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ORIGINAL ARTICLE

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Do blood cells mimic gene expression profile alterations known to occur in muscular adaptation to endurance training ?

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Abstract Exercise is known to upregulate mRNA synthesis for carnitine palmitoyl transferase1 (CPT1) and possibly also other mitochondrial carnitine acyltransferases in muscle tissue. The aim of this study was to test whether such an adaptation of oxidative metabolism in skeletal muscle is a systemic process and consequently, also affects other cells. Messenger RNA levels of five genes [carnitine palmitoyl transferases 1 and 2 (CPT1 and CPT2), carnitine acetyltransferase (CRAT), carnitine palmitoyltransferase 2 (CPT2), microsomal carnitine palmitoyltransferase (GRP58) and organic cation transporter (OCTN2)] were determined with quantitative real time polymerase chain reaction (PCR) in blood cells and in muscle biopsy samples from six cross country skiers before and 6 months after a high volume/low intensity exercise training, when training had elicited a significantly slower rate of lactate accumulation. Quantitative real time PCR showed that levels of mRNA in blood cells correlated significantly (CPT1B: $P < 0.001$) with those in muscle tissue from the same donors. After 6-months training, there was a 15-fold upregulation of CPT1B mRNA, a six to ninefold increase of CRAT mRNA, of CPT2 mRNA, GRP58 mRNA, and of OCTN2 mRNA. The observation of a concordant stimulation of CPT1, CPT2, CRAT, GRP58 and OCTN2 transcription in blood cells and muscle tissue after 6-month-endurance training leads the hypothesis of a common stimulation mechanism other than direct mechanical stress or local chemical environment, but rather humoral factors.

Keywords Adaptation to endurance training · mRNA levels · Muscle · Blood cells · Oxidative metabolism · Carnitine acyltransferases

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Introduction

Endurance training has been shown to increase fat oxidation both at rest and during exercise. Long-term training (at least > 31 days) produces an increase in muscle oxidative potential and a glycogen sparing that is associated with improving endurance capacity (Phillips et al. 1996). According to the present knowledge, one of the central adaptations in the course of endurance training is a relative decrease in carbohydrate utilization and an increase in fatty acid oxidation in muscle tissue during exercise (Jeukendrup 2002). The difference in fatty acid oxidation rate between trained and untrained individuals is accompanied by an enhanced transport of fatty acid (FA) into the mitochondria (Sidossis et al. 1998) which depends on expression levels of carnitine palmitoyl transferases 1 and 2 (CPT1 and CPT2, McGarry and Brown 1997).

A local increase of activity and mRNA expression of CPT1 in single human skeletal muscle and a concomitant increase in mitochondrial protein concentration has been described in response to training, (Pilegaard et al. 2000; Tunstall et al. 2002; Hawley 2002).

These changes along with increased capillary supply have been interpreted as a shift in trained muscle to a

greater reliance on fat as a fuel with a concomitant reduction in glycolytic flux and a tighter control of acid–base status (Pilegaard et al. 2000; Tunstall et al. 2002; Hawley 2002).

Furthermore, it is known that skeletal muscle metabolic activity modifies serum lipids and lipoproteins and also the hormonal profile (Tikkanen et al. 1999), but systemic effects in relation to training are scarcely investigated.

However, particularly in relation to anthracycline induced oxidative stress, which also affects enzymes of fatty acid metabolism such as CPTs, changes in gene expression profiles of blood cells are in line with cardiac gene expression profiles (Brown et al. 2002). This indicates that gene expression profiles of mononuclear blood cells may be useful in monitoring and predicting not just cardiological parameters affected by oxidative stress and effects on carnitine transport but also physiological changes in skeletal muscle thus, potentially obviating the need for muscle biopsies in assessing intramuscular changes of gene expression.

Aim of the present study was to test the hypothesis that adaptive processes with moderate long-term endurance exercise induce an increased transcription of genes encoding several mitochondrial carnitine acyltransferases as well as one human specific microsomal carnitine acyltransferase and the organic cation transporter OCTN2 which mediates uptake of L-carnitine across the plasma membrane (Tamai et al. 2001), in muscle and blood cells.

The rationale for this investigation of parallelism between gene expression of key enzymes in the oxidative cycle of blood and muscle cells was (1) to validate the replacement of muscle biopsies with peripheral blood sampling in assessing muscular gene expression, and (2) to gain insight into possible direct influences of endurance training on systemic gene expression levels, by mechanisms other than changes in the local mechanical and/or chemical environment in the muscle.

Compared to muscle biopsy, blood sampling is much less invasive and thus, more practicable. Further, we investigated whether blood cells can mimic some of the expression profile alterations known to occur in muscular adaptation to endurance training.

Materials and methods

Subject characteristics

Six male endurance trained junior cross-country skiers volunteered to participate in the study. All subjects had finished their competitive season without major health problems. The subjects entered the study in May at the end of a 3-week period with no training after the end of the competitive season. Training status and level of condition of the subjects was low compared to the level of condition at the beginning of the past competitive season. None of the subjects got injured or sick during observation period. Anthropometric and physiological data of the athletes are summarized in (Table 1). All subjects had a regular endurance training experience of a minimum of 2 years and competed on local or national level. Subjects were weight stable throughout the study period. Mean VO_{2max} was 58.7 ml/kg/min at the beginning. The observation period lasted from May till November 2001, which is 6 months. Our test design consisted of two treadmill-tests for each participant, one at the beginning and one at the end of the training period. Muscle and blood samples were taken after informed consent during routine health- and performance tests. All subjects were familiar with the examined test procedures

Treadmill testing (TT)

Each participant underwent two treadmill tests, one at the beginning and one at the end of the training period. The treadmill test was performed as a ramp test on a motorized treadmill (HP Cosmos Sports& medical GmbH, Traunstein, Germany). The ramp test consisted of an initial workload of 8 km.$^{h\text{-}1}$ with an increment of 2 km.$^{h\text{-}1}$ every 3 min at a grade of 0%. The test ended with voluntary termination due to subjective exhaustion.

Capillary blood samples from the earlobe were taken after completion of each stage, immediately after the end of the test and after 2, 3 and 6 min during the recovery period for analysis of blood lactate (LA). Heart rate (HR) was determined by means of a chest-belt telemetry monitor (Polar, Kempele, Finland) which was transmitted and also recorded at the end of each stage. Blood LA was determined with the full enzymatic method utilizing Eppendorf (ESAT 6661, (Eppendorf, Hamburg, Germany). The plasma lactate response (PLR) was fitted to an exponential function calculated according to $LA = a * e^{bxv} + c$ (where vx = running velocity; a, b are fitting parameters).

Table 1 Anthropometric and physiological data of the athletes before and after 6 months of endurance training

	Before training	After training
Age	16.6 ± 1.8	
Body weight (kg)	62.7 ± 11	65.2 ± 10.8
VO_{2max} ml/kg/min	58.7 ± 2.8	64.4 ± 9.3
HR max (beats/min)	195.3 ± 12.1	199.3 ± 13.8
HR at P2	157.3 ± 11.7	162.5 ± 7.1
HR at P4	180.8 ± 14	183.3 ± 11.3
Max LA	11.1 ± 2.4	9.7 ± 1.4
Max P, km/h	18.7 ± 1	18.7 ± 1.6
P2, km/h	10.9 ± 1.5	12.6 ± 1.1*
P4, km/h	14 ± 1.1	15.5 ± 1.1*

VO_{2max}, maximal oxygen consumption
HR Heart rate
LA Lactic acid
P2 Performance at 2 mmol lactate
P4 Performance at 4 mmol lactate
Values are means ± SE ($n = 6$ subjects)
*Significantly different from before training $P < 0.05$

Finally, the workloads (P) and HR at fixed blood lactate levels of 2 (P2) and 4 (P4) mmol/l were calculated by extrapolation from the workload-lactate over heart rate graph using above function. To assess VO_{2max} and to monitor pulmonary gas exchange, breath-by-breath measurement of expired and inspired air was done by an automated gas analyzer system (Oxycon alpha, Jäger GmbH, Würzburg, Germany).

All tests were performed within 3–10 days at approximately the same hour (between 9 a.m. and 11 a.m.). Subjects were instructed to comply with a similar nutrition intake during the last 36 h before each test. The day before each test no intensive or extensive physical exercise was allowed.

Six months later, after having undergone high volume/low intensity training, the ramp test was repeated using the same protocols.

Workloads at both lactate levels were compared and correlated with the changes in mRNA transcriptional rates of several enzymes.

Training protocol

Over the 6 months, the subjects followed a low-intensity training programme with a minimum of seven 90–120 min sessions per week. Weekly training volume was between 8 h and 15 h with an average of 12 h per week. To prevent overtraining, blood urea nitrogen and resting heart rate were examined twice a week. A 3-week training period with moderate increases in training volume, intended to induce physiological overloading without overtraining, was followed by 1 week with progressive reductions in training volume (70–80%). The intensity of the exercise was individually set according to the results of the treadmill test. Individual training workload was determined before the first training by determination of the individual HR at fixed lactate levels and at the ventilatory threshold (33). More than 85% of the total training load was performed below the ventilatory threshold respectively below HR at fixed lactate levels of 2 mmol/l. To monitor and adjust the training intensity according to training improvements, heart rate monitors (Polar, Kempele, Finland) were used for all training sessions. Blood lactate concentrations were measured (Eppendorf ESAT 6661, Hamburg, Germany) during training. The mean lactate concentration from monthly, up to 40, measurements per subject was 1,7 mmol/l. The training program consisted of running (50%), cycling (25%, Cycling device: Bikerrace, Technogymm Gambettola, Italy), and skating with a professional ski roller device (25%).

Muscle biopsies

Muscle biopsies were taken from the right musculus vastus lateralis at the midthigh level at baseline and 6 months later at the end of the training period. Sampling was performed 1-cm distally and 1-cm proximally to baseline sampling, respectively. The overlying skin was anesthetized. Local anesthesia was applied (with 1% lidocaine), and sampling was done through an incision using a 5-mm Bergström needle (Bergström et al. 1975). A suction device, in conjunction with the biopsy needle was used to create a negative pressure while sampling, which allowed a larger sample specimen. Samples were immediately frozen in liquid nitrogen, aliquots of homogenized muscle tissue were frozen in 4 M guanosine isothiocyanate (GTC) for mRNA preparation. Biopsies were carried out for all subjects at one day between 5 p.m. and 8 p.m. at the beginning and at the end of the observation period. The subjects were instructed to comply with a similar nutrition intake during the last 24 h before each biopsy. On the day of the biopsies, no intensive or longer lasting extensive physical exercise was allowed. All biopsies were carried out without any complications.

Blood samples

Venous blood samples were collected into plain evacuated tubes from a forearm vein with minimal stasis after approximately 10 min of rest in a sitting position between 8 a.m. and 9 a.m., after an overnight fast and at least 24 h from the last workout. Blood was immediately centrifuged and then stored at −20°C before analysis. Biopsies and blood sampling were performed with time interval no longer than 14 days to the treadmill-test of each subject.

Analysis of mRNA expression

All mRNA analyses were made either from 4-ml peripheral blood or from homogenized muscle tissue collected in EDTA tubes. After separation of cell free plasma for L-carnitine determination, the mononuclear cells were enriched by density gradient centrifugation using Ficoll–Hypaque separation medium (density according to fraction index: 1.077) and washed several times in PBS. Aliquots of 1–10 million cells were frozen in 4 M GTC for preparation of mRNA and cDNA for subsequent rt-PCR.

Isolation of mRNA and preparation of cDNA was carried out according to standard procedures using commercially available kits. Quantitative reverse transcriptase polymerase chain reaction (rtRT-PCR) (Weghofer et al. 2001; Karlic et al. 2001: Karlic et al. 2002) was carried out using the LightCycler System (Roche) which allows amplification and detection. Dilutions of 200–2 ng of cDNA were used in each assay. Evaluation of the transcription rate was done by calculating the number of copies of the analyzed gene per 100 copies of

beta-actin which showed the lowest standard deviations per amount of cDNA and therefore, was used as an internal standard. Details of primers and reaction conditions are shown in Table 2.

Analysis of plasma carnitine

Perchloric acid extracts of plasma were used for assaying free carnitine and short-chain acylcarnitine. The carnitine esters were saponified and assayed as free carnitine by a radioenzymatic method (Lohninger et al. 1990), with two modifications: HEPES instead of TRIS buffer, and N-ethylmaleimide instead of tetrathionate.

Analysis of plasma lipids

Free fatty acids, free cholesterol, cholesteryl esters and triglycerides were determined directly from total lipid extract by capillary gas chromatography, as described previously (Lohninger et al. 1990). In brief, for FFA determination the programmed temperature vaporizer (PTV) injector was heated from 40°C (sample introduction) to 190°C. In a second gas chromatographic run, the PTV injector was heated from 60°C (sample introduction) to 400°C enabling the determination of the other lipid classes, except phospholipids.

Statistics

All data are presented as means standard deviation (SD) ± SE. A nonparametric ranking sum test was used to detect significant differences between unpaired (Mann–Whitney) and paired (Wilcoxon) data before and after the training period (2-tailed testing). A $P < 0.05$ was considered significant. Pearson correlation coefficients (r) were calculated between the transcription rates in blood cells and muscle before and after the training period.

Results

Subject characteristics

The exercise training did not result in significant changes in body weight and body composition (Table 1). There was no increase in VO_{2max} and no significant change in heart rates. The workloads at both fixed lactate levels (P2 and P4) increased significantly during the 6-month-training period. AverageP2 was increased by 1.5 $km.^{h\text{-}1}$; AverageP4 was increased by 1.5 $km.^{h\text{-}1}$ from May until November. The maximal running speed ($km.^{h\text{-}1}$) of mean 18.7 $km.^{h\text{-}1}$ did not change over the 6-month-training period.

Plasma lipid profile and carnitine profile

The levels of free, total, short-chain acyl carnitine and triacylglycerol were generally in the lower reference range and showed no training induced changes (Table 3).

In contrast, plasma levels of free FA were in the upper range and diminished after 6-month training significantly (Table 3), whereas the composition of FFA elicited significant changes.

At the start of endurance exercise training the composition of FFA is rather comparable to those of adipose tissue, whereas after 6-months training the portion of those FA, known to be the main products of endogenous FA synthesis (palmitic- and palmitoleic FA), increased significantly ($P = 0.001$) and the portion of oleic acid on total FA was significantly reduced ($P = 0.001$).

Expression of carnitine acyltransferases and OCTN2 in blood and muscle

Transcript levels of CPT1B, CPT 2, CRAT, OCTN2 and the microsomal carnitine acyltransferase GRP58 are

Table 2 Primers and PCR conditions

PCR-primer	Sequence	PCR-product-size	Annealing temp/time (°C/s)	Extension temp/time (°C/s)	Acquisition temp/time (°C/s)
ß-Actin (1,488–1,777)	S: 5′-TgccATccTAAAAgccAc-3′ A: 5′-TcAAcTggTcTcAAgTcAgTg-3′	285 bp	64/5	72/34	83/1
CPT1A (242–540)	S: 5′-ccTTccAAcTcATTcAg-3′ A: 5′-ccAggATccTcTgcATcTg-3′	298 bp	64/5	72/34	87/1
CPT1A (891–1,138)	F: 5′-cATTcAggcAgcAagAgc-3′ A′-cAgcAgccgcccATcATg-3′	247 bp	64/5	72/34	87/1
CPT1B (2,961–3,309)	S: 5′-ggTgAAcAgcAAcTATTATgTc-3′ A: 5′-ATccTcTggAAgTgcATc-3′	348 bp	62/6	72/34	87/1
CPT 2 (241–414)	S: 5′-gggAAgggAAgggAgAcgAg-3′ A: 5′-ccAAgAcAcTgcgTcAggAc-3′	173 bp	63/5	72/34	92/1
CRAT (343–518)	S: 5′-gAAgcccTTcTccTT-3′ R: 5′-cTccccTAcAccTccTgAg-3′	175 bp	64/5	72/34	91/1
GRP58 (571–841)	S: 5′-cccTcAcATgAcAgAAgAc-3′ A: 5′-cTccTgcATgAcAAAcTTc-3′	270 bp	64/5	72/34	83/1
OCTN2	S: 5′-TccAAgTcAcAcAAggATg-3′ A: 5′-TcccTAgAggAAggTggTg-3′	246 bp	62/5	72/34	86/1

Table 3 Plasma carnitine, triacylglycerols and free fatty acids (FFA) levels and the portion (%) of a single FA on total FFAs of athletes before and after 6 months of endurance training

	Before	After	*P*
Free carnitine (μmole/l)	33.0 ± 9.5	33.7 ± 8.9	NS
Total carnitine (μmole/l)	40.1 ± 9.9	42.0 ± 5.8	NS
Short chain acyl carnitine (μmole/l)	7.1 ± 4.7	8.2 ± 5.5	NS
Triacylglyceroles	41.3 ± 12.8	54.5 ± 27.2	NS
Total free fatty acids (g/l)	88.9 ± 42.3	55.6 ± 18.9	$P < 0.05$
% Palmitic acid (C16:0)	19.0 ± 2.0	36.0 ± 2.7	$P < 0.001$
% Palmitoleic acid (C16:1)	8.2 ± 1.4	10.4 ± 1.1	$P < 0.001$
% Stearic acid (C18:0)	11.6 ± 1.6	10.8 ± 1.1	NS
% Oleic acid (C18:1n9)	48.4 ± 2.3	30.1 ± 3.2	$P < 0.001$
% Linoleic acid (C18:2n6)	11.2 ± 3.1	10.5 ± 3.0	NS
% Linolenic acid (C18:3n3)	1.6 ± 0.7	2.2 ± 0.9	NS

shown in Fig. 1a–e. The expression rate of the liver isoform of CPT1, CPT1A had doubled after training in blood cells. In the muscle specimens, no transcription was detected. After 6-months training CPT1B expression showed a 13–15 fold increase in transcription rates (Fig. 1a) both in blood cells and in muscle tissue.

Carnitine palmitoyl transferases 2 expression was significantly higher in peripheral blood and muscle indicating a six to eightfold increase in transcription rate (Fig. 1b).

The mean expression rate of CRAT showed a nine- to tenfold increased mRNA expression (Fig. 1c).

Transcript levels of OCTN2 mRNA are shown in Fig. 1d. After 6-months training OCTN2 mRNA expression indicated a sevenfold increase in transcription rate (Fig. 1d).

Transcript levels of the microsomal carnitine palmitoyltransferase are shown in Fig.1f. After 6-months training, GRP58 mRNA expression showed a six to eightfold increase (Fig. 1e).

Correlation of transcriptional rates between blood and muscle samples

Data from the first measurements of blood cells and muscle samples correlated significantly for CPT1B (Fig. 2a), for CPT2 (Fig. 2b), for CRAT (Fig. 2c), for OCTN2 (Fig. 2d). Considering data from second measurements of blood cells and muscle samples, there was a significant correlation with r-values for CPT1B, 0.997 ($P < 0.001$), CRAT 0.916 ($P = 0.10$) and OCTN2 0.997 ($P < 0.001$). No significant correlation between BLOODT2 and MUSCLET2 was found for GRP58 with *r*-values of 0.594 ($p = 0.214$).

Discussion

The results of the present study show that long-term moderate endurance exercise training is effective in increasing the expression of mitochondrial carnitine acyltransferases as well as GRP58 and OCTN2 both in muscle and blood cells. This indicates that training influences signaling processes which induce a systemic upregulation of oxidative metabolism via increasing the expression and activity of key enzymes.

Our results indicate that blood cells can mimic some of the expression-profile alterations known to occur in muscular adaptation to endurance training (see correlations under results). These data strongly suggest a common mechanism of transcriptional control.

The concept that endurance exercise training induced metabolic adaptation mechanisms are not intrinsically limited to the skeletal muscle can be further supported by the finding that the relative amount of CPT1A-mRNA (liver isoform of CPT1, which is not expressed in adult skeletal muscle) was also increased to the same order of magnitude as the muscle isoform CPT1B in mononucleated blood cells.

However, it is known that (1) active muscle is not solely responsible for elevated lactate and (2) training increases leg lactate clearance, decreases whole body and leg lactate production at a given moderate-intensity power output, and increases both whole body and leg lactate clearance at a high relative power output (Bergman et al. 1999a, b). In a recent report, it was shown, that even a minimal amount of physical activity, namely low-intensity endurance exercise of 3 h a week over 3 months lead to marked changes in the expression of genes coding for key enzymes in fat metabolism in human skeletal muscle (Schrauwen et al. 2002).

During exercise, several different fat sources are available for fat metabolism:

(1) Plasma fatty acids from lipolysis of triglycerides in adipose tissue, and (2) fatty acids released from circulating VLDL triglycerides by the action of lipoprotein lipase (LPL), and finally (3) intramuscular triglyceride depots. It appears possible that different types of cells from other tissues including mononuclear blood cells respond in a similar manner to exercise-induced release of lipids from depots as it is known for muscle cells.

It is proposed that hematopoietic progenitors and stem cells in the muscle tissue are derived of mesenchymal stem cells of bone marrow origin. A hematopoietic stem cell may be capable of differentiating into a mature liver cell, muscle tissue or even neurons. Similarly, muscle stem cells may be capable of producing mature blood cell populations (reviewed by Lemischka 2001; Graf 2002). The results of the present study show that exercise capacity during the same intensity of high vol-

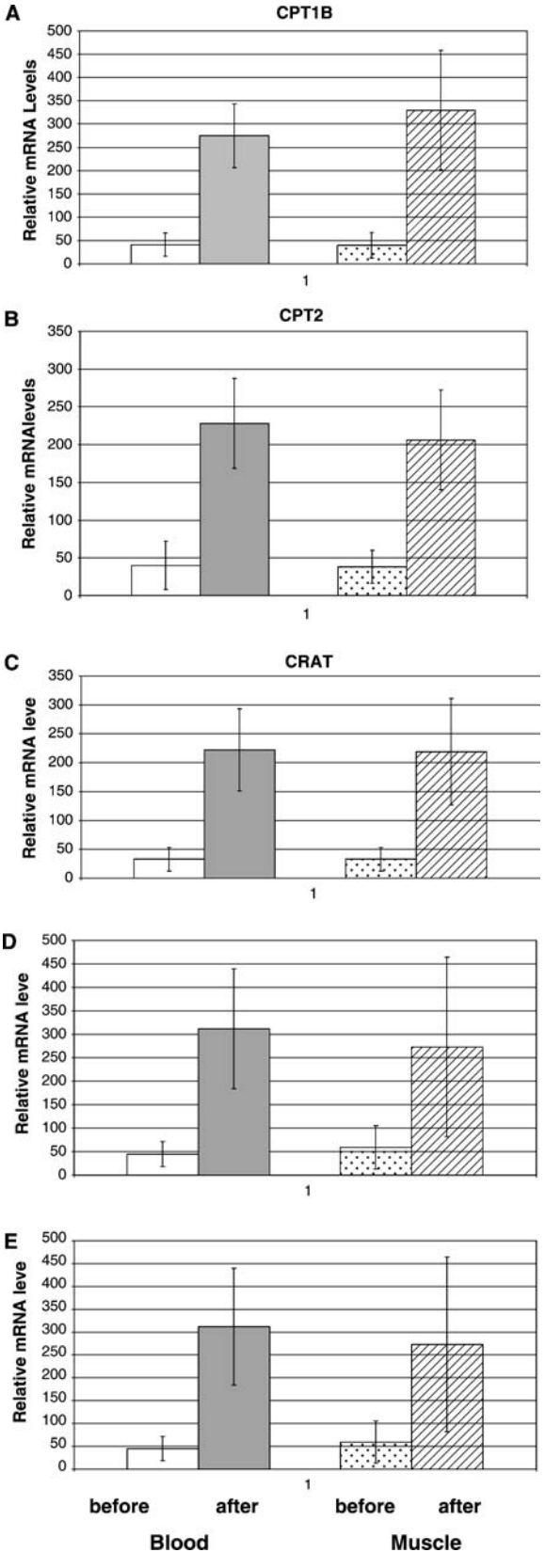

Fig. 1 Relative mRNA levels as a percentage of standard gene (ß-actin) transcripts (= gene copies/ 100 copies ß-actin) before and after 6-month-endurance training in mononuclear blood cells and muscle tissue. Data represent mean values. Values after training were all significantly higher than values before training ($P < 0.05$). **a** Carnitine palmitoyltransferase 1B, muscle isoform; **b** carnitine palmitoyltransferase 2, **c** carnitine acetyltransferase (CRAT); **d** organic cation transporter (OCTN2); **e** microsomal carnitine palmitoyltransferase

ume training was increased after a 6-months program of endurance exercise training as a major adaptation. This view is supported by significantly lower lactate levels on a treadmill test of the same intensities ($P < 0.05$). Thus, training was effective in accumulation of lactate at a slower rate with increasing exercise intensity whereas other physical parameters remained constant (Table 1)).

Furthermore, no significant changes in serum lipid profiles could be detected in this study (Table 3). As triglyceride levels before and after training remained constant in the lower normal range of average, a relationship between CPT expression and triglyceride level as reported by Tikkanen et al. (1999) could not be established in this study.

The data presented in this long-term study are confirmed by data from short-term studies showing that this increased capacity for lipid oxidation is accompanied by an increased expression of CPT 1B (Pilegaard et al. 2000; Tunstall et al. 2002) and activity of CRAT (Spina et al. 1996), although Tunstall et al. (2002) showed that genes involved transcriptional control of CPT1B, namely, peroxisome proliferator-activated receptor (PPAR) alpha, PPAR gamma, PPAR gamma coactivator 1, sterol regulatory element-binding protein-1c were unaltered by exercise. This discrepancy was also found by Lapsys et al. (2000) who reported that inter-individual variations of PPAR expression in muscle were up to threefold whereas mRNA concentrations of CPT1B had a range of 13-fold variation between individuals. This is also confirmed by this study where the exercise associated increase of CPT1B transcripts was 15-fold after a 6-month-training program. Thus, it appears possible that the high inter-individual variations in expression of CPT1B of freely randomly chosen voluntary donors may reflect inter-individual variations in physical activity.

It is known that a network of endocrine factors including thyroxine and steroid hormones which is influenced by endurance training (Tikkanen et al. 1999) also plays a role in regulating gene expression of CPT1B and CRAT (Guzman et al. 1991; Campbell et al. 2001; Chiu et al. 1997; Chiu et al. 1999) thus, explaining our observation that training affects metabolic genes of blood cells in a comparable manner as muscle tissue. However, it cannot be completely excluded that infiltrating white blood cells might have contributed to the striking correlation between white blood cells and muscle tissue as observed in this study.

In addition, the mRNA expression of the microsomal isoform of CPT1 was stimulated six to eightfold in both blood- and muscle cells ($r = 0.951$). There is strong evidence that this enzyme is involved in lipid synthesis as a

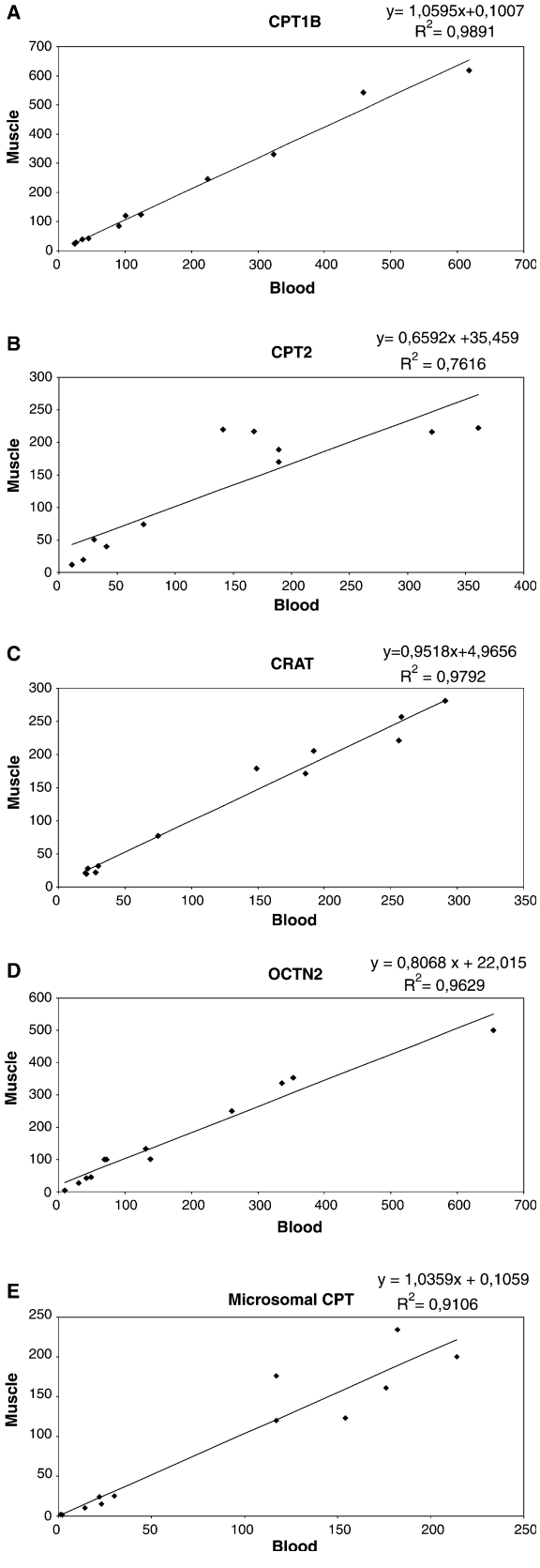

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Fig. 2 Correlation between relative mRNA levels as a percentage of standard gene (ß-actin) transcripts (= gene copies/ 100 copies ß-actin) before (= values below 100) and after 6-month-endurance training (= values over 100) in mononuclear blood cells and muscle tissue. **a** Carnitine palmitoyltransferase 1B, muscle isoform; **b** carnitine palmitoyltransferase 2, **c** carnitine acetyltransferase (CRAT); **d** organic cation transporter (OCTN2); **e** microsomal carnitine palmitoyltransferase

part of the FA shuttle mechanism from the cytoplasm to the microsomes. This relative increase in mRNA content of the microsomal CPT1 is in accordance with findings showing that increased muscle activity induces both palmitate oxidation and esterification into triacylglycerols and phospholipids (Bonen et al. 1999).

Presently, few data are available concerning the endocrine regulation of CPT2 and OCTN2, but the similarities of gene expression patterns in blood and muscle indicate that these genes might also be regulated by systemic factors. This suggestion is also supported by our previous finding showing that these genes are downregulated in blood cells of elderly persons as compared to adults (Karlic et al. 2002).

However, it appears also possible that enhanced fat oxidation after exercise training is also associated with the genes involved in regulating carnitine uptake by OCTN2 in blood cells and muscle across the plasma membrane and besides the known transport across the mitochondrial membrane (CPT 1B). Previous observations (Uenaka et al. 1996) showed that expression of more than 100 genes in different organs is affected in OCTN2-deficient knockout mice. A compensation of the defect in carnitine uptake in these mice by supplementation of high doses of L-carnitine reversed expression profiles of more than 100 genes of oxidative metabolism as well as carbohydrate- and nitrogen metabolism in these animals to the state of wild type animals.

A decrease in free carnitine availability has been discussed to be responsible for a downregulation of CPT1 during high-exercise training, which appears to be effected by, a decrease in cellular pH (Jeukendrup 2002) or hypoxic conditions as indicated by data from high altitude training, although soleus muscle appears to be less affected by hypoxia than e.g., extensor digitorum longus (Kennedy et al. 2001, Bigard et al. 1991).

There are several potential regulators of fat oxidation: for downregulation, the regulator is malonyl-CoA concentration, which is formed from acetyl-CoA, catalyzed by the enzyme acetyl-CoA carboxylase (ACC), which in turn will inhibit carnitine palmitoyl transferase I (CPT I). Another possible mechanism is accumulation of acetyl-CoA that will result in acetylation of the carnitine pool, reducing the free carnitine concentration. This could theoretically reduce FA transport into the mitochondria. There is also some recent evidence that CPT I activity is inhibited by small reductions in pH that might be observed during exercise at high intensities (Jeukendrup 2002).

On the other hand, it is known that exercise induces a decline in malonyl-CoA before muscle glycogen depletion and before onset of hypoglycemia (Winder and Hardie 1999). This is preceded by an exercise inducible

activation of AMP-activated protein kinase (AMPK) which phosphorylates and inhibits ACC2. This mechanism has the potential to prevent inhibitory effects of insulin and glucose on the rate of fatty acid oxidation (Winder and Holmes 2000). Although these enzymes were not analyzed in this study, it appears possible that these enzymes are also expressed at similar levels in blood and muscle cells as shown for carnitine acyltransferases.

Correlation of the established marker for aerobic capacity, namely, citrate synthase with CPT1 remains to be established and will be subject to further studies.

In conclusion, we found a concomitant stimulation of CPT1B, CRAT, GRP58 and OCTN2 transcription in blood cells and muscle tissue after a longer period of endurance training. Our data indicate that adaptation of oxidative metabolism in skeletal muscle associated to systemic processes in blood cells. Therefore, gene expression profiles derived from blood cells might be useful to evaluate the effects of endurance training and dietary interventions due to ease of access.

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