implications for treatment with 5-fluorouracil. *J Clin Oncol* 1994, **12**, 2035–2042.
29. Marquet R, Westbroek DL, Jeekel J. Interferon treatment of a transplantable rat colon carcinoma. *Int J Cancer* 1984, **38**, 689–692.
30. Marinelli A, Pons DHA, Kuppen PJK, Vreeken JAC, Tjaden UR, van de Velde CJH. Isolated liver perfusion (ILP) vs hepatic artery infusion (HAI) with 5-Fluorouracil (5FU) and mitomycin C (MMC) in a rat model. *Proc Am Ass Cancer Res* 1990, **31**, 429.
31. Peters GJ, Laurensse E, Leyva A, Pinedo HM. Tissue homogenization using a microdismembrator for the measurement of enzyme activities. *Clin Chim Acta* 1986, **158**, 193–198.
32. Spears CP, Shahinian AH, Moran RC, Heidelberger C, Corbett TH. *In vivo* kinetics of thymidylate synthetase inhibition in FUra-sensitive and -resistant murine colon adenocarcinomas. *Cancer Res* 1982, **42**, 450–456.
33. van der Wilt CL, Pinedo HM, Smid K, Peters GJ. Elevation of thymidylate synthase following 5-fluorouracil treatment is prevented by the addition of leucovorin in murine colon tumours. *Cancer Res* 1992, **52**, 4922–4928.
34. Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* 1989, **24**, 148–154.
35. Berne MHO, Gustavsson BG, Almersjö O, Spears CP, Frösing R. Sequential methotrexate/5FU: FdUMP formation and TS inhibition in a transplantable rodent colon adenocarcinoma. *Cancer Chemother Pharmacol* 1986, **16**, 237–242.
36. Chu E, Koeller DM, Johnston PG, Zinn S, Allegra CJ. Regulation of thymidylate synthase in human colon cancer cells treated with 5-fluorouracil and interferon-γ. *Mol Pharmacol* 1993, **43**, 527–533.
37. Chu E, Koeller DM, Casey JL, Drake JC, Chabner BA, Elwood PC, *et al.* Autoregulation of human thymidylate synthase messenger RNA translation by thymidylate synthase. *Proc Natl Acad Sci USA* 1991, **88**, 8977–8981.
38. Weber G. Biochemical strategy of cancer cells and the design of chemotherapy: G.H.A. Clowes memorial lecture. *Cancer Res* 1983, **43**, 3466–3492.
39. Johnston PG, Drake JC, Steinberg SM, Allegra CJ. Quantitation of thymidylate synthase in human tumors using an ultrasensitive enzyme-linked immunoassay. *Biochem Pharmacol* 1993, **45**, 2483–2486.
40. Aherne GW, Hardcastle A, Newton R. Measurement of human thymidylate synthase (hTS) in cell lines using ELISA. *Ann Oncol* 1992, **3** (Suppl. 1), 77.
41. Horikoshi T, Danenberg KD, Stadlbauer THW, Volkenandt M, Shea LCC, Aigner K, *et al.* Quantitation of thymidylate synthase, dihydrofolate reductase, and DT-diaphorase gene expression in human tumors using the polymerase chain reaction. *Cancer Res* 1992, **52**, 108–116.
42. Aigner KR. Isolated liver perfusion: 5-year results. *Reg Cancer Treat* 1988, **1**, 11–20.
43. Marquet RL, Jeekel J. Combined effect of 5-fluorouracil and interferon on experimental liver metastases of rat colon carcinoma. *J Cancer Res Clin Oncol* 1985, **109**, 156–158.
44. Busch ORC, Slooter GD, Jeekel J, Marquet RL. Effect of levamisole and 5-fluorouracil in immune status and tumor growth in a rat model of colon carcinoma (CC531) in the rat. *Proc Am Ass Cancer Res* 1993, **34**, 457.
45. Houghton JA, Williams LG, Loftin SK, Cheshire PJ, Morton CL, Houghton PJ, Dayan A, Jolivet J. Factors that influence the therapeutic activity of 5-fluorouracil [6RS] leucovorin combinations in colon adenocarcinoma xenografts. *Cancer Chemother Pharmacol* 1992, **30**, 423–432.

**Acknowledgements**—This study was supported by the Dutch Cancer Society (Koningin Wilhelmina Fonds by grants IKA-VU 88-20 and IKW 88-07). These experiments were partly performed at the KDL (clinical animal laboratory) of the Free University.

Pergamon

*European Journal of Cancer* Vol. 31A, No. 5, pp. 760–765, 1995
Elsevier Science Ltd
Printed in Great Britain
0959–8049/95 $9.50+0.00

0959-8049(95)00086-0

# Coenzymes $Q_9$ and $Q_{10}$ in Skeletal and Cardiac Muscle in Tumour-bearing Exercising rats

**P. Daneryd, F. Åberg, G. Dallner, L. Ernster, T. Scherstén and B. Soussi**

**Physical exercise increases metabolic rate, and induces both adaptational biogenesis of mitochondria in skeletal muscle and an increase in antioxidant capacity. The onset of experimental anorexia and cachexia can be delayed by voluntary exercise. As skeletal muscle is the main target for cancer cachexia, we determined the levels of coenzymes $Q_9$ and $Q_{10}$ in skeletal muscle from tumour-bearing exercising rats, and compared them to those of sedentary tumour-bearers and controls. Both tumour-bearing groups had increased levels of coenzymes $Q_9$ and $Q_{10}$ in the anterior tibial muscle ($P < 0.05$ for exercised animals). In the soleus muscle, only the tumour-bearing exercising animals demonstrated an increase in the levels of both coenzymes ($P < 0.05$). In cardiac muscle, the presence of tumour and exercise reduced the levels of coenzymes below that of sedentary controls. Exercise counteracted the anaemia in the tumour-bearing host ($P < 0.05$). In conclusion, the increase in antioxidant capacity in skeletal muscle indicates a defence mechanism in the tumour-bearing hosts which is augmented by physical exercise.**

**Key words: exercise, cancer, ubiquinone, energy metabolism**
*Eur J Cancer*, Vol. 31A, No. 5, pp. 760–765, 1995

## INTRODUCTION

PHYSICAL TRAINING may increase the aerobic metabolic rate by up to 10-fold [1], and one of the most obvious adaptations is an increased biogenesis of mitochondria in skeletal muscles [2, 3]. Most experimental studies on physical training describe the effects of forced physical exercise, i.e. exhaustive exercise or endurance training, which reduces respiratory control and thus increases the production of oxygen free radicals [4]. The levels of antioxidant systems in blood increase in response to training load [5, 6], and exercise capacity is highly dependent on contents of antioxidants in skeletal muscle [7]. Oxygen free radicals in excess of the antioxidant capacity induce subcellular and cellular damage, and experimental settings with forced physical exercise might thus not be optimal for evaluation of the anabolic effects of physical exercise on muscle tissue in health and disease.

The mevalonate pathway, which is present in all tissues, synthesises the end-products cholesterol, dolichol and coenzyme Q (ubiquinone; 2,3-dimethoxy-5-methyl-6-decaprenyl benzoquinone), and also participates in the isoprenylation of proteins. The initial portion of the pathway is common, and thereby the biosynthesis of coenzyme Q is related to that of cholesterol and dolichol. A terminal regulation of the biosynthesis of these lipids has also been confirmed.

Coenzyme Q has two major functions in the cell: it is a redox lipid serving an obligatory component of the mitochondrial respiratory chain, and the concentration in the inner mitochondrial membrane is thought to be a restricting factor in electron transfer [8]. In reduced form, coenzyme Q is an antioxidant, which explains its presence in all cellular membranes.

The biosynthesis of coenzyme Q is influenced both by physiological and pathophysiological conditions. The content is increased in skeletal muscle of exercised rats to a greater extent than other components of the mitochondrial respiratory chain [9]. In preneoplastic liver nodules, the contents are increased, probably as a protection against oxygen free radicals [10]. In contrast, the contents are substantially decreased in developed hepatocellular cancer, thus indicating an insufficient antioxidant capacity [11].

Physical exercise increases total haemoglobin content in order to keep the haemoglobin concentration constant. Owing to dilution from an increase in plasma volume, and possibly also from mechanical destruction, a lower haemoglobin concentration is sometimes found as the result of exercise [12]. In addition, oxygen delivery to the tissues is increased through an increase in 2,3-diphosphoglycerate (2,3-DPG), which shifts the oxygen dissociation curve. In solid cancers, the haemoglobin concentration is decreased [13].

We have shown that tumour-bearing rats subjected to voluntary physical exercise demonstrate delayed onset of anorexia and cachexia [14], as well as improved oxidative capacity and energy charge in skeletal muscle [15]. As the levels of antioxidants have been shown to be altered in various tissues in the tumour-bearing host [16], the aim of the present study was to determine whether voluntary physical exercise can influence the levels of coenzymes $Q_9$ and $Q_{10}$ and thus the antioxidant capacity in muscle tissue, which is the main target for cancer cachexia.

Correspondence to B. Soussi.
P. Daneryd, T. Scherstén and B. Soussi are at the Bioenergetics Group, Department of Surgery, Sahlgrenska Hospital, University of Göteborg, Göteborg; and F. Åberg, G. Dallner and L. Ernster are at the Department of Biochemistry, Arrhenius Laboratories for the Natural Sciences, Stockholm University, Stockholm, Sweden.
Revised 20 Feb. 1995; accepted 22 Feb. 1995.

## MATERIALS AND METHODS

*Animal and tumour model*

The experimental model has been previously described in detail [14]. Briefly, the experiments were performed in growing female Wistar Furth rats (B&K Universal AB, Sollentuna, Sweden). A total of 80 animals were divided into four equal groups, matched for body weight: tumour-bearing exercising animals (TBE), tumour-bearing sedentary animals (TBS), non-tumour-bearing exercising animals (CE), and non-tumour-bearing sedentary animals (CS). After adaptation to the cages, all animals were anaesthetised and the animals in the tumour groups were implanted subcutaneously with a transplantable Leydig cell sarcoma, which does not metastasise or penetrate into the adjacent tissue [17]. The amount of tumour tissue which was transplanted leads to tumours that are palpable after 7–10 days and death after 40–45 days due to anorexia and cachexia. The control animals were sham operated. All animals were killed on day 30 after the surgical procedures. The animals allocated to exercise were individually housed in cages open to the interior of a free-moving, wire-bottomed and non-motorised running-wheel, while the other animals were individually housed in standard cages. All animals had free access to a standard balanced diet and tap water. Body weight, food intake and distance run were recorded regularly. The experimental model was approved by the animal ethical committee of the University of Göteborg, Sweden.

*Sample collection*

On day 30 after tumour implantation or sham operation, all animals were killed. They were anaesthetised with sodium pentobarbital (ACO, Solna, Sweden) intraperitoneally (40–50 mg/kg body weight).

In the experiment on skeletal and cardiac muscles (10 animals/group), a cross-section biopsy was taken from one calf, and immediately homogenised and frozen, and then a cross-section biopsy was taken from the heart and prepared the same way. The tumours were dissected free and weighed. The animals were then killed by bleeding.

In the experiment for analysis of haemoglobin in whole blood (10 animals/group), sternotomy was performed as the only procedure, and blood was collected by cardiac puncture.

*Coenzymes $Q_9$ and $Q_{10}$*

Homogenates for analysis of coenzymes $Q_9$ and $Q_{10}$ were prepared from fresh calf muscle tissue (soleus, tibial, plantar, gastrocnemius and EDL). The homogenates were then frozen and stored until analysis as previously described [18]. Aliquots of the homogenates were extracted with petroleum ether:methanol, 3:2. After phase separation, the upper petroleum ether phase was removed and the methanol phase re-extracted with more petroleum ether. The two petroleum ether phases were pooled, evaporated under $N_2$ at 60°C and the residue was dissolved in *n*-hexane. This mixture was injected on to a HPLC column with a prefilter. The column employed was a $C_{18}$ reversed phase (Hewlett-Packard, ODS 3 μm), and separation was accomplished with a convex gradient consisting of solvent A (methanol:water, 9:1) and solvent B (methanol:isopropanol:*n*-hexane, 2:1:1). The separation was completed within 30 min. The absorption of the eluate was monitored at 275 nm with low attenuation. Coenzyme $Q_6$ was used as an internal standard (Figure 1).

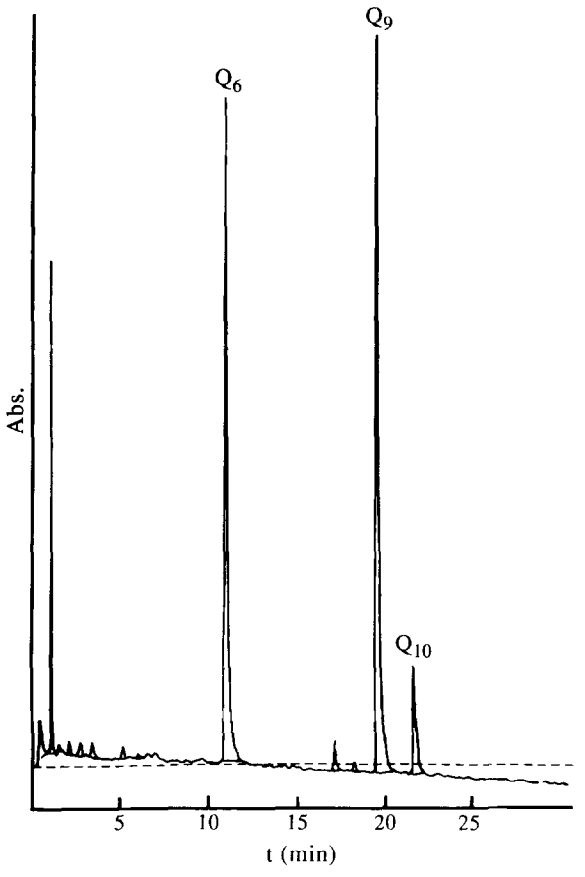


**Figure 1. Representative HPLC pattern of coenzyme Q analysis in muscle extract.**

Protein content in the homogenates was determined according to Lowry [19].

*Chemicals*

Coenzymes $Q_6$, $Q_9$ and $Q_{10}$ were obtained from Sigma Chemical Co. (St Louis, Missouri, U.S.A.). All other chemicals used were of analytical grade.

*Haemoglobin*

Haemoglobin concentration in whole blood was analysed with a commercially available kit (Reflotron, Boehringer Mannheim, Germany) [20].

*Statistical analyses*

The values are presented as means and standard errors of the mean. Statistical significance was tested with ANOVA and the Mann–Whitney *U*-test. A $P$ value of less than 0.05 was considered significant.

## RESULTS

*Body weight, food intake and physical activity*

The results were in accordance with those presented in the methodological article on this experimental model [14], the only exception being that the amount of physical activity was approximately 20% higher in both the exercised groups in the present experiment.

Briefly there were no notable differences between the TBE and the TBS animals concerning total body weight (including tumour weight) throughout the experiment. Both groups of exercising animals had increased food intake compared to that of their sedentary controls after the fourth day of the experiment ($P < 0.05$). The two exercising groups differed as to daily distance run from day 20 onwards, with a gradual decrease in the TBE group, but still with the ability to run remaining, despite a growing tumour impact (Table 1).

*Tumour weights*

Lower tumour weights were recorded in the exercising group as shown in Table 1 ($P < 0.05$).

*Coenzymes $Q_9$ and $Q_{10}$*

The results are presented as contents of coenzymes $Q_9$ and $Q_{10}$ (μg/g) protein in skeletal and cardiac muscle (Table 2) and of coenzymes $Q_9$ and $Q_{10}$ (μg/g) muscle tissue wet weight (Table 3).

In skeletal and cardiac muscle, as well as in other tissues of the rat, the major form of coenzyme Q has nine isoprene units in the side chain while less than 10% has a decaprenol side-chain [18] (Tables 2 and 3).

In the anterior tibial muscle, the TBE group showed a 55% increase in coenzyme $Q_9$ and an 82% increase of coenzyme $Q_{10}$ as compared to CS animals ($P < 0.05$). TBS animals showed a smaller increase of both coenzymes in the tibial muscle (19 and 33%, respectively).

In the soleus muscle, the TBE animals had the highest values and the TBS animals the lowest values for both coenzymes, with a 42% difference between groups for coenzyme $Q_9$ ($P < 0.05$) and 47% for coenzyme $Q_{10}$ (non-significant).

In the cardiac muscle, all four groups had higher levels of both coenzymes, as compared to the tibial and soleus muscles. The TBE and TBS groups had slightly decreased levels of both coenzymes in the cardiac muscle as compared with the CS group. The CE animals had 23% less coenzyme $Q_9$ and 28% less coenzyme $Q_{10}$ than their sedentary controls ($P < 0.05$).

Despite the extensive fluctuations of total coenzyme Q levels among the various groups, the coenzyme $Q_9/Q_{10}$ ratios did not change significantly under the different conditions (Table 4). However, the coenzyme $Q_9/Q_{10}$ ratios showed minor variations in the muscle tissues analysed, increasing from cardiac to soleus and to tibial muscle. The importance of this finding is unclear since no functional differences are known to exist between the two coenzyme Q homologues.

*Haemoglobin*

Both tumour-bearing groups had lower haemoglobin concentrations in whole blood than the non-tumour-bearers, but the TBE group demonstrated a higher value than the TBS group ($P < 0.05$) (Table 1).

## DISCUSSION

Forced physical exercise can cause oxidative damage to tissues in the unaccustomed experimental animal [4]. A number of studies have indicated that graded endurance training can improve the antioxidant status in blood [5, 6, 21, 22] and skeletal muscle [7, 22, 23], and thus improve the ability to withstand oxidative stress, but even in pretrained animals, oxidative damage occurs if the threshold for antioxidant capacity is passed [2].

Rats subjected to voluntary physical exercise exhibit interindividual differences as to running ability [14, 24]. This is not taken into account when forced physical exercise is applied in order to standardise the experimental procedure. Moreover, increased need of dietary intake of the antioxidants vitamins C and E has been postulated [25]. Experimental settings with

*Table 1. Tumour weight, body weight differences, food intake, running activity and haemoglobin levels*

| | TBE | TBS | CE | CS |
|---|---|---|---|---|
| Tumour weight (g) | 19.8 ± 4.9† | 34.7 ± 5.3 | — | — |
| % tumour weight (tumour weight/body weight−tumour weight) | 11.8 ± 2.9† | 20.0 ± 3.2 | — | — |
| Body weight difference, last 24 h (%/day) | 1.9 ± 0.4*−¤ | 1.2 ± 0.3 | 0.5 ± 0.1 | 0.2 ± 0.0 |
| Food intake last 24 h (g/100 g body weight/day) | 6.6 ± 0.4*† | 5.1 ± 0.2‡\| | 10.3 ± 0.2§ | 6.9 ± 0.1 |
| Running distance, last 24 h (m/day) | 2899 ± 1341* | — | 8639 ± 729 | — |
| Haemoglobin in whole blood (g/l) | 117.4 ± 3.7*†=¤ | 98.8 ± 5.5‡\| | 140.4 ± 2.9 | 143.8 ± 2.2 |

$n$ = 10/group.
$P < 0.05$: *TBE versus CE. †TBE versus TBS. ‡TBS versus CS. §CE versus CS. |TBS versus CE. =¤TBE versus CS. TBE, tumour-bearing exercising animals; TBS, tumour-bearing sedentary animals; CE, control exercising animals; CS, control sedentary animals.

*Table 2. Contents of coenzymes $Q_9$ and $Q_{10}$ in anterior tibial, soleus and cardiac muscle expressed as coenzyme Q (μg/g) protein in muscle tissue*

| Muscle | Coenzyme | TBE | TBS | CE | CS |
|---|---|---|---|---|---|
| Anterior tibial | $Q_9$ | 505.2 ± 59.0*=I | 388.7 ± 42.1 | 338.4 ± 8.6 | 326.5 ± 12.9 |
| ($n$ = 10/group) | $Q_{10}$ | 32.9 ± 5.4*=I | 24.1 ± 3.4 | 19.4 ± 0.3§ | 18.1 ± 0.8 |
| | $Q_9 + Q_{10}$ | 537.7 ± 64.0*=I | 412.7 ± 45.3 | 357.7 ± 8.8 | 344.6 ± 13.7 |
| Soleus | $Q_9$ | 257.9 ± 25.2† | 181.0 ± 8.6‡\| | 225.6 ± 8.5 | 206.0 ± 6.8 |
| ($n$ = 10/group) | $Q_{10}$ | 21.0 ± 3.1 | 14.3 ± 0.9 | 16.2 ± 1.3 | 16.1 ± 0.9 |
| | $Q_9 + Q_{10}$ | 278.4 ± 28.1† | 195.2 ± 9.3‡\| | 241.2 ± 9.1 | 222.1 ± 7.8 |
| Cardiac | $Q_9$ | 1272.4 ± 58.8 | 1305.9 ± 62.8\| | 1120.2 ± 44.1§ | 1377.0 ± 33.5 |
| ($n$ = 5/group) | $Q_{10}$ | 124.9 ± 4.7* | 129.1 ± 4.8\| | 103.5 ± 3.7§ | 132.3 ± 3.3 |
| | $Q_9 + Q_{10}$ | 1397 .3 ± 63.2 | 1434.9 ± 67.6\| | 1223.7 ± 47.7§ | 1509.3 ± 36.7 |

$P < 0.05$: *TBE versus CE. †TBE versus TBS. ‡TBS versus CS. §CE versus CS. |TBS versus CE. =ITBE versus CS. See Table 1 for abbreviations.

*Table 3. Contents of coenzymes $Q_9$ and $Q_{10}$ in anterior tibial, soleus and cardiac muscle expressed as coenzyme Q(μg/g) muscle tissue wet weight*

| Muscle | Coenzyme | TBE | TBS | CE | CS |
|---|---|---|---|---|---|
| Anterior tibial | $Q_9$ | 94.1 ± 9.0\| | 75.6 ± 7.8 | 72.7 ± 2.6 | 65.9 ± 1.4 |
| ($n$ = 10/group) | $Q_{10}$ | 6.1 ± 0.9\| | 4.7 ± 0.6 | 4.2 ± 0.1‡ | 3.7 ± 0.1 |
| | $Q_9 + Q_{10}$ | 100.1 ± 9.8\| | 80.3 ± 8.4 | 76.8 ± 2.8 | 69.6 ± 1.8 |
| Soleus | $Q_9$ | 39.3 ± 1.7† | 33.1 ± 1.4§ | 40.4 ± 1.8 | 37.1 ± 1.9 |
| ($n$ = 10/group) | $Q_{10}$ | 3.1 ± 0.3 | 2.6 ± 0.2 | 2.9 ± 0.3 | 2.9 ± 0.2 |
| | $Q_9 + Q_{10}$ | 42.3 ± 2.0† | 35.7 ± 1.5§ | 43.2 ± 2.0 | 40.0 ± 2.1 |
| Cardiac | $Q_9$ | 238.5 ± 11.3 | 233.6 ± 6.9 | 218.3 ± 5.1‡ | 246.0 ± 8.5 |
| ($n$ = 5/group) | $Q_{10}$ | 23.4 ± 0.9* | 23.1 ± 0.6§ | 20.2 ± 0.3‡ | 23.6 ± 0.9 |
| | $Q_9 + Q_{10}$ | 261.9 ± 12.1 | 256.7 ± 7.5§ | 238.5 ± 5.4‡ | 269.6 ± 9.4 |

$P < 0.05$: *TBE versus CE. †TBE versus TBS. ‡CE versus CS. §TBS versus CE. |TBE versus CS. See Table 1 for abbreviations.

*Table 4. $Q_9$:$Q_{10}$ ratios (for μg/g protein in muscle tissue)*

| Muscle | TBE | TBS | CE | CS |
|---|---|---|---|---|
| Anterior tibial ($n$ = 10/group) | 16.4 ± 1.0 | 16.7 ± 0.6 | 17.5 ± 0.3 | 18.1 ± 0.2 |
| Soleus ($n$ = 10/group) | 13.1 ± 0.9 | 12.6 ± 0.4 | 14.8 ± 1.8 | 12.8 ± 0.4 |
| Cardiac ($n$ = 5/group) | 9.4 ± 0.2 | 9.3 ± 0.1 | 10.0 ± 0.1 | 9.6 ± 0.0 |

See Table 1 for abbreviations.

forced physical exercise cause a decrease in food intake [26]. In our experimental model, the animals run voluntarily and food intake is increased as compared to sedentary animals [15]. Consequently, we found it important to study possible modifications in the endogenous antioxidant status in tumour-bearing animals subjected to voluntary instead of forced exercise.

Coenzyme Q is an important redox component of the respiratory chain, located in the inner mitochondrial membrane, but quantitative estimation of this lipid has demonstrated its presence in all intracellular membranes, including the outer mitochondrial membrane. It has also been shown that this lipid is not solely synthesised in the inner mitochondrial membrane, but also elsewhere [27]. In addition to its role as a component of the respiratory chain in mitochondria, the reduced form of coenzyme Q (i.e. ubiquinol) acts as an antioxidant, inhibiting lipid peroxidation [9]. This provides an explanation for the presence of coenzyme Q in all cellular membranes. It is unknown whether functional compartmentalisation of this lipid occurs in mitochondria in order to fulfil the two functions.

The rates of biosynthesis of the different products of the mevalonate pathway are independent, which might make it easier for the cell to adapt to different physiological situations. Experiments with administration of peroxisomal proliferators as well as carcinogens have revealed an increase in endogenous synthesis of coenzyme Q without any effects on the other lipids of the other mevalonate pathway. Because of the low concentration of the lipid in the blood, no substantial exchange between tissues seems to occur. Thus, all tissues must synthesise their own coenzyme Q in appropriate amounts, and the mevalonate pathway is the main or exclusive source of this lipid [28]. Only a few per cent of dietary coenzyme Q is taken up, and this appears in the blood with no substantial uptake in various tissues. Nevertheless, dietary supplementation with coenzyme Q has been shown to improve antioxidant status in clinical studies [29–32].

Increases in free radical formation, as induced by exercise, are expected to induce cell defense mechanisms at one or several subcellular locations. No subcellular fractionation was performed in the present experiments, but other studies have not been able to demonstrate compositional changes in the mitochondrial membrane, despite increase in total content of coenzyme Q as seen in preneoplastic liver nodules [10], or decrease in total content of this lipid as seen during ageing [9].

Our findings that the TBE animals exhibit the highest values for both coenzymes, $Q_9$ and $Q_{10}$, are unexpected, especially in comparison with the CE animals. Even the TBS animals show a tendency towards increased contents of coenzymes $Q_9$ and $Q_{10}$ in the anterior tibial muscle. Food intake is increased in tumour-bearing animals as a result of exercise [14], but as uptake of dietary coenzyme Q is negligible, the increase in muscle tissue must indicate an enhanced endogenous synthesis as a defence mechanism. This is emphasised by the fact that even the sedentary tumour-bearers have increased contents, despite the lowest food intake of all groups [14].

The oxidative metabolism in the mostly fast twitch, glycolytic anterior tibial muscle has been found to be more susceptible to exogenous and endogenous stimuli than the mostly slow twitch oxidative soleus muscle, which is in accordance with our previous findings [15]. Consequently, a more extensive oxygen free radical formation in muscle tissue of the anterior tibial type would be anticipated. In fact, this muscle responded with more extensive formation of coenzyme Q than did the soleus muscle, which emphasises the relationship between formation of oxygen free radicals and the content of coenzyme Q.

Furthermore, our results showed that physical exercise can reverse anaemia in the tumour-bearing host subjected to physical exercise. We have made the same finding in relation to an adenocarcinoma in the same experimental setting (unpublished data). The increased haemoglobin content not only increases the oxygen delivery to organs and tissues, but also the antioxidant capacity in the blood. Guilivi and Davies have recently presented evidence that haemoglobin plays an antioxidant role, as continuous reduction of hydrogen peroxide is obtained when methaemoglobin ($X\text{-}Fe^{III}$) is oxidised to ferrylhaemoglobin ($X\text{-}Fe^{IV}\text{-}OH$) [33].

An impairment in electron transfer between cytochrome c and cytochrome oxidase is seen in mitochondria with reduced levels of coenzyme Q from skeletal muscle [34]. The same is seen when the cardiolipin content is reduced in the reperfused state after ischaemia [35, 36], but endurance training on a treadmill enhances the activity of cytochrome c oxidase [37]. The present findings are thus in accordance with our previous results showing that the reduced activity of cytochrome c oxidase in skeletal muscle from the tumour-bearing host can be normalised by voluntary physical exercise [15]. It remains to be determined whether the content of coenzyme Q is directly related to these effects or if they represent parallel changes.

The question arises as to whether coenzymes $Q_9$ and $Q_{10}$ are present in two distinct pools with different functions. The coenzyme $Q_9/Q_{10}$ ratios were not influenced despite modifications in the total amount of coenzyme present. These results indicate that the two forms of coenzyme Q in the different types of muscle tissue do not appear to be involved in different functions, and that their levels are regulated in a coordinated manner.

In conclusion, the increased levels of coenzymes $Q_9$ and $Q_{10}$ in skeletal muscle reflect an increased antioxidant capacity. As a substrate deviation to the tumour-bearing host in response to exercise has been postulated [14, 15], the increased antioxidant capacity might be part of a defence mechanism. However, the detailed mechanisms remain to be elucidated.

1. Jenkins RR. Free radical chemistry—relationship to exercise. *Sports Med* 1988, 5, 156–170.
2. Davies KJA, Packer L, Brooks GA. Biochemical adaptation of mitochondria, muscle, and whole-animal respiration to endurance training. *Arch Biochem Biophys* 1981, **209**, 539–554.
3 Holloszy JO. Biochemical adaptations in muscle. Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J Biol Chem* 1967, **242**, 2278–2282.
4. Davies KJA, Quintanilha AT, Brooks GA. Free radicals and tissue damage produced by exercise. *Biochem Biophys Res Comm* 1982, **107**, 1198–1205.
5. Duthie GG, Robertson JD, Maughan RJ, Morrice PC. Blood

antioxidant status and erythrocyte lipid peroxidation following distance running. *Arch Biochem Biophys* 1990, **282**, 78–83.
6. Robertson JD, Maughan RJ, Duthie GG, Morrice PC. Increased blood antioxidant systems of runners in response to training load. *Clin Sci* 1991, **80**, 611–618.
7. Lang JK, Gohil K, Packer L, Burk RF. Selenium deficiency, endurance exercise capacity, and antioxidant status in rats. *J Appl Physiol* 1987, **83**, 2532–2535.
8. Lenaz G, Battino M, Castellucci C, *et al.* Studies on the role of ubiquinone in the control of the mitochondrial respiratory chain. *Free Rad Res Commun* 1990, **8**, 317–327.
9. Beyer RE, Ernster L. The antioxidant role of coenzyme Q. In Lenaz G, Barnabei O, Rabbi A, Battino M, eds. *Highlights in Uniquinone Research*. London, Taylor & Francis, 1990, 191–213.
10. Olsson JM, Eriksson LC, Dallner G. Lipid compositions of intracellular membranes isolated from rat liver nodules in Wistar rats. *Cancer Res* 1991, **51**, 3774–3780.
11. Eggens I, Elmberger PG. Studies on the polyisoprenoid composition in hepatocellular carcinomas and its correlation with their differentiation. *APMIS* 1990, **98**, 535–542.
12. Hunding A, Jordal R, Paulev PE. Runner's anemia and iron deficiency. *Acta Med Scand* 1981, **209**, 315–318.
13. Sears DA. Anemia of chronic disease. *Med Clin N Am* 1992, **76**, 567–579.
14. Deneryd P, Hafström L, Karlberg I. Effects of spontaneous physical exercise on experimental cancer anorexia and cachexia. *Eur J Cancer Clin Oncol* 1990, **26**, 1083–1088.
15. Daneryd P, Karlberg I, Scherstén T, Soussi B. Cytochrome c oxidase and purine nucleotides in skeletal muscle in tumour-bearing exercising rats. *Eur J Cancer* 1992, **28A**, 773–777.
16. Westman NG, Marklund SL. Copper- and zinc-containing superoxide dismutase and manganese-containing superoxide dismutase in human tissues and human malignant tumors. *Cancer Res* 1981, **41**, 2962–2966.
17. Mordes JP, Rossini AA. Tumor-induced anorexia in the Wistar rat. *Science* 1981, **213**, 565–567.
18. Åberg F, Appelkvist E-L, Dallner G, Ernster L. Distribution and redox state of ubiquinones in rat and human tissues. *Arch Biochem Biophys* 1992, **295**, 230–234.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951, **193**, 265–275.
20. Price CP, Koller PU. A multicentre study of the new Reflotron system for the measurement of urea, glucose, triacylglycerols, cholesterol, γ-glutamyltransferase and haemoglobin. *J Clin Chem Clin Biochem* 1988, **26**, 233–250.
21. Gleeson M, Robertson JD, Maughan RJ. Influence of exercise on ascorbic acid status in man. *Clin Sci* 1987, **73**, 501–505.
22. Gohil K, Rothfuss L, Lang J, Packer L. Effect of exercise training on tissue vitamin E and ubiquinone content. *J Appl Physiol* 1987, **63**, 1638–1641.
23. Beyer RE, Morales-Corral PG, Ramp BJ, *et al.* Elevation of tissue coenzyme Q (ubiquinone) and cytochrome c concentrations by endurance exercise in the rat. *Arch Biochem Biophys* 1984, **234**, 323–329.
24. Rodnick KJ, Reaven GM, Haskell WL, Sims CR, Mondon CE. Variations in running activity and enzymatic adaptations in voluntary running rats. *J Appl Physiol* 1989, **66**, 1250–1257.
25. Packer L, Almada AL, Rothfuss LM, Wilson DS. Modulation of tissue vitamin E levels by physical exercise. *Ann NY Acad Sci USA* 1989, **570**, 311–321.
26. Applegate EA, Upton DE, Stern JS. Food intake, body composition and blood lipids following treadmill exercise in male and female rats. *Physiol Behav* 1982, **28**, 917–920.
27. Kalén A, Norling B, Appelkvist E-L, Dallner G. Ubiquinone biosynthesis by the microsomal fractions from rat liver. *Biochem Biophys Acta* 1987, **926**, 70–78.
28. Elmberger PG, Kalén A, Appelkvist E-L, Dallner G. *In vitro* and *in vivo* synthesis of dolichol and other main mevalonate products in various organs of the rat. *Eur J Biochem* 1987, **168**, 1–11.
29. Guarnieri C, Muscari C, Manfroni S, Caldarera I, Stefanelli C, Pretolani E. The effect of treatment with coenzyme $Q_{10}$ on the mitochondrial function and superoxide radical formation in cardiac muscle hypertrophied by mild aortic stenosis. *J Mol Cell Cardiol* 1987, **19**, 63–71.
30. Kamikawa T, Kobayashi A, Yamashita T, Hayashi H, Yamazaki N. Effects of coenzyme $Q_{10}$ on exercise tolerance in chronic stable angina. *Am J Cardiol* 1985, **56**, 247–251.
31. Takeo S, Tanonaka K, Tazuma Y, Miyake K, Murai R. Possible mechanism by which coenzyme $Q_{10}$ improves reoxygenation-induced recovery of cardiac contractile force after hypoxia. *J Pharmacol Exp Ther* 1987, **243**, 1131–1138.
32. Zuliani U, Bonetti A, Campana M, Cerioli G, Solito F. The influence of ubiquinone (Co $Q_{10}$) on the metabolic response to work. *J Sports Med* 1989, **29**, 57–62.
33. Giulivi C, Davies KJA. A novel antioxidant role for hemoglobin. *J Biol Chem* 1990, **265**, 19453–19460.
34. Lee CP, Martens ME, Peterson PL, Hatfield JS. Cytochrome oxidase and neuromuscular diseases. In Papa S, Chance B, Ernster L, eds. *Cytochrome Systems*. New York, Plenum Press, 1987, 407–414.
35. Soussi B, Idström J-P, Scherstén T, Bylund-Fellenius A-C. Cytochrome c oxidase and cardiolipin alterations in response to skeletal muscle ischaemia and reperfusion. *Acta Physiol Scand* 1990, **138**, 107–114.
36. Soussi B, Bylund-Fellenius A-C, Scherstén T, Ångström J. $^1$H-NMR evaluation of the ferri-cytochrome c – cardiolipin interaction: effect of superoxide radicals. *Biochem J* 1990, **265**, 227–232.
37. Soussi B, Idström J-P, Scherstén T, Bylund-Fellenius A-C. Kinetic parameters of cytochrome c oxidase in rat skeletal muscle: effect of endurance training. *Acta Physiol Scand* 1989, **135**, 373–379.

**Acknowledgements**—We thank Anette Arvidsson, Margareta Jonsson, Lilian Karlsson and Kerstin Lagerwall for their technical assistance. This work was supported by the Swedish Medical Research Council (project nos. 536 and B 90-17X-07184-06-A), the Göteborg Medical Society, the Sahlgrenska Hospital Foundation, the Assar Gabrielsson Foundation, The King Gustav V Jubilee Clinic Cancer Research Foundation in Göteborg and the University of Göteborg.