver, brain, kidney and spinal cord). IGF-I was calculated using a rat IGF-I radioimmunoassay kit (DSL, USA).

2.6. Sample preparations for carnitine and protein assays

Tissue samples, mentioned above, were prepared by applying the following procedure. Muscle was homogenized, with approximately 50 mg of tissue in 99 volumes (1% homogenate) of cold diluted water, using a sonicator (Fisher Scientific Co., USA) at approximately 50–60 Hz for 20 s. Liver and muscle were homogenized with approximately 50 mg of each tissue in 1.5 ml of cold water and homogenized with the same settings mentioned above. One volume (0.1 ml) of tissue extract was added to 9 volumes (0.9 ml) of 50 mmol/L KOH

and allowed to stay overnight at room temperature. Then, the concentration of NCP(non-collagen protein) was measured using a Bio-Rad protein assay kit (BIO-RAD Co., USA) that operated based on the Bradford method (Bradford, 1976).

2.7. Carnitine assay

Carnitine concentrations, in serum and in tissues (from skeletal muscle, liver), were measured using a modified radioisotopic method (Cederblad and Lindstedt, 1972; Sachan et al., 1984). A 100 μl sample was hydrolyzed with 200 μl of 0.5 N KOH and then centrifuged at 1500 ×*g* for 10 min. ASAC (acid-soluble acyl carnitine) and NEC(non-esterified carnitine) were determined in the supernatant. AIAC(acid insoluble acyl carnitine) was determined in the pellet. A 150 μl aliquot of the supernatant was neutralized with 1 M KHCO3 to measure NEC. 100 μl of supernatant, prior to ASAC assay, was hydrolyzed with 0.5 N KOH and neutralized with PCA/MOPS-II(perchloric acid /4-morpholinepropanesulfonic acid Sodium Salt) solution. The pellet containing AIAC was washed with 0.6 M PCA, then hydrolyzed in 0.5 N KOH for 60 min in a water bath at 60 °C, and neutralized by PCA/MOPS-I. The reaction mixture, containing (1 M MOPS buffer, 0.1 M potassium ethyleneglycoltetraacetate, 0.1 M sodium tetrathionate, 0.1 mM [^{14}C]-acetyl CoA from Amersham), was diluted with water up to 100 μl and added to the 100 μl neutralized sample, then incubated with carnitine acetyl transferase (Sigma Chemical Co., USA) in a water bath at 37 °C for 30 min. At the end of incubation, 200 μl of the incubation mixture was transferred onto a mini-column and eluted with two portions of 500 μl of water. Then, each sample was transferred to a 20 ml scintillation vial and the radioactivity of the samples was measured with a liquid scintillation counter, Beckman LS-380.

2.8. RNA preparation and RT-PCR

Total RNA from skeletal muscle (gastrocnemius) and from liver was isolated using a modified guanidinium thiocyanate-phenol-chloroform extraction procedure. Total RNA was quantified by measuring the absorption at 260 and 280 nm. ACC, ACS and CPT-I mRNA expression levels were measured through RT-PCR(the reverse transcriptase-PCR method). The RT-PCR was conducted using 1 μg denaturated RNA and random hexamer primers as described by the manufacturer (One-step RT-PCR kit from ABgene, USA). The cDNA product was amplified in a 50 μl final volume containing each of the primers (of ACC, ACS and CPT-I) and RT-PCR Master Mix (thermoprime plus DNA polymerase, and an optimized reaction buffer, dNTP mix and $MgCl_2$.

2.9. Statistical analysis

Significance of the differences between the samples was determined by 2-way analysis of variance (ANOVA) using SAS software version 8 (SAS Institute, USA). Differences within the four groups were separated using Duncan's multiple range test. The accepted level of significance was $P<0.05$. Results were expressed as mean±S.D.

3. Results

3.1. Effects of human growth hormone and/or exercise on body weight and body composition

Compared to the control group, body weight gain was significantly lower in the X group, significantly higher in the G group, and the GX group had intermediate weight gains that were not significantly different from C and X. Food consumption was greatly increased in all groups more so than in the C group (Table 1). The highest food consumption was evident in the groups given with GH. The total muscle (soleus and plantaris R and L), were higher in G group than in control group (Table 2).

3.2. Effects of human growth hormone and/or exercise on lipid profiles

GH combined with exercise significantly decreased serum triglyceride ($P<0.01$). GH administration, both alone and with exercise training, decreased total cholesterol and exercise training resulted in an increased HDL-cholesterol concentrations (Table 3).

3.3. Effect of growth hormone and/or exercise on insulin, leptin and IGF-I concentrations

In G and GX groups, serum insulin and leptin concentrations were higher than in the control group (Table

Table 1
Body weight gain, food consumption, and exercise performance

	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Initial body weight (g)	296.88±16.23[2)]	305.88±10.64	304.88±15.82	300.12±16.90	NS	NS	NS
Body weight gain (g)	32.50±4.95[b3)]	59.00±12.73[a]	14.00±2.94[c]	22.00±6.48[b,c]	0.0059	0.0011	NS
Food consumption (g/day)	13.46±1.06[c]	16.11±0.13[a]	14.35±0.21[b]	15.75±0.01[a]	0.0062	NS	NS

[1)]The degree of significance resulting from the 2-way ANOVA is shown through the effects of growth hormone (G) administration, exercise (X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX human growth hormone administration combined with resistance exercise group.

Table 2
Percent body weight of heart, muscles and abdominal fat (%)

	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Heart	0.309±0.042[b]	0.395±0.071[a]	0.333±0.019[b]	0.324±0.026[b]	0.0362	NS	0.0057
Total soleus and plantaris R and L	0.290±0.025[b]	0.341±0.053[a]	0.308±0.017[a,b]	0.310±0.026[a,b]	0.0434	NS	0.0409
Abdominal fat	1.413±0.577	1.878±0.529	1.495±0.265	1.780±0.664	NS	NS	NS

[1)]The degree of significance resulting from the 2-way ANOVA is shown with the effects of growth hormone (G) administration, exercise(X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; Soleus R, soleus right; Soleus L, soleus left; Plantaris R, plantaris right; Plantaris L, plantaris left.

4). The exercise only group had increased IGF-I concentrations in the kidney and liver, but growth hormone appeared to counteract the effect of exercise on IGF-I in the kidney (Table 5).

3.4. Effect of growth hormone and/or exercise on carnitine concentrations

The NEC concentration of serum was highest in the GH administered group. Conversely, TCNE tended to be higher in the groups given with growth hormone, but only the GX group had significantly higher TCNE than controls, suggesting an interaction between exercise and GH, and in this case, some synergy. Muscle carnitine concentrations were higher in the G group than in any other groups (Table 6).

3.5. Effect of growth hormone and/or exercise on mRNA expression of CPT-I, ACS and ACC

The mRNA levels of hepatic ACS (Fig. 1) in the exercise groups were higher than in the C group. On the contrary, the mRNA levels of muscle ACS in the X group were lower than the control and the effects were counteracted by the combination. The mRNA levels of ACS from both liver and muscle tissues, in the GX group, were higher compared to the G group. The mRNA levels of hepatic ACC, in both the X and GX groups, were higher compared to the C group. However, there was no detectable change in muscle ACC mRNA levels among the groups (Fig. 2). The mRNA levels of liver CPT-I were mostly up-regulated in the exercised group than in all treatment groups. However, there were no clear differences in muscle composition between the control and the G and X groups. The CPT-I expression, in the GX group, was unexpectedly low (Fig. 3).

4. Discussion

The results of our study reflect chronic metabolic regulations rather than acute effects of growth hormone and exercise. This is due to the fact that all final measurements were made with approximately a 48 h lapse between terminating the animals and the most recent growth hormone administration (and/or exercise training). Most other studies of growth hormone administration have evaluated its effects on either young athletes or the frail

Table 3
Lipid profiles in serum and liver

	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Serum							
TG (mg/dL)	70.79±8.25[2)b3)]	98.30±5.98[a]	92.13±14.62[a]	63.59±14.75[b]	NS	NS	0.0077
TC (mg/dL)	87.97±12.67	133.95±12.13	110.35±19.83	123.87±12.85	NS	NS	NS
HDL-C (mg/dL)	71.66±13.58	73.64±8.70	66.45±12.20	71.02±7.24	NS	NS	NS
LDL-C (mg/dL)	36.00±17.95	39.27±6.77	36.78±14.98	38.23±9.01	NS	NS	NS
Atherogenic Index	0.80±0.20	0.99±0.32	0.70±0.25	0.76±0.12	NS	NS	NS
Liver							
TG (mg/g)	12.85±2.02	12.65±2.30	15.63±1.82	16.44±3.29	NS	NS	NS
TC (mg/g)	2.54±0.66[a]	1.58±0.49[b]	2.26±0.87[a,b]	1.65±0.41[b]	0.018	NS	NS
HDL-C (mg/g)	0.30±0.08[b]	0.30±0.04[b]	0.41±0.08[a]	0.35±0.07[a,b]	NS	0.0286	NS

[1)]The degree of significance resulting from the 2-way ANOVA is shown with the effects of growth hormone (G) administration, exercise(X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; TG, triglyceride; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; Atherogenic Index means the ratio of total cholesterol-HDL-C/HDL-C.

Table 4
Insulin, glucose and leptin concentrations in serum

	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Insulin (μIU/ml)	11.34±2.93[2)b3)]	16.16±3.32[a]	9.70±2.84[b]	15.51±2.67[a]	0.003	0.0364	NS
Leptin (ng/ml)	2.46±0.42[b]	3.50±0.69[a]	1.97±0.47[b]	3.25±1.04[a]	0.0001	0.0475	NS
Glucose (mg/dl)	115.29±12.42[a]	110.42±9.20[b]	101.79±5.09[b]	100.28±10.03[b]	NS	0.0035	NS

[1)]The degree of significance resulting from the 2-way ANOVA is shown with the effects of growth hormone (G) administration, exercise(X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with endurance resistance exercise group; GX, human growth hormone administration combined with endurance resistance exercise group.

and elderly, however, this study employed an animal model to evaluate how the effects of growth hormone and/or exercise on 1 year old rats, which corresponded to a stage of life just prior to the full effects of aging.

Aging is associated with metabolic changes, which are affected by both GH and exercise and result in alterations in body composition (Gillette-Guyonnet et al., 2003). This study revealed, however, some contradictory results. For examples GH administration can increase (Castillo et al., 2003) or decrease (Thompson et al., 1998) body weight. Interestingly, when elderly women were administered with GH, fat mass and lean body mass were not significantly affected, but GH combined with endurance training improved them (Lange et al., 2001). The results of our study demonstrated that GH administration correlated to higher muscle weight as a percent of body weight. This result may be explained by the fact that both GH and exercise together stimulate protein synthesis (Sonksen, 2001) resulting in an increase in muscle weight. Endurance exercise alone could improve body composition (Miyatake et al., 2002). However, compared to the controls, the results of this study verify that endurance resistance exercise did not affect muscle weight or the percent abdominal fat of body weight, but in contrast, reduced the effect of GH on muscle weight.

Effects of GH administration and/or endurance resistance exercise on lipid profiles are uncertain and conflicting results have been reported (Viidas et al., 2003). Growth hormone deficient patients exhibit lower HDL-cholesterol and higher TC (total cholestrol), TG and LDL-cholesterol than healthy subjects (Colao et al., 2001). However, when the growth hormone deficiency was reversed with GH for 12 months, it did not alter lipid profiles (Colao et al., 2002). We previously confirmed that 3 months of swimming exercise by middle-aged women decreased serum LDL-cholesterol and total lipid (Cha et al., 1995) and also resulted in a decrease in total triacylglycerol, LDL-cholesterol and VLDL-cholesterol concentrations. Glycogen is a primary intramuscular energy source in skeletal muscle, but skeletal muscles also utilize fatty acids from triglyceride during prolonged exercise. Similar to glycogen supercompensation, skeletal muscle from trained human subjects exhibit 2–2.5 times higher lipid content than that from untrained subjects (IKeda et al., 2002). We verified that exercise increases hepatic HDL-cholesterol and TG concentrations in serum. We previously assumed that exercise facilitated increased liver TG for the purpose of supercompensation to provide a labile source of energy from fatty acid.

Insulin is essential for the anabolic action of GH (Sonksen, 2001). Daily injections of GH to growth hormone deficient adults increased serum insulin (Spina et al., 2004) and glucose levels (Johansson et al., 2003). Body weight (kg) and body fat (%) all increase linearly with age (Narro et al., 2003). Some studies suggest that there is a negative relationship between

Table 5
IGF-I concentrations in serum and various tissue

	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Serum (ng/ml)	590.86±92.83[2)ab]	495.52±87.8[b3)]	535.10±85.37[b]	653.33±105.74[a]	NS	NS	0.0078
Brain (ng/g)	4.24±0.36	5.79±0.01	4.04±0.76	4.92±0.41	0.0097	NS	NS
Kidney (ng/g)	18.45±7.53[b]	15.23±2.85[b]	46.37±2.93[a]	23.17±4.66[b]	0.0083	0.0056	0.0192
Liver (ng/g)	15.57±0.37[b]	13.05±2.72[b]	28.58±6.29[a]	25.80±7.94[a]	NS	0.0004	NS
Muscle (ng/g)	ND	0.005±0.00	ND	0.002±0.00	–	–	–
Spinal cord (ng/g)	2.75±0.84	2.31±0.90	2.26±1.60	2.30±0.69	NS	NS	NS

[1)]The degree of significance resulting from the 2-way ANOVA is shown with the effects of growth hormone (G) administration, exercise(X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; ND, no-detected.

Table 6
Carnitine concentrations in serum, liver and skeletal muscle

Variables	Non-exercise		Exercise		Statistical significance[1)]		
	C	G	X	GX	G	X	G×X
Serum (nmol/ml)							
NEC	28.32±6.11[2)b3)]	37.67±11.41[a]	27.97±3.85[b]	41.07±7.01[a]	0.0001	NS	NS
ASAC	21.23±5.10[a,b]	19.76±6.93[b]	27.03±3.62[a]	23.71±4.68[a,b]	0.0141	0.0162	NS
AIAC	0.33±0.39	1.14±1.35	0.61±0.74	0.27±0.25	NS	NS	0.0472
TCNE	50.33±5.91[b]	59.77±10.63[a,b]	55.61±7.15[a,b]	64.76±10.69[a]	0.0001	0.0001	0.0012
Acyl/Free	0.78±0.33[a,b]	0.65±0.32[b]	0.99±0.11[a]	0.52±0.26[b]	0.0033	NS	NS
Liver (nmol/mg NCP)							
NEC	3.82±2.08[2)]	2.92±0.77	3.46±0.55	3.47±0.74	NS	NS	NS
ASAC	0.67±1.32	0.16±0.11	0.15±0.23	0.41±0.29	NS	NS	NS
AIAC	0.48±1.04	0.15±0.16	0.03±0.06	0.24±0.25	NS	NS	NS
TCNE	4.98±1.67[a3)]	3.57±0.44[b]	3.65±0.53[b]	4.13±0.72[a,b]	NS	NS	0.0362
Acyl/Free	0.08±0.07	0.30±0.38	0.06±0.08	0.20±0.15	NS	NS	NS
Skeletal muscle (nmol/mg NCP)							
NEC	11.78±3.16	16.83±7.42	11.25±2.47	13.50±2.13	NS	NS	NS
ASAC	0.39±0.46[b]	1.98±0.70[a]	0.75±0.75[b]	0.96±0.63[b]	0.0142	NS	0.0185
AIAC	0.24±0.65	0.86±1.93	0.45±1.09	0.00±0.00	NS	NS	NS
TCNE	12.42±3.64	14.15±2.03	12.45±2.18	15.39±4.22	NS	NS	NS
Acyl/Free	0.05±0.07[b]	0.39±0.27[a]	0.12±0.14[b]	0.06±0.04[b]	0.044	0.0492	0.0014

[1)]The degree of significance resulting from the 2-way ANOVA is shown with the effects of growth hormone (G) administration, exercise (X), and the interaction of growth hormone administration and exercise (G×X) being expressed as the numerical value or as not significant (NS) when $P>0.05$.
[2)]All values are means±SD.
[3)]Values with different superscripts in the same row are significantly different ($P<0.05$). C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; NEC, non esterified carnitine; ASAC, acid-soluble acylcarnitine; AIAC, acid-insoluble acyl carnitine; TCNE, total carnitine; Acyl/Free ratio, (ASAC+AIAC)/NEC; NCP, non-collagen protein.

circulating growth hormone and leptin (Hoybye et al., 2003). Our results verified that circulating insulin and leptin levels were increased by GH injection without affecting serum glucose concentrations. However, exercise significantly decreased insulin and leptin concentrations. Circulating glucose concentration was only lower in X group compared to the control group. The increase in insulin suggests that an insulin resistant state was induced by GH as previously seen by other investigators when using exogenous GH administration to animals with normal GH status (Dominici et al., 2005). If there was, indeed, an insulin resistant state induced by GH, it may have exacerbated by exercise increasing the production of endogenous GH. Interestingly, aging effects on IGF-I were different between mouse and human. According to microarray analysis of IGF related genes, transcriptional regulation exhibited different patterns between mouse(IGF-I) and human (IGF-IA) (Kobayashi et al., 2004). Unlike in human, mouse IGF-I was transcriptionally up-regulated with increasing age.

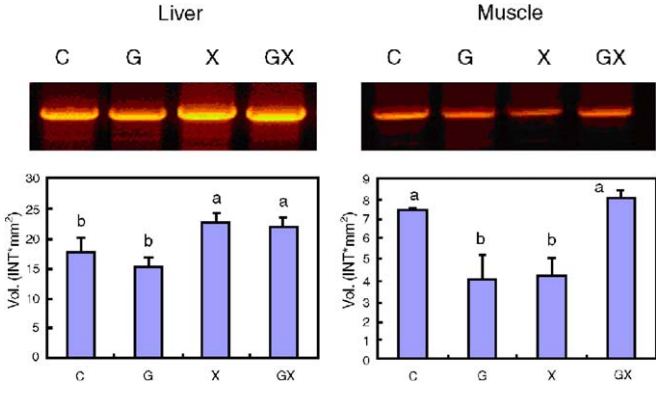

Fig. 1. ACS mRNA levels. C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with endurance exercise group; INT, intensity. The error bars show the standard deviations of the means. Values with different letters are significantly different ($P<0.05$).

As a whole, serum insulin concentrations could be increased to maintain IGF-I homeostasis because insulin is a positive regulator of leptin production (Saladin et al., 1995). In this study, both growth hormone administration and growth hormone combined with exercise failed to significantly affect body and abdominal fat pad weight gain. Therefore, increased circulating leptin levels in the GH treated animals may have been due to the increased serum insulin resulting from increased food intake.

Carnitine biosynthesis is affected by diet, age, exercise and hormonal status of the animal (Cha et al., 1999). Our results demonstrate that GH administration resulted in higher serum NEC and TCNE; whereas exercise had only a slight effect on total carnitine levels, and increased ASAC at the expense of NEC. It has been suggested that the ratio of acyl/free carnitine may provide a useful marker for changes in carnitine metabolism (Bohles et al., 1994). This ratio can be used as a screening system to detect alterations in mitochondrial metabolism (Bohles et al., 1994). Thus, we suggest that resistance exercise facilitated an increase in mitochondrial carnitine metabolism and, thereby, increased carnitine transport through the blood to other organs which are entirely dependent on carnitine uptake from the blood (Costell and Grisolia, 1993).

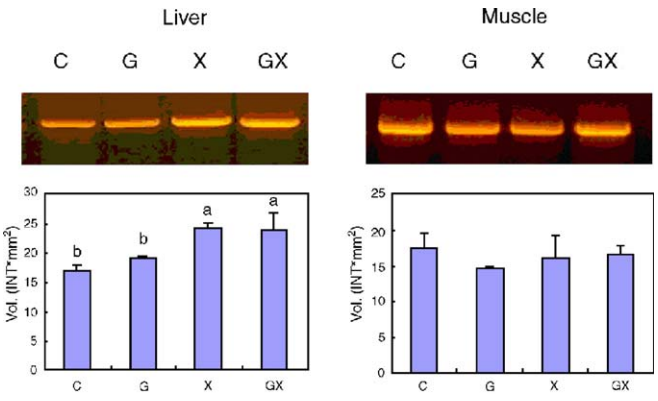

Fig. 2. ACC mRNA levels. C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; INT, intensity. The error bars show the standard deviations of the means. Values with different letters are significantly different ($P<0.05$).

GH administration causes a noticeable increase in carnitine concentrations in tissues. Our results demonstrate that resistance exercise does not affect GH action on carnitine. Furthermore, administration of GH to aged rats enhanced carnitine concentration in tissues possibly by incorporating acyl-carnitine into the formulation.

The results from this study on mRNA expression of CPT-I, ACS and ACC confirmed that there were no differences in hepatic ACS mRNA levels with GH administration compared with the C group. In exercised animals with and without GH, however, ACS expression increased. The ACS products are utilized in both anabolic and catabolic processes.

ACC catalyses the biotin dependent conversion of acetyl-CoA to malonyl-CoA (Beswick and Kennelly, 1998) and transcriptional regulation of ACC activity could be affected by diet and hormones (Mao et al., 2003). The ACC product, malonyl-CoA, is a major regulator of CPT-I activity. The CPT-I mRNA expression and activity decreases with age (Odiet et al., 1995). The regulation of CPT-I gene expression appears to have a high flux control over-oxidation which accounts for changes in CPT-I activity under a wide range of physiological conditions (Eaton, 2002). In a previous study, GH treatment was responsible for reducing the total activity of ACC in adipose tissue. Interestingly, ACC activity and mRNA levels are lower in old rats than in young rats (Nogalska et al., 2003). We determined that hepatic ACC mRNA expression was higher in the X and GX groups compared with the C group and effects of any group on ACC mRNA levels in muscle. Our results reveal that GH administration does not influence hepatic ACC mRNA expression, whereas exercise increases hepatic ACC mRNA levels, which suggests that ACC mRNA expression may be influenced more by endurance resistance exercise than by GH administration. Normally, when ACC activity is increased CPT-I is decreased (Chien et al., 2000) since the product of ACC inhibits CPT-I. In this study the mRNA expression of both CPT-I and ACC was higher in exercised animals and CPT-I expression was lower in the GX animals with intermediate values for both the G and X groups. This process could be explained as a counter reaction, against the GH effect after exercise, which reduces the capacity of fatty acid oxidation.

GH and exercise both have the potential to improve body composition and to slow down age-related sarcopenia. Most studies elsewhere have investigated the effects of these two effects independently and in either young athletes or frail elderly people. This study evaluated the independent and combined effects exercise training and GH administration on body composition and assessed their effects on energy metabolism in female rats undergoing the early stages of aging. In the present protocol, acute effects of GH and exercise were avoided to permit evaluation of chronic effects by accessing metabolic indices 2 days after terminating exercise training and GH injection, thereby evaluating permanent rather than transient effects. GH resulted in significant increases in body weight gain during the study period which was apparently due to greater increases in muscle mass. However, there was no noticeable difference in muscle mass between the exercise only group and the exercise group which was also administered with GH. This implausible result suggests that IGF-I could be the key mediator that counteracts the anabolic effects of exercise. Furthermore, IGF-I concentrations were concurrently increased in both groups administered with GH. GH may be inducing insulin resistance in our experiment. That seems likely since both insulin and leptin were also increased in both groups administered with GH (Table 4). If an insulin resistant state was induced, the normal anabolic effects of insulin would be compromised which could, in turn, counteract the anabolic effects of exercise on muscle growth. This is supported by evidence presented by Dominici et al. (Dominici et al., 2005) of impaired insulin receptor function in rats given excess growth hormone.

In contrast, animals supplemented with GH had modestly higher carnitine concentrations in serum and heart and skeletal muscles. The group subjected to combined treatments, however, exhibited the lowest concentration of muscle CPT-I. The increased total mass of muscle, between the growth hormone only group and GH with exercise group, was not obvious. This

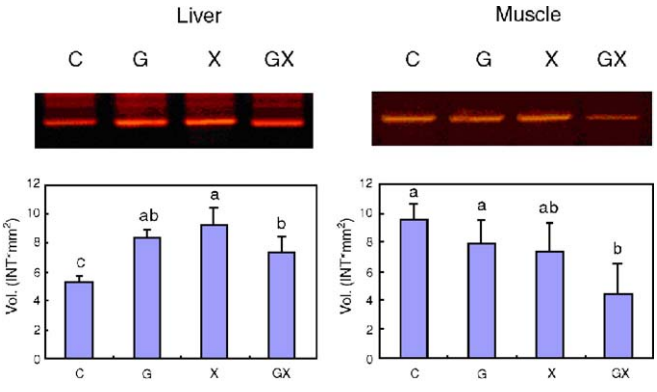

Fig. 3. CPT-I mRNA levels. C, saline administration group; G, human growth hormone administration group; X, saline administration combined with resistance exercise group; GX, human growth hormone administration combined with resistance exercise group; INT, intensity. The error bars show the standard deviations of the means. Values with different letters are significantly different ($P<0.05$).

data suggests that conventional wisdom on exercise is not consistent with rat hormonal dynamics shown here, because calories burned during exercise is a relatively minor benefit in reducing fat masses in our animal model system. However, the major benefits of exercise are not only metabolic but also hormonal in nature.

In conclusion, exercise combined with GH treatment did not yield positive synergic effects. These results are strikingly similar to results in humans by Yarasheski et al. (Yarasheski et al., 1995) in which resistance exercise combined with GH had no additive benefit on muscle mass or strength. Therefore, this study suggests that there is little, if any, benefit in combining GH administration and exercise, and that GH administration may induce insulin resistance.

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