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Insulin Sensitivity, Hormonal Levels and Skeletal Muscle Protein Metabolism in Tumour-bearing Exercising Rats*

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We have previously shown that spontaneous physical exercise can delay onset of experimental anorexia and cachexia, and retard tumour growth; we now report the effects on insulin sensitivity, hormonal levels and skeletal muscle protein metabolism. Insulin sensitivity determined with a euglycaemic hyperinsulinaemic clamp revealed a normalised glucose disposal rate in tumour-bearing exercising (TBE) versus sedentary (TBS) animals (TBE 15.55 ± 2.71 versus TBS 2.47 ± 2.12 mg/kg/min; $P < 0.05$). Both TBE and TBS animals had decreased levels of corticosterone during the clamp. Serum levels of insulin during tumour progression were unaffected by exercise, but the insulin : glucagon ratio increased and the progressive decrease in rT_3 was attenuated. The concentration of glucagon decreased in both tumour-bearing groups during the experiment, while TBE animals showed a relative reduction in corticosterone. Capacity for skeletal muscle protein synthesis, expressed as RNA : protein ratio, was normalised in TBE animals in two tumour protocols (TBE 5.9 ± 0.6 versus TBS 4.7 ± 0.3; TBE 2.9 ± 0.4 versus TBS 1.8 ± 0.2; $P < 0.05$, respectively). Incorporation rate of ^{14}C-phenylalanine into skeletal muscle protein was increased in the TBE group *in vitro* and *in vivo*. In the postexercise period, protein degradation evaluated by tyrosine release *in vitro* was increased, but decreased over time. This study has confirmed a positive skeletal muscle protein balance in exercising tumour-bearing animals, partly explained by the increased insulin sensitivity. This conclusion was further supported by the less catabolic pattern indicated by hormonal levels.

Key words: cancer, exercise, insulin resistance, insulin, glucagon, thyroid hormones, corticosterone, proteins
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INTRODUCTION

WASTING OF skeletal muscle tissue is a predominant feature of progressive malignant disease [1, 2]. This is the combined effects of inanition, altered protein metabolism and inactivity [1]. As to protein metabolism in skeletal muscle, there is evidence indicating a decreased synthesis rate [3, 4], and the degradation rate has been shown to be either unaffected [5] or increased [6]. This altered metabolic state contrasts with reports on increased whole body protein turnover, previously described in viscera and the immune system [1, 7, 8].

As physical exercise is a powerful stimulus for an anabolic state in skeletal muscle, successful attempts to counteract the catabolic state in cancer have been made with forced physical exercise in an experimental model [9]. In an experimental model with spontaneous physical exercise, we have recently reported a better preserved body composition in the tumour-bearing exercising host and an increased capacity for protein synthesis in skeletal muscle [10]. We have also found a decrease in tumour weight, despite an early increase in food intake in the tumour-bearing exercising animals. These tumour-bearing animals in

the early tumour growth phase even produced an increased amount of spontaneous physical exercise compared with the non-tumour bearers.

The aim of the present study was to evaluate the effects of voluntary exercise on the alterations of four hormones regulating substrate flow and skeletal muscle metabolism, and on the peripheral insulin resistance usually seen in cancer cachexia. The effects of exercise on skeletal muscle protein metabolism, under the distant influence of a tumour, were also studied.

MATERIALS AND METHODS

Animal and tumour model

A total of 222 female Wistar Furth rats, with initial body weights of 125–150 g, were allocated to study groups and corresponding control groups, matched for body weights: tumour-bearing exercising animals (TBE); tumour-bearing sedentary animals (TBS); non-tumour-bearing exercising animals (CE); and non-tumour-bearing sedentary animals (CS). The animals in each tumour group were subcutaneously implanted with either a Leydig cell sarcoma (LTW) or with a nitrosoguanidine-induced adenocarcinoma (NGW). The tumours do not metastasise and are well defined [11, 12], inducing similar types of anorexia and cachexia, although the time course with NGW is faster [10, 13].

After adaptation to the cages, implantation with 1.5 mm^3 of viable tumour tissue in each flank, or a sham operation with the same volume of 0.9% NaCl was performed under ether anaesthesia. The animals allocated to exercise were individually housed in cages open to the interior of a freely moving, wire-bottomed and non-motorised running wheel with a diameter of 33 cm (UNO Roestvaststaal BV, Arnhem, Holland). These animals had free access to the running wheels throughout the experiment. The other half of the animals were individually housed in standard cages. All animals had free access to a balanced diet (EWOS—ALAB Brood Stock Feed for Rats and Mice, ALAB, Sollentuna, Sweden) and tap water. Room temperature was maintained at 22 ± 1°C and the light : dark cycle was 12 : 12 h. Body weight, food intake and running distance were recorded every fourth day. At sacrifice, after blood samples and biopsies were taken, the tumours were dissected free and weighed, and the animals were killed by bleeding.

The experimental model was approved by the Animal Ethical Committee of the University of Göteborg, Sweden.

Insulin sensitivity

Protocol. The protocol with the NGW tumour was used and 22 animals were divided into the four groups of TBE, TBS, CE and CS.

Euglycaemic hyperinsulinaemic clamp. Insulin sensitivity was evaluated as previously described [14]. On day 15 after implantation or sham operation, rats, anaesthetised with sodium pentobarbital (ACO, Solna, Sweden) intraperitoneally (50 mg/kg), were provided with one catheter in the left carotid artery for blood sampling and one in the right jugular vein for infusion of glucose and insulin. After a 30 min stabilisation period, 5 mU/kg/min of neutral human insulin (Human Actrapid, 40 U/ml; Novo, Copenhagen, Denmark) was continuously infused. A 10% glucose solution in physiological saline was then infused, guided by repeated measurements of blood glucose in order to maintain euglycaemia. Insulin sensitivity was expressed as glucose disposal rate (GDR), i.e. mg glucose/kg body weight/min.

At t = 0 and 60 min of insulin infusion, blood samples were taken for analysis of insulin with a double antibody radioimmunoassay (Pharmacia Insulin RIA, Pharmacia Diagnostics, Uppsala, Sweden). Immediately after the stabilisation period (at t = 0, i.e. prior to insulin infusion), a blood sample was taken for analysis of corticosterone (RSL Corticosterone ^{125}I Kit for Rats and Mice, ICN Biomedicals Inc., Costa Mesa, California, U.S.A.).

Peripheral hormonal concentrations

Protocol. Peripheral hormonal levels were only measured in animals with the NGW tumour as there is the risk of paracrine secretion of hormones from the tumour derived from Leydig cells [15]. Blood samples for hormonal analyses were taken, without previous fasting, on days 10 and 20 from a total of 80 animals, i.e. from 20 in each group (TBE, TBS, CE, CS). The animals were anaesthetised with ether on day 10 and sodium pentobarbital intraperitoneally (i.p.) on day 20. All blood samples were drawn between 10 and 12 am.

Hormonal analysis in serum or plasma. The hormones were analysed with radioimmunoassay kits: Pharmacia Insulin RIA (Pharmacia Diagnostics, Uppsala, Sweden), ^{125}I Glucagon RIA Kit (Cambridge Diagnostics, Billerica, Massachusetts, U.S.A.), RSL Corticosterone ^{125}I Kit for Rats and Mice (ICN Biomedicals Inc., Costa Mesa, California, U.S.A.), Biodata Reverse T_3 Kit (Biodata S.p.A., Rome, Italy).

Protein metabolism in skeletal muscle

Protocols. The LTW tumour was chosen for the studies on skeletal muscle protein metabolism, in order to prolong the training regimen. For determination of RNA and protein contents, biopsies were also taken in an experiment using the NGW protocol. A total of 100 animals were studied with 25 in each group (TBE, TBS, CE, CS).

On day 28 (or day 20 in the NGW protocol) after implantation or sham operation, without previous fasting, all animals were anaesthetised with sodium pentobarbital i.p. as described above.

RNA and protein contents in skeletal muscle. A cross-section biopsy was taken from the quadriceps femoris muscle on one side and immediately frozen in liquid N_2 for analysis of protein [16] and RNA [17, 18].

Skeletal muscle protein synthesis in vitro. At 3 h postexercise, each extensor digitorum longus muscle (EDL) was rapidly dissected free without touching and incubated at 37°C for 3 h in saturated (O_2 : CO_2 95 : 5) Basal Medium Eagle (FLOW Laboratories, Glasgow, U.K.) with HEPES 20 mmol/l (FLOW Laboratories) and L-[^{14}C(U)]-phenylalanine (NEC-284E; Du Pont de Nemours, Dreiech, Germany) at a concentration of 98.17 nmol/ml [5, 19]. Incubation was terminated on ice by a three-fold washing with trichloroacetic acid (TCA). Lipids were extracted sequentially with chloroform-methanol (1 : 1 vol/vol),

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followed by ethanol/acteone (1 : 1 vol/vol), and finally in pure ether. The muscles were dried, weighed and then completely dissolved in Soluene 100 (Packard Instrument Co., La Grange, IL, U.S.A.). The radioactivity was counted in Instagel (Canberra Packard, Meriden, Connecticut, U.S.A.). Incorporation rate was given as nmol phenylalanine/mg acid-precipitable protein/h.

Skeletal muscle protein synthesis in vivo. In order to study the integrated skeletal muscle protein synthesis over time, i.e. the last days of the experiment, the animals were anaesthetised with ether, and Alzet micro-osmotic pumps model 1003D (Alza Corporation, Palo Alto, California, U.S.A.) were implanted subcutaneously by midscapular incisions. Each pump was filled with 20 pCi of L-[U-^{14}C]-phenylalanine (CFB 70; Amersham, Buckinghamshire, U.K.) in 0.9% NaCl and handled according to the manufacturer. At sacrifice, 36 h after pump implantation, cross-section biopsies were taken from each quadriceps femoris muscle and frozen in liquid N_2 for counting of radioactivity [5] and determination of protein content [16]. The pumps were weighed to verify release of the amino acid solution. Incorporation was expressed as dpm/mg skeletal muscle protein.

Skeletal muscle protein degradation in vitro. A tyrosine release system was employed as described elsewhere [20]. To evaluate the immediate postexercise effects on protein degradation, muscles were taken at 3 and 6 h postexercise, respectively, in two separate experiments. The EDL muscles from each animal were rapidly dissected free without touching, weighed and placed in separate vessels containing saturated (O_2 : CO_2 95 : 5) Krebs–Ringer bicarbonate buffer supplemented with glucose. Cycloheximide (130 μg/ml) was added to inhibit recycling of tyrosine. The EDL muscles were incubated simultaneously for 2 h; one at 4°C and the other at 37°C. After incubation, the muscles were homogenised with an Ultra-Turrax and the proteins were precipitated with TCA. The tyrosine concentration in the supernatant was determined fluorometrically [21], and the degradation rate was expressed as nmol tyrosine/g skeletal muscle wet weight/h.

Table 1. Tumour weight relative to body weight minus the tumour weight ("carcass weight") in LTW (day 28) and NGW (day 20) protocols. Animals from clamp experiments not included (i.e. day 15 in NGW protocol)

	TBE		TBS
LTW-protocol (n = 25/group)	13.4 ± 1.0	$P < 0.05$	16.4 ± 0.9
NGW-protocol (n = 25/group)	11.8 ± 1.7		13.6 ± 2.1

TBE, tumour-bearing exercising animals.
TBS, tumour-bearing sedentary animals.

Statistics

All values are presented as mean ± S.E.M. Statistical significance was evaluated with ANOVA and Student's t-test. A P-value less than 0.05 was regarded as significant. Correlation coefficients were evaluated according to Pearson.

RESULTS

Body weight, food intake, physical activity and tumour weight

There were no significant differences between the TBE and the TBS animals in total body weight throughout the experiment. Both groups of exercising animals (TBE and CE) had increased food intake compared with their sedentary controls (TBS and CS) from the fourth day after tumour implantation or sham operation. The TBE animals had increased food intake compared with the TBS animals throughout the experiments. As to the daily distance run, there were differences between the two exercising groups, with and without tumour, from day 20 onwards in the LTW protocol, and from day 16 in the NGW protocol. The TBE animals showed a gradual decrease in running, but preserved an ability for physical exercise despite a growing tumour impact. Lower means of relative tumour weights, i.e. tumour/"carcass", were recorded in the exercising animals both in the LTW and in the NGW protocol (Table 1).

EDL muscle weight

The EDL wet weight relative to body weight was significantly increased in the TBE group (44 ± 2) compared with the TBS group (40 ± 1) in the LTW protocol (n = 10/group; $P <$ 0.05).

Euglycaemic hyperinsulinaemic clamp

Insulin sensitivity, expressed as glucose disposal rate (GDR), was significantly reduced in the TBS group compared with the other three groups, respectively (Table 2). The TBE group showed a normalised insulin sensitivity. Insulin concentration

Table 2. Insulin sensitivity in NGW protocol determined by hyperinsulinaemic euglycaemic clamp and expressed as glucose disposal rate (mg/kg/min). Concentrations of insulin (mU/l) and corticosterone (ng/ml) in serum during clamp, and data on food intake (g/100 g body weight/day), body weight difference (%/day) and running activity (m/day) for the last 24 h prior to clamp are given

	TBE (n = 5)	TBS (n = 6)	CE (n = 6)	CS (n = 5)
Glucose disposal rate (mg/kg/min)	15.55 ± 2.71†	2.47 ± 2.12‡‖	16.24 ± 2.58	11.76 ± 1.16
Insulin at t = 0 min (mU/l)	20.8 ± 2.9†¶	35.8 ± 4.1	30.2 ± 3.2	30.8 ± 1.5
Insulin at t = 60 min (mU/l)	109.0 ± 15.4*	115.0 ± 30.9	70.7 ± 2.9	75.0 ± 3.1
Corticosterone at t = 0 min (ng/ml)	453 ± 97*	413 ± 78‡‖	798 ± 40	733 ± 102
Food intake last 24 h (g/100 g body weight/day)	5.98 ± 0.39*†¶	2.68 ± 0.73‡‖	9.74 ± 0.31§	7.51 ± 0.15
Body weight difference last 24 h (%/day)	1.32 ± 0.21*†	−1.09 ± 0.85	0.47 ± 0.19	0.71 ± 0.09
Running distance last 24 h (m/day)	12 493 ± 1787*		22 086 ± 1 619	

Mean ± S.E.M.
TBE, tumour-bearing exercising animals; TBS, tumour-bearing sedentary animals; CE, non-tumour-bearing exercising animals; CS, non-tumour-bearing sedentary animals.
*TBE versus CE, †TBE versus TBS, ‡TBS versus CS, $P < 0.05$; §CE versus CS; ‖TBS versus CE; ¶TBE versus CS.

at $t = 0$ min was significantly lower in the TBE group than in the two sedentary groups, respectively ($P < 0.05$). At $t = 60$ min, both tumour-bearing groups showed higher insulin values than the non-tumour-bearers, but a significant difference was reached only between the two exercising groups ($P < 0.05$). Corticosterone concentrations during clamp were reduced in both groups of tumour-bearing animals compared with their respective non-tumour-bearing controls ($P < 0.05$).

Insulin

As shown in Table 3, there were no significant differences in serum insulin between the four groups on day 10. All groups showed an increase throughout the experiment, which was significant in the non-tumour-bearing groups, and on day 20, both tumour-bearing groups had significantly lower levels than the sedentary non-tumour-bearers ($P < 0.001$ respectively). There were negative correlations between insulin concentration in the TBE group and absolute ($r = -0.67$), $P < 0.05$) and relative tumour weights ($r = -0.69, P < 0.05$).

The insulin : glucagon ratios increased from days 10 to 20 in both tumour-bearing groups. In both non-tumour-bearing groups, there were significant positive correlations between the insulin : glucagon ratio and body weight both on day 10 (CE $r = 0.77, P < 0.01$; CS $r = 0.87, P < 0.01$) and on day 20 (CE $r = 0.77$, $P < 0.01$; CS $r = 0.64$, $P < 0.05$). These correlations were not found in the tumour-bearing animals.

Glucagon

The plasma concentrations of glucagon are shown in Table 3. No significant differences were found between the two tumour-bearing groups or between the two non-tumour-bearing groups. Both groups of tumour-bearing animals showed a decrease in glucagon levels from days 10 to 20 with significant differences compared with either control group (CE or CS) on day 20 ($P < 0.001$). In both non-tumour-bearing groups, there were negative correlations between glucagon concentration and body weight on day 10 (CE $r = -0.66$, $P < 0.05$; CS $r = -0.70$, $P < 0.05$) and on day 20 (CE and CS $r = -0.73, P < 0.05$).

rT_3

The concentrations of rT_3 in the TBE, CE and CS groups remained essentially unchanged throughout the experiment, while there was a significant decrease in the TBS group (Table 4). On day 20, both the TBE and the TBS group had significantly lower concentrations of rT_3 than the CS group ($P < 0.05$ and $P < 0.01$, respectively). In all groups there were significant inverse correlations between the hormonal level and total body weight on both days 10 and 20 (CE and CS: $P < 0.01$ respectively on both days; TBE and TBS $P < 0.01$ respectively on day 10 and $P < 0.05$, respectively, on day 20).

Table 3. Concentrations of insulin in serum (mU/l) and glucagon in plasma (ng/l) in NGW protocol

	TBE	TBS	CE	CS
Insulin (mU/l)				
Day 10 (n = 10/grp)	9.7 ± 0.7†	10.2 ± 0.8	10.6 ± 0.6	11.0 ± 0.4
Day 20 (n = 10/grp)	11.9 ± 0.9*§	12.0 ± 0.7‡	14.6 ± 1.0	17.0 ± 0.9
Glucagon (ng/l)				
Day 10 (n = 10/grp)	696 ± 128	694 ± 107¶	614 ± 111	863 ± 55
Day 20 (n = 10/grp)	486 ± 35‖††	460 ± 28¶**	920 ± 56	904 ± 23

Mean ± S.E.M.
*TBE versus CE, †TBE versus CS, $P < 0.05$; ‡TBS versus CS, §TBE versus CS, $P < 0.01$; ‖TBE versus CE, ¶TBS versus CS, **TBS versus CE, ††TBE versus CS, $P < 0.001$.

Table 4. Concentrations of rT_3 (pg/ml) and corticosterone (ng/ml) in serum in NGW protocol

	TBE	TBS	CE	CS
rT_3 (pg/ml)				
Day 10 (n = 10/grp)	57 ± 6	67 ± 4	70 ± 6	70 ± 5
Day 20 (n = 10/grp)	50 ± 5‡	46 ± 5*†	64 ± 7	66 ± 5
Corticosterone ng/ml				
Day 10 (n = 10/grp)	319 ± 55	183 ± 42	203 ± 55	175 ± 42
Day 20 (n = 10/grp)	358 ± 36‖§	474 ± 46†	652 ± 51	560 ± 45

Mean ± S.E.M.
*TBS versus CS, †TBS versus CE, ‡TBE versus CS, $P < 0.05$; §TBE versus CS, $P < 0.01$; ‖TBE versus CE, $P < 0.001$.

Corticosterone

The concentrations of corticosterone are shown in Table 4. The TBE animals showed the highest value on day 10, and this remained essentially unchanged, while the other groups had a progressive increase, and became the relatively lowest value on day 20. On day 20, the levels in the tumour-bearing groups were significantly reduced compared with the CS group ($P < 0.001$, respectively).

RNA and protein contents in skeletal muscle

As seen in Table 5, the RNA : protein ratios were increased in the quadriceps femoris muscle in TBE compared with TBS animals in both tumour protocols ($P < 0.05$).

Skeletal muscle protein synthesis in vitro

Both groups of exercising animals had increased protein synthesis rate compared with their sedentary controls (Table 6).

Table 5. RNA : protein ratios in quadriceps femoris muscle in LTW and NGW protocols

	RNA : protein × 10^{-3}	Ratio to CS animals
LTW protocol (n = 5/grp)		
TBE	5.9 ± 0.6*	1.05
TBS	4.7 ± 0.3	0.84
CE	6.8 ± 0.5	1.21
CS	5.6 ± 0.6	1.00
NGW-protocol (n = 5/grp)		
TBE	2.9 ± 0.4*	1.21
TBS	1.8 ± 0.2†	0.75
CE	3.0 ± 0.4	1.25
CS	2.4 ± 0.5	1.00

Mean ± S.E.M.
*TBE versus TBS, †TBS versus CE, $P < 0.05$.

Table 6. Skeletal muscle protein synthesis rate in LTW protocol estimated by incorporation rate of ^{14}C-phenylalanine into extensor digitorum longus (EDL) in vitro (nmol phe/g protein/h) and into quadriceps femoris (dpm/mg protein) in vivo

	Incorporation rate of ^{14}C-phenylalanine into protein in EDL muscle *in vitro*	
n = 5/grp	nmol phenylalanine/ g protein/h	Ratio to CS animals
TBE	47.8 ± 4.6	0.97
TBS	41.5 ± 3.7	0.84
CE	53.3 ± 2.0	1.08
CS	49.2 ± 2.4	1.00
n.s.		
	Incorporation of ^{14}C-phenylalanine into protein in quadriceps femoris muscle during 36 h *in vivo*	
n = 5/grp	dpm/mg protein	Ratio to CS animals
TBE	27.2 ± 5.0*§	0.82
TBS	23.1 ± 2.1‡	0.70
CE	46.8 ± 2.2†	1.41
CS	33.1 ± 1.1	1.00

Mean ± S.E.M.
*TBE versus CE, †CE versus CS, ‡TBS versus CE, §TBE versus CS; $P < 0.05$.

However, significance was reached only when calculation was based on the values for each separate biopsy instead of averages of both muscles ($P < 0.05$, data not shown).

Skeletal muscle protein synthesis in vivo

Both groups of exercising animals had increased values as compared with their sedentary controls as seen in Table 6.

Skeletal muscle protein degradation in vitro

The two exercising groups had increased numerical values for degradation rate as compared with their respective sedentary controls at 3 h postexercise (Table 7). At 6 h postexercise, these numerical differences were reversed (Table 7).

DISCUSSION

We have previously reported a delayed onset of anorexia and cachexia in tumour-bearing animals after spontaneous physical exercise as compared with tumour-bearing sedentary animals [10, 13]. Important findings were a retarded tumour growth, despite an early increase in food intake, an early increase in spontaneous physical activity and an increased capacity for protein synthesis and oxygen consumption in skeletal muscle. The data on body weights, food intake and running activity in the present study were in accordance with our previous results [10, 13].

Table 7. Skeletal muscle protein degradation rate in LTW protocol estimated by release rate of tyrosine from EDL in vitro (nmol tyr/g wet weight/h)

n = 10/grp	nmol tyrosine/g wet weight/h	Ratio to CS animals
Release rate of tyrosine from EDL muscle at 3 h postexercise		
TBE	121.1 ± 14.1	1.02
TBS	98.7 ± 7.5	0.83
CE	126.2 ± 8.9	1.06
CS	119.2 ± 8.6	1.00
n.s.		
Release rate of tyrosine from EDL muscle at 6 h postexercise		
TBE	87.6 ± 4.5	0.98
TBS	97.5 ± 11.4	1.09
CE	75.3 ± 6.9	0.84
CS	89.7 ± 6.3	1.00
n.s.		

Mean ± S.E.M.

Our animal model, with an entirely voluntary situation in order to reduce the stress imposed on the animal, is different from most experimental models within this field [10]. The emphasis on minimised stress in this animal model limits the possibility of evaluating metabolic alterations in each animal repeatedly over a longer period of time. Animal behaviour is clearly affected by anaesthesia, interruption of exercise and removal of food (Daneryd, unpublished data).

The altered metabolism of insulin in the tumour-bearing host is characterised by low-to-normal levels in the fasted state, insufficient plasma concentration in the fed state and a pancreatic insensitivity to glucose stimulation [22]. In addition, a peripheral resistance to insulin has been proven [2, 23]. Exogenous insulin within or above physiological levels has been shown to temporarily reverse cancer anorexia and cachexia in rats [24], but neither direct nor indirect effects could be found in mice [22]. In the present study, both tumour-bearing groups showed decreased concentrations of insulin on day 20. No significant differences were found between the two tumour-bearing groups, despite an early increase in food intake in the exercising rats, or between the two non-tumour-bearing groups. This shows that the adaptive changes in insulin concentration cannot be overcome by physical exercise.

A normalised peripheral sensitivity to insulin was demonstrated in tumour-bearing exercising animals in the present study. The organ primarily responsible for the increased sensitivity to insulin seen in exercised animals is skeletal muscle, and not the liver [25, 26]. As insulin plays a fundamental role, in synergy with other factors, in the regulation of protein turnover in skeletal muscle [27], physical activity might improve, directly or indirectly, the metabolic alterations associated with insulin. This is of importance in progressive malignant disease, as skeletal muscle tissue is the main target organ for wasting. The lower concentrations of corticosterone in both tumour-bearing groups during the euglycaemic clamp indicate that insulin resistance is mainly caused by other factors.

An increased plasma concentration of glucagon, which parallels increased tumour burden, has been found in the tumour-bearing rat early in cachexia, and the catabolic effect(s) might be augmented by a decreased concentration of insulin [28]. Hypoglycaemia has been shown in tumour-bearing rats [28] and mice [29], and this has been regarded as the immediate cause of death in experimental cancer [29]. These reports contrast with the more common finding of hyperglycaemia, which parallels resistance to insulin in cancer patients [30]. In the present study, we found reduced concentrations of glucagon on day 10 in the tumour-bearing groups and the exercised non-tumour-bearing group. On day 20, this reduction was greater in the tumour-bearing groups, with significant differences, compared with both groups of non-tumour-bearers, respectively. The insulin : glucagon ratio was increased in both tumour-bearing groups during the experiment, but decreased in the non-tumour-bearing exercising group. This indicates that the training regimen caused different kinds of metabolic adaptations in the two groups of exercising animals.

Progressive tumour growth is associated with a decrease in the concentrations of circulating thyroid hormones, and exogenous supplementation of T_4 can only reduce the degree of anorexia and has no significant effect on cachexia [31]. Thus, the reduction can be an adaptation to malnutrition [31]. Induction of hypothyroidism inhibits local and metastatic tumour growth in rats [32], and thyroxine treatment has the opposite effects in mice [33]. Thyroid hormones are important for the regulation of protein turnover in skeletal muscle [34], and the principal effect is thought to be at the level of RNA turnover [27]. In the present study, the tumour-bearing sedentary animals showed decreased values of rT_3 during tumour progression, while the values remained unchanged in the other three groups. These differences between the tumour-bearing groups could be expected, as the low thyroid hormonal state found in tumour-bearing animals has been essentially explained by food restriction and malnutrition, while the tumour-bearing exercising animals in our study had an increased food intake and a delayed onset of cachexia. Thus, physical exercise could modify the adaptive state with a reduced thyroid function in the tumour-bearing host.

Increased production of adrenergic hormones and corticosteroids, as reflected by increased urinary excretion, has been shown in malignant disease both in rodents [35] and in man [36]. These hormonal alterations are part of the explanation for tissue wasting in malignant disease. Plasma levels of corticosteroids are elevated in response to stress and exercise, but the extent is dependent on the type of exercise. Humans secrete predominantly cortisol while rodents almost exclusively secrete corticosterone, which was measured in plasma in this study. The tumour-bearing exercising animals showed an essentially unchanged value during the experiment, but exhibited the highest values on day 10 and the lowest on day 20. This might reflect the increased running activity in the early part of the experiment [10]. The relative reduction of corticosterone during the experiment reflects adaptational changes in order to preserve body mass. Taken together with the decreased concentration of glucagon throughout the study, the changes indicate that this experimental setting is not a stressful event.

Protein metabolism in skeletal muscle in the tumour-bearing sedentary animal is characterised by a depressed synthesis [3–6] and unaltered [5] or increased degradation [4, 6], even if the time course of skeletal muscle wasting differs between animal tumour models. Spontaneous physical exercise can partly counteract these changes as evidenced by a better preserved body composition and an increased RNA : protein ratio in skeletal and cardiac muscle, indicating an increased capacity for protein synthesis [10]. In the present study, protein metabolism was evaluated in the quadriceps femoris muscle, composed of both red and white fibres, and in the extensor digitorum longus muscle (EDL), which is mainly white. The training regimen clearly affects EDL as evidenced by an increase in wet weight and by changes in concentrations of most purine nucleotides as well as in energy charge in predominantly white muscle [13].

In the present study, exercise increased the capacity for protein synthesis in quadriceps femoris muscle, as demonstrated by an increase in RNA : protein ratio. Protein synthesis in EDL muscle *in vitro* was slightly increased in both groups of exercising animals. Protein synthesis was also evaluated *in vivo* with continuous administration of ^{14}C-phenylalanine by osmotic pumps over a 36 h period. Exercise increased incorporation of amino acid into proteins in quadriceps femoris muscle in both groups, but significance was reached only in the non-tumour-bearing animals. This could be due to increased recycling of the amino acid and/or entrapment in the tumour.

The tumour-bearing exercising animals had an increased rate of protein degradation in EDL muscle *in vitro* at 3 h after exercise, compared with the sedentary tumour-bearers, and a rate comparable to that of the exercising non-tumour-bearers. 6 h after exercise, the differences were reversed, and there was a tendency towards a decrease in degradation rate in both groups of exercising animals, indicating an anabolic state.

These combined findings on skeletal muscle protein metabolism indicate that net synthesis of skeletal muscle protein is positive in exercising animals. Despite the postexercise effects on skeletal muscle protein metabolism [37, 38], we found differences between the groups, which strongly supports our hypothesis that physical exercise improves skeletal muscle protein metabolism in tumour-bearing animals.

In conclusion, this study confirms that skeletal muscle protein balance is positive in the tumour-bearing exercising rat. The tumour-induced adpative pattern in the anabolic hormones insulin and rT_3 is partially modified as exercise attenuates the progressive decrease in rT_3. An increase in insulin : glucagon ratio, a normalised peripheral insulin sensitivity and a relative decrease in corticosterone, which all parallels a decrease in physical activity, reflect adaptive mechanisms to preserve body mass in a state of competition between the metabolic demands of the tumour and those of the host.

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Disregulation of Urokinase Plasminogen Activator Gene in Breast Cancer

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Disregulation of urokinase plasminogen activator (uPA) was assessed in 134 breast cancer specimens. Overexpression of uPA was determined by immunohistochemistry using the specific monoclonal antibody, #394. Gene amplification was assessed by differential polymerase chain reaction, using primers designed to amplify a 111 bp segment of the uPA gene. Overexpression of uPA was detected in 33% of breast cancers, including 4 of 21 *in situ* carcinomas, 7 of 14 lobular and 2 of 10 tubular carcinomas. Overexpression of uPA did not correlate with the presence or absence of oestrogen receptors. uPA gene amplification was not detected in any cancer. We conclude that uPA gene amplification is not a major mechanism instigating uPA overexpression in breast cancer, and that overexpression is likely to be controlled by other mechanisms.

Key words: breast carcinoma, urokinase plasminogen activator, gene amplification, gene overexpression
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INTRODUCTION

OVEREXPRESSION OF the serine protease,urokinase plasminogen activator (uPA) in epithelial cells has been linked to aggressive behaviour in breast cancer [1, 2]. The biochemical action of the enzyme may promote metastasis by degradation of proteins, such as collagen, in the extracellular tumour stroma [3]. The conversion of plasminogen to plasmin by uPA is thought to be a critical step in this degradation [4]. Previous studies of breast cancers with immunohistochemistry using a monoclonal antibody directed against human uPA have shown overexpression