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Expression of Metabolic and Myogenic Factors during two Competitive Seasons in Elite Junior Cyclists

Expressionsveränderungen metaboler und myogener Faktoren während zwei Wettkampfsaisons in Junioren-Radrennfahrern

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Summary

- **We assessed whether** the seasonal training pattern of endurance athletes produces corresponding adaptations of mitochondrial, angiogenic, and myogenic processes in skeletal muscle and whether these relate to the training volume and metabolic load during exercise.
- **Aerobic performance** and content per actin of markers of mitochondrial respiration (NDUFA9, SDHA, UQCRC1, COX4AI, ATP5A1, UCP3), myogenic regulators (myogenin, myoD, tenascin-C) and MyHC were determined in m. vastus lateralis of ten male elite junior cyclists (17.3±0.2 years) after the preparation and competition phase over two seasons. Effects were assessed with a repeated measures ANOVA at a 5% significance level.
- **Energy spent in training** was comparable in the preparation and competition phase of the first season (9354 and 9825 MET x min/week), despite a 14%-reduced training volume, but decreased thereafter to 6800 MET x min/week. Average mitochondrial protein content changed in anti-cyclical pattern to VO_{2peak}, capillarisation, satellite cell and myonuclear number; being 22% and 45% higher after the preparation than the preceding competition phase in season 1 and 2. The content of tenascin-C (+34%), and myogenin (+166%), increased transiently after the second preparation phase.
- **The findings** emphasize a role of seasonal metabolic load for adjustments in muscle protein expression with training of junior cyclists and suggest that the capacity for mitochondrial biogenesis may become exhausted at the end of the competition phase when muscle enters a regeneration program.

KEY WORDS:
Competition, Training, Plasticity, Molecular Biology

Zusammenfassung

- **Wir verfolgten die Fragestellung,** inwiefern das saisonale Trainingsmuster von Ausdauersportlern Anpassungen mitochondrialer, angiogener und myogener Prozesse im Skelettmuskel beeinflusst, und ob diese auf das Trainingsvolumen und den Energieverbrauch zurückzuführen sind. Zehn männliche Junioren-Radrennfahrer (17,3±0,2 Jahre) wurden über zwei Wettkampfsaisons begleitet.
- **Die aerobe Leistungsfähigkeit** und der Gehalt pro Aktin von Markern der mitochondrialen Atmungskette (NDUFA9, SDHA, UQCRC1, COX4AI, ATP5A1, UCP3), myogener Regulatoren (Myogenin, MyoD, Tenascin-C), sowie Myosinschwerketten (MyHC) im M. vastus lateralis wurden nach der jeweiligen Vorbereitungs- und Wettkampfphase bestimmt. Effekte wurden unter einem 5%-Signifikanzniveau mittels einer ANOVA für Wiederholungen untersucht.
- **Der wöchentliche Energieverbrauch** in der Vorbereitungs- und Wettkampfphase der ersten Saison war trotz 14% tieferem Trainingsvolumen vergleichbar (9354 vs. 9825 MET x min / Woche), fiel danach aber auf 6800 MET x min/Woche. Der durchschnittliche Gehalt mitochondrialer Proteine verringerte sich jeweils nach der Wettkampfphase antizyklisch zur VO_{2peak}, zur Kapillarisierung sowie zu der Zahl der Muskelzellkerne. Der Gehalt von Tenascin-C (+34%), und Myogenin (+166%), war transient nach der zweiten Vorbereitungsphase erhöht und korrelierte mit dem Trainingsvolumen und dem Energieverbrauch bei höchster Intensität in der vorangehenden Trainingsphase (r=0.65).
- **Die Ergebnisse** unterstreichen die Rolle der saisonalen metabolen Belastung für Anpassungen der Muskelproteinexpression während des Trainings von Junioren-Radrennfahrern und deuten an, dass die Fähigkeit zur mitochondrialen Biogenese am Ende der Wettkampfsaison erschöpft ist und der Muskel zu diesem Zeitpunkt in ein Regenerationsprogramm eintritt.

SCHLÜSSELWÖRTER:
Wettkampf, Training, Muskelplastizität, Molekularbiologie

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Introduction

Competitive cyclists subject themselves to a high training volume that follows a seasonal pattern; where extensive phases with long rides in winter to spring dominate the preparative phase of training, while intensive types of exercise during races impact on the athlete during the competition phase from spring to late summer (30). In consequence of training and competition, endurance athletes demonstrate adjustments in energy supply lines and underlying gene expression in exercised muscles (14, 29). The implication of the two former processes in the progression of cycling performance and the seasonal patterning of training in the well-trained endurance athlete is little understood (19, 29).

Studies with non-specifically trained, adult subjects show that distinct adaptations, which improve local aerobic performance, occur in the course of a few weeks of stationary bicycle training in knee extensor muscle, m. vastus lateralis (15, 24). Main adaptations in this regard include increases in mitochondrial volume density and capillarisation, that enhance the capacity for aerobic metabolism (5, 10, 15). By contrast, few changes are observed for the cross sectional area and distribution of slow type muscle fibers, that could improve the economy of repeated muscle contractions (5, 24). Regulation of metabolic pathways with bicycle-type endurance training is supported by increased expression of mitochondrial proteins and gene transcripts being associated with the four electron transport complexes, as well as the coupled ATP synthase complex (5, 10, 23). Transcript level alterations in vastus lateralis muscle of untrained subjects indicate, that also a myogenic gene program is activated during recovery from bicycle-type endurance exercise (2, 24). Interestingly, these regulatory adjustments can be observed despite the virtually unchanged, cross sectional area and distribution of fiber types, with short term endurance training (5, 24). This observation relates to the observation, that satellite cell proliferation is increased in m. vastus lateralis of bicycle athletes during the pre-competitive season, before their fusion with muscle fibers is enhanced during the subsequent competitive season (13). In rodents, certain myogenic transcription factors, i.e. myogenin, appear essential for the expression of muscle-specific metabolic enzymes in rodents such as mitochondrial factors (16, 25). The findings raise the possibility, that myogenic processes are set in motion in exercised muscle to sustain the elevated protein turnover subsequent to the increased metabolic load and wear-and-tear of muscle with extensive exercise. Regarding the adjustments in muscle structure and mass, the duration and intensity of exercise and the consequent energy expenditure, may represent an important additional factor (18, 27).

We set out a study to address molecular regulation of mitochondrial and myogenic processes with endurance training of competitive cyclists and asked, whether this would show a seasonal pattern that is graded by energy expenditure during the preparatory and competitive phase(s). Towards the end, we recruited a group of elite junior cyclists, which typically demonstrate a considerable adaptive potential (8) and investigated the content of selected mitochondrial proteins and myogenesis associated proteins in m. vastus lateralis. On one hand, this comprised markers for electron transport complex I (NDUFA9), complex II (SDHA), complex III (UQCRC1) and complex IV (COX4I1), as well as a marker for the coupled ATP synthesis in complex V (ATP5A1) and the uncoupling of this process (UCP3). On the other hand, this included myogenic factors myogenin, myoD and tenascin-C, which are regulated by exercise (4, 11, 12, 25). Subsequently, we tested their relationship to the metabolic load (or energy expenditure) during training and reported alterations in satellite cells and myonuclear number, fiber types and capillarisation in a larger population of subjects (13). We thereby hypothesized, that expression changes of myogenic and mitochondrial proteins would relate to the time and energy spent in the different intensity zones of training, and show a seasonal pattern.

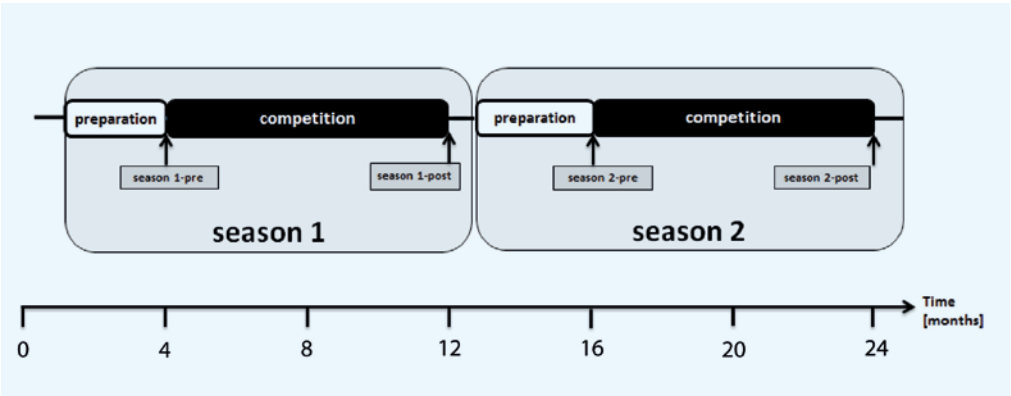

Figure 1

Overview of the experimental design. Subjects entered the study after ≥ 5 years of prior performance orientated endurance training and competition. Four tests (indicated by boxes with arrows), each comprising an exercise test and biopsy were carried out on elite junior cyclists over two seasons, each of which was comprised of a preparation and a competition phase.

Materials and Methods

Ethics

This longitudinal study was approved by the Institutional Ethics Committee (German Sport University Cologne, Cologne, Germany) which was conducted in accordance with the Declaration of Helsinki. Subjects were recruited from several regional junior cycling teams. Inclusion criteria were male gender and active participation in competitive cycling events at the national level as a junior (i.e. 17-18 years of age) under a licence by the International Cycling Union (UCI). Exclusion criteria included drug consumption and contraindications for endurance training such as cardiovascular, metabolic and inflammatory disorder or any signs of infection. Participants and their guardians gave their written informed consent after having been informed of the experimental protocol and potential risks involved.

Design

The cyclists entered the study after a preparation phase of three months and then passed in the 8-month of the competition phase, which was followed by a ~4 week 'regenerative phase' before the phases were repeated over a second season, which were performed based on the instructions by the coaches (Fig. 1). The cyclists underwent tests and an assessment of the health status in a physical examination instructed by the formalities of the UCI: In the preparation phase, the test session took place in February several days before the start of the competition phase (from the month of February to October). During the duration of the study, both in the preparation and competition phases, the subjects maintained their regular exercise training and competition program. Within one week after the end of the competition phase all subjects performed a post-season testing. The same procedure was repeated over a second season for the subjects, which continued the study into the second season. All subjects were familiar with the general laboratory and exercise testing procedure. Subjects completed a log-book of the training load for the first season and the preparation phase of the second season. Biopsies were subjected to immunohistochemical and biochemical analysis. ›

Table 1

Biometric data and physiological characteristics of the cyclists at baseline. Values refer to mean±standard error. Season 1 pre=time point before the competitive phase of the first season. N=10 (except satellite cell number where N=8).

PARAMETER	SEASON 1-PRE
BMI (kg/m²)	20.9±0.5
Body mass (kg)	69.7±1.6
VO_{2peak} (ml/min/kg)	64.9±1.6
VO_{2peak} (ml/min)	4.5±0.2
Peak power (W)	346.4±10.6
Skeletal Muscle	
Leg muscle mass (kg)	20.9±0.4
Fiber Type Distribution	
Type I [%]	63.3±1.5
Type II [%]	36.7±1.5
Minor Fiber Diameter	
Type I [µm]	66.9±2.4
Type IIA [µm]	69.0±2.8
Type IIX [µm]	62.6±1.5
myonuclear number [per mm fiber]	119.2±16.1
satellite cell number [per mm fiber]	13.7 ±1.0
capillary-to-fiber ratio:	2.28±0.12
capillary density [mm-2]:	306.0±3.68

Table 2

Changes in physiological characteristics of the cyclists with preparation and competition. Values refer to mean±standard error of the differences vs. season 1 pre. N=10. *denotes p-values<0.05 vs. season 1 pre (post-hoc test of Fisher for a repeated ANOVA). Season 1 pre=time point before the competitive phase of the first season; Season 1 post=time point after the competitive phase of the first season; Season 2 pre=time point before the competitive phase of the second season; Season 2 post=time point after the competitive phase of the second season.

VS. SEASON 1-PRE	SEASON 1-POST	SEASON 2-PRE	SEASON 2-POST
BMI (kg/m²)	1.4±3.4%	3.4±4.0%	0.5±5.3%
body mass (kg)	1.3±3.1%	5.2±3.8% *	3.3±4.4%
VO_{2peak} (ml/min/kg)	7.9±3.5% *	0.2±4.1%	-4.0±3.3%
VO_{2peak} (ml/min)	11.1±6.0%	6.7±6.1%	0.0±6.3%
Peak power (W)	-4.3±4.4%	0.0±4.5%	0.5±4.6%

Table 3

Changes in muscle characteristics of the cyclists after the first competitive season.Values refer to mean±standard error of the differences vs. season 1 pre N=10 (except satellite cell number where N=8). *, and ** denote p<0.05 and p<0.01, respectively (post-hoc test of Fisher for a repeated ANOVA).

PARAMETER	DIFFERENCE VS. SEASON 1-PRE
Leg muscle mass (kg)	0.5±2.7
Fiber Type Distribution	
Type I [%]	-9.5±5.9%
Type II [%]	16.3±8.9
Minor Fiber Diameter	
Type I [µm]	-3.1±4.9%
Type IIA [µm]	-8.3±5.1%
Type IIX [µm]	1.9±3.7%
myonuclear number	125.4±14.3%**
satellite cell number	-22.9±10.8%**
capillary-to-fiber ratio	20.6±6.7%**
capillary density [mm-2]	18.9±4.0%**

Subjects

Ten active, well-trained, junior male competitive cyclists (age, 17.3±0.2 years; height, 182.8±2.3cm; means±SE) participated in this study. Further biometric data and physiological characteristics of the cyclists at baseline are shown in table 1. All athletes were national level road cyclists and they had an exercise volume between 13.500-19.500 kilometers per year with at least five years competition experience.

Training

Each subject trained alone and in groups during the preparation and competition phase. The training in the preparation phase consisted in sport-specific long slow-distance type-training. Cyclists carried out their structured regular conditioning and competition program without additional training interventions or alteration by the authors, except for the testing sessions.

The study participants were requested to keep a record of their daily training and competition data. This was quantified in terms of the training volume and intensity as recorded, based on measurement of heart rate (Polar S710i, Polar, Kempele, Finland). The time spent in cyclists' individual training zones was documented in a modified classification scheme for physical activity, based on relative exercise intensities. The recorded intensities covered ten zones ranging from moderate (i.e. <70% of peak oxygen uptake (VO_{2peak})), to hard (i.e. 70–90% of VO_{2peak}) and very hard (i.e. >90% of VO_{2peak}) intensity as estimated, based on the relationship between heart rate and VO_2 (7). For practical reasons, the duration of exercise was recorded for the races without separation of intensities and the different intensity zones of the hard and very hard training zone, each, were combined and referred to as high and very high intensity zones of exercise. For comparison of the proportion of training being spent at relative intensities between the training phases, weekly total and relative exercise time were calculated. Energy expenditure was estimated as metabolic equivalents (METs) based on the time spent in the different intensity zones of exercise from standard tables of oxygen consumption, individual VO_{2peak} and the conversion factor 3.5 ml/min/kg=1MET.

Test Sessions

Cyclists reported to the laboratory where they underwent a test battery under standardized conditions consisting of anthropometric measures, an incremental exercise test and a muscle biopsy 3 days later. All subjects were instructed to refrain from strenuous exercise for 48 h preceding the tests.

All cyclists were briefed to ensure a high carbohydrate diet, which they usually maintain when preparing for a race. All cyclists were asked to refrain from alcohol and tobacco at least 24 h before testing and to avoid the intake of coffee, tea as well as food and drinks enriched with processed sugar for at least 8 h prior to tests.

On the test day, a fasting period of at least three hours was incorporated before the start of the measurements. Body and leg muscle mass were recorded using bioelectrical impedance analyzer (BC-418, Tanita Corporation, Tokyo, Japan). Afterwards all subjects performed an incremental exercise test to volitional exhaustion in order to assess VO_{2peak} and PPO on an electromagnetically braked cycle ergometer (SRM-Ergometer, Schoberer Rad Messtechnik GmbH, Jülich, Germany). The incremental cycling test started at 100 W, increased by 40 W every 5 min at constant cadences between 80–90 r/min throughout the test.

Table 4

Correlation between in muscle and exercise characteristics of the cyclists over two years of training. R-values and p-values of correlations which met the criteria of r≥0.65, p≤0.05 (Pearson correlation).

PARAMETER 1	PARAMETER 2	R-VALUE	P-VALUE
Correlations between Muscle Parameters			
Mitochondrial protein	Satellite cell number	-0,651	0,049
Fast MyHC	Capillary-to-fiber ratio	-0,824	0,023
Slow MyHC	Actin	0,854	0,007
MyoD	Leg muscle mass	0,835	0,01
MyoD	PPO	-0,701	0,008
MyoD	Relative VO_{2peak}	0,784	0,002
Capillary density	Satellite cell number	-0,653	0,01
Correlations to Training Volume			
Tenascin-C	VOL high intensity preceding	0,65	0,014
Myonuclear number	VOL moderate intensity preceding	-0,688	0,001
Myonuclear number	VOL very high intensity preceding	0,747	0,001
Capillary-to-fiber ratio	VOL total intensity preceding	-0,682	0,001
Capillary-to-fiber ratio	VOL moderate intensity preceding	-0,676	0,001
Capillary density	VOL very high intensity preceding	0,75	0,001
Myonuclear number	VOL very high intensity proeceeding	-0,849	0,001
Mitochondrial protein	VOL very high intensity proeceeding	0,835	0,048

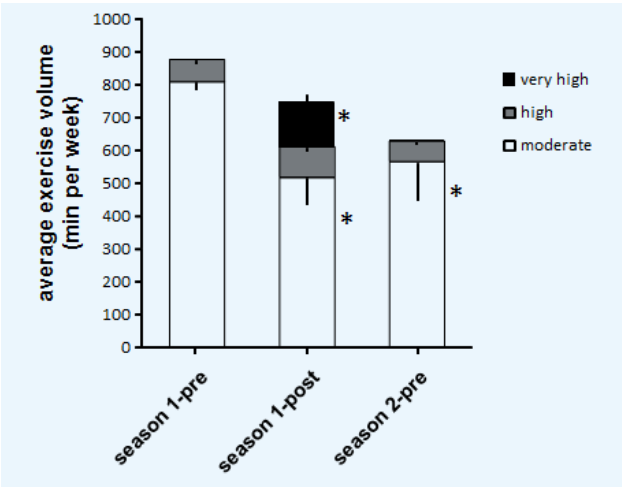

Figure 2

Training volume and intensity in the training phases. Bar graph visualizing the cyclists' average weekly exercise volume (in minutes) spent in the different intensity zones of training and competition during the preparation phases (season 1-pre and season 2-pre) and competitive phase (season 1-post) of the two seasons. Training data were not reported during second competitive phase (season 2-post). *p<0.05 vs. season 1-pre, post hoc test of Fisher (ANOVA).

Muscle Biopsies

After local anesthesia (1% lidocaine), percutaneus needle biopsies (60-95 mg) were taken from m. vastus lateralis at one-third (±2cm) of the distance between patella and anterior superior iliac spine as previously described (13). After removal from the leg, muscle biopsy samples were quickly freed from visible blood and fat, and sectioned into two portions. The larger portion was embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands), immediately frozen in liquid nitrogen-cooled isopentane and stored at -80°C. The other portion was fixed in aldehyde and embedded in adhesive.

Immunohistochemistry and Morphometry

Muscle fiber types, minor fiber diameter, myonuclear number and satellite cell number were quantified on serial transverse cryosections of the collected biopsies, as previously described for a subset of the athletes (13).

Biochemistry

Detection of proteins was carried out using immunoblotting of total homogenates and signal detection with enhanced chemoluminescence (SuperSignal West Femto, Pierce) as described, but with the modification, that proteins were separated on 10%-Mini-Protean-TGX Precastgels (BioRad, cat. 456-8026) and blotted onto the nitrocellulose (BioRad, cat. 170-4158) for the detection using a Turbo-System (Biorad) (5). A paired design was applied with all samples from the same animal being loaded in adjacent lanes of the same gel. The following proteins were assessed using the following primary antibodies: tenascin-C (B28.13, gift of Prof. Matthias Chiquet, University of Berne); myoD-C20 (Santa Cruz, sc-304), myogenin-F5D (Santa Cruz, sc-12732), Anti-OxPhos Complex Kit (NDUFA9, SDHA, UQCRC1, COX4I1, ATP5A1; Invitrogen, cat.457999), UCP3 (Millipore, #AB3046) and actin (Sigma, cat. A2172). Signals were recorded using a Chemidoc system PXi system (Syngene) and the intensity of the respective band quantified with Quantity One software (Bio-Rad, Life Science Research, Hercules, CA, USA). The signal intensity of the respective band was estimated with the 'volume rectangular tool' and was corrected versus the background of a band of equal height and size (area) from an empty sample lane. Background-corrected data were standardized to actin signal, normalized to the mean values of the samples before training for the respective gel. Normalized values of the detected mitochondrial proteins were highly correlated and therefore also combined to reveal average mitochondrial protein content per actin protein.

Statistics

All statistical tests were carried out with SPSS software (IBM version 22). First a Shapiro-Wilks test to verify, whether the data were normally distributed for all phases of the training (i.e. season 1-pre, season 1-post, season 2-pre, season 2-post). Effects were verified with a Friedman ANOVA or ANOVA with repeated measures for the repeated factor 'training phase' depending on whether the hypothesis of a normal distribution could be rejected at p<0.05. The test statistics (comprising χ- or F-values, significance (p) and effect size (η^2) were recorded for each test. For non-parametric data, effects were localized post-hoc with a Friedman ANOVA for the two training phases to be compared. For normally distributed data, effects were localized with a Fisher test. Post hoc differences were also localized in the case, when the main effect of the ANOVA test did not reach statistical significance in order to assign effects between training phases. Results are expressed as mean±standard errors. Linear relationships were verified with Pearson correlations, whether they reached a level of r≥0.65, p≤0.05.

Results

Subjects

The biometric data, physiological values, and muscle characteristics of the ten elite junior cyclists at baseline are given in table 1.

Seasonal Effect of Training

The time spent in the moderate, high and very high intensity zones of exercise significantly differed between the different training phases of the cyclists (Fig. 2). In consequence, the ›

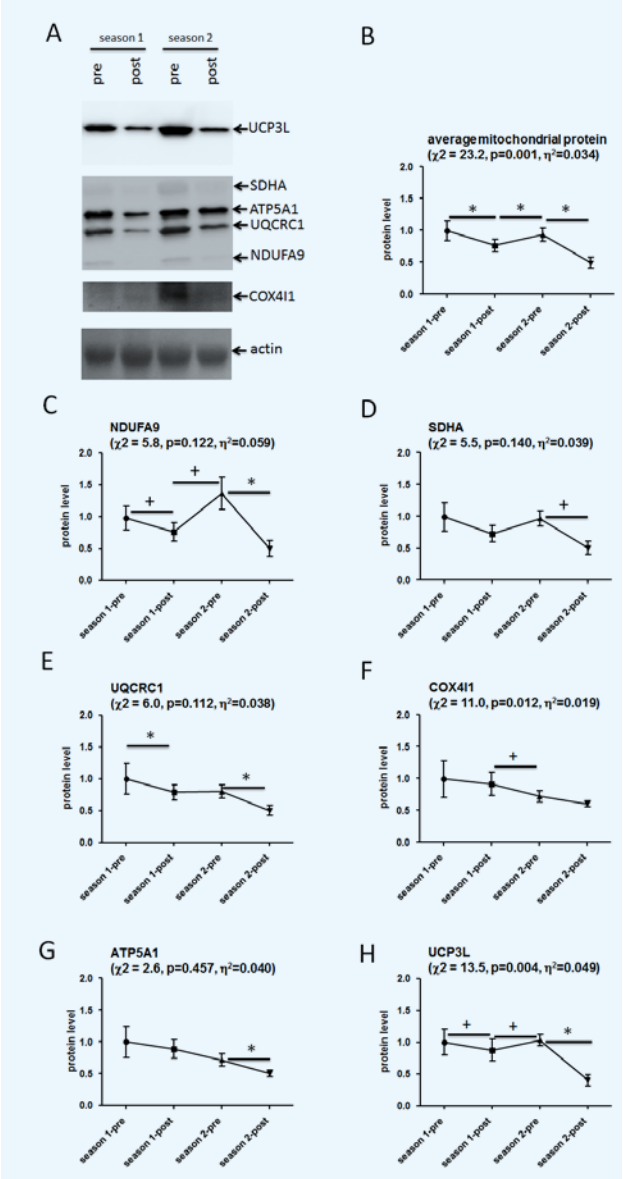

Figure 3

Mitochondrial protein concentration in m. vastus lateralis. A) Composite panel visualizing detection of selected mitochondrial proteins at four time points of season in ten elite junior cyclists as detected in immunoblots and actin loading control (bottom). B-H) Line graphs summarizing the mean ±SE in the content of average (B) or individual mitochondrial proteins (C-H) per actin. The corresponding values of the test statistics, i.e. χ-value; significance, p; and effect size, η^2, are given in the heading of each figure panel. df=3 for all comparisons. *and + denote $p<0.05$ and $0.05\leq p<0.10$, respectively, for the indicated comparison (Friedman ANOVA).

average energy expended per week of training differed between the training phases. Energy expenditure reached 9354±485 and 9825±404 METs x min/week, respectively, in the preparation and competition phase of the first season, and the fell to 6800±917 METs x min/week in the preparation phase of the second season. On average, the cyclists spent an equivalent of their basal metabolism during exercise (i.e. 0.9 METs).

The changes in physiological values of the cyclists with the two years of training are given in table 2. Body mass-related peak oxygen uptake, but not peak power, body mass or leg muscle mass, was increased after the competition phase of the first season. Body mass was increased after the preparation phase of the second season.

Fiber size, based on measurements of the minor fiber diameter, and fiber type distribution, were not altered after the first competition phase (Table 3). Myonuclear number was increased by 125% after the first competition phase while the number of satellite cells was decreased by 23%. Capillary-to-fiber ratio and capillary density was increased after the competition phase by 21% and 19%, respectively.

Muscle Protein Expression

Six mitochondrial proteins encoding respiratory chain constituents (NDUFA9, SDHA, UQCRC1, COX4I1, ATP5A1) and UCP3L were detected (Fig. 3A) and their average content per actin protein was analyzed. Average mitochondrial protein content showed a zigzag pattern, being lowered after each of the competition phases while recovering in the preparation phase in-between (Fig. 3B). This pattern was essentially reflected by the expression of the individual proteins. Thereby the fall in NDUFA9, SDHA, UQCRC1, ATP5A1 and UCP3L after the last competitive season was most prominent (Fig. 3H). Sarcomeric actin, slow and fast myosin expression was not significantly affected by competition (Fig. 4). Fast type myosin heavy chain content showed a trend for a reduction after the first competitive season (Fig. 4D). The myogenic factors, myogenin and myoD, and the large tenascin-C isoform being implicated in muscle regeneration (11), were amply expressed in m. vastus lateralis (Fig. 5). Myogenin and tenascin-C demonstrated an increase after the preparation phase of the second compared to the first season, and decreased thereafter during the competition phase (Fig. 5B,D).

Correlations

The content of mitochondrial proteins were highly inter-related (data not shown). MyoD was correlated to indices of maximal aerobic performance, i.e. PPO, relative VO_{2peak}, and leg mass (Table 4). Mitochondrial protein content and fast myosin heavy chain content were negatively correlated to satellite cell number.

Tenascin-C content, myonuclear number and capillary density correlated positively to the time spent in the high and very high intensity in the preceding training phase (Table 4). Conversely, tenascin-C and myonucler number correlated negatively to the total time spent in training during the proceeding training phase. Mitochondrial protein correlated with the training volume spent in the very high intensity zone in the proceeding training season. Qualitatively similar relationships were found for the energy spent in training (data not shown).

Discussion

Pronounced increases in mitochondrial and capillary density of knee extensor muscle have been documented to take place within a few weeks in young untrained subjects with endurance training on stationary bicycles (10, 17). Considerably less is understood regarding the adjustments of well-trained cyclists to endurance stimuli and particularly competition. In order to fill this gap, we monitored the adjustments in competitive performance and muscle composition of young elite cyclists after the preparation and competition phase over two seasons in relation to training volume and energy expenditure. Our investigation into the adjustments of metabolic and myogenic processes in m. vastus lateralis therefore fills the gap of knowledge on mechanisms of muscle plasticity between elite sportsmen and beginners, and this has practical implications.

Current training concepts are based on physiological measures of performance and mainly weighed by success in competition (30). This is especially true for professional cyclists,

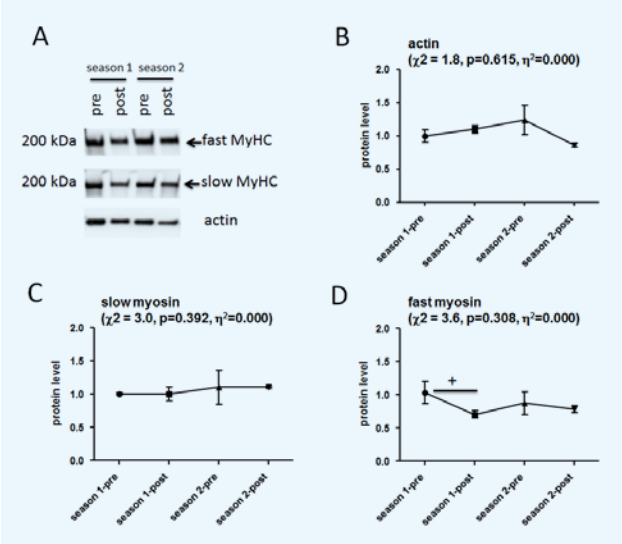

Figure 4

Expression of contractile proteins. Composite panel visualizing the detection of sarcomeric actin, slow and fast type myosin heavy chain in immunoblots (A, indicated by arrows). B-D) Line graphs visualizing the mean±SE for the detected proteins at each of the four time points, for the ten elite junior cyclists. The corresponding values of the test statistics, i.e. χ-value; significance, p; and effect size, η_2, are given in the heading of each figure panel. df=3. * and + denote p<0.05 and 0.05≤p<0.10, respectively, for the indicated comparison (Friedman ANOVA). N=10.

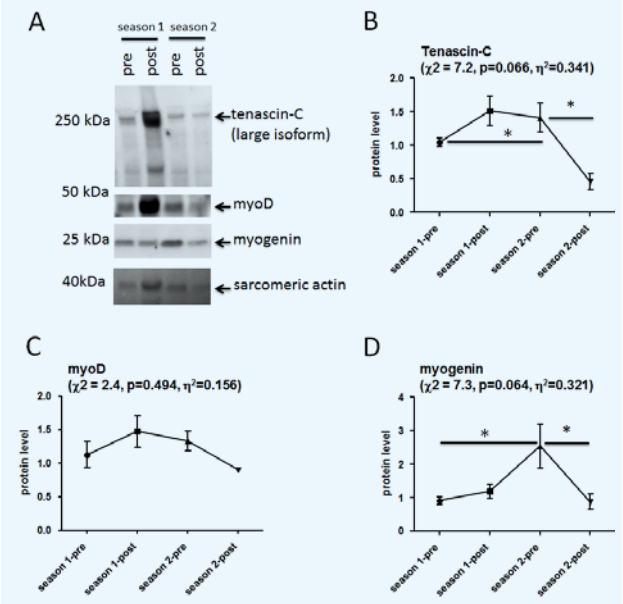

Figure 5

Myogenic factor expression. A) Composite panel visualizing the detection of tenascin-C, myoD, myogenin in immunoblots (indicated by arrows) relative to molecular weight markers (left) and the loading control of sarcomeric actin (bottom). B-D) Line graphs visualizing the mean±SE for the detected regulators of myogenesis (tenascin-C, myoD, myogenin) at each of the four time points, for the ten elite junior cyclists. The corresponding values of the test statistics, i.e. χ-value; significance, p; and effect size, η^2) are given in the heading of each figure panel. df=3. * and + denote p<0.05 and 0.05≤p<0.10, respectively, for the indicated comparison (Friedman ANOVA).

which typically adhere to conservative types of training with high volumes and little time to recover during competition (7, 17). Molecular biological tests in exercised muscle have been suggested as possible venue to refine training, because they resolve adaptations in more detail than the course measurement of ergospirometry (5, 20). Here we identify, that altered expression of mitochondrial proteins, and tenascin-C was detectable after specific time points of the seasonal training (Fig. 3B, 5B), and correlated to parameters that are relevant to training (i.e. the volume spent in different intensity zones of training, Table 4). As well we identify that protein content of the myogenic factor, myoD, whose expression was unaffected at the investigated time points (Fig. 5C) correlated to leg mass, relative VO_{2peak} and power output. These findings provide first evidence for the sensitivity of molecular measures for monitoring training-induced adjustments in the population of elite junior cyclists.

Adaptations in skeletal muscle mitochondria and capillaries with endurance training, have been suggested to attenuate after a period of endurance training (9, 22). This ceiling effect is supported by measurements of gene transcripts in m. vastus lateralis, which show that the magnitude of transcript level alterations during recovery from exercise is reduced after 6 weeks of endurance training (2, 24). In contrast, our data emphasize, that adaptations in mitochondria and capillaries still occur in skeletal muscle of well-trained subjects, which expend a near equivalent of basal metabolism during their endurance training.

Interestingly, we find opposite patterns of level adaptations for mitochondrial proteins with competition and preparation. Thereby the content of the combined mitochondrial proteins was reduced after the more intense competition phase and increased after the extensive type of training during the preparation phase (Fig. 3). This finding contrasts with the classic view that endurance training per se elevates the content of mitochondrial protein in exercised muscle. It raises the question, whether the synthesis and degradation of mitochondria is out of balance with competitive types of endurance exercise balance as previously reported (21). In this regard, it is of interest, that the content of mitochondrial protein correlated to the time and energy spent in very high intensity zones in the proceeding phase (table 4). This hints that local aerobic capacity in knee extensor muscle of the studied cyclists did affect the selected duration of exercise in high intensity zones. Notably, these zones were only attained during the competition phase. It remains to be assessed, whether the identified relationships may reflect the influence of a high mitochondrial content on the athlete's choice to select high intensity zones during high training.

The concentration of mitochondria per muscle fiber volume is understood to represent a critical parameter for maximal aerobic capacity and economy of locomotion (28). Especially uncoupling protein 3 has been argued as a critical factor for cycling efficiency (19). From this perspective, the distinct reduction in the content of the large UCP3 isoform, UCP3L, and average mitochondrial protein content after each competition phase (Fig. 3B, 3H) is intriguing. It may suggest a reduced local aerobic capacity in m. vastus lateralis. In this regard, it is of interest to consider that VO_{2peak}, which integrates muscle oxygen consumption, cardiovascular and lung function (6), was increased after the first competition phase being investigated. The increase in VO_{2peak} occurred concomitantly with an increase in capillary density and capillary-to-fiber ratio, which did correlate to the volume spent in very hard and moderate intensity, respectively, in the preceding training phase (Table 4). Muscle capillarisation is understood to contribute, in conjunction with mitochondrial content, to an important degree to maximal ›

oxygen uptake at the muscle level (6). Thereby an increase in capillarisation is thought to improve oxygen exchange capacity (26). Thus seasonal variations in whole body aerobic capacity in the studied cyclists were related to opposing adaptations of capillaries and mitochondria.

The identified phasic level alterations in mitochondrial protein in m. vastus lateralis at the end of the preparation and competition phase (Fig. 3) have bearing on the interpretation of current practice in endurance sports. The observed down-regulation of mitochondrial protein at the end of the competition phase effectively suggests a reduced capacity of the studied vastus muscle to respond to the repeated impact of high intensity endurance exercise with an elevated capacity for oxygen-dependent energy metabolism in mitochondria. The concomitantly increased VO_{2peak} implies, that potential deficits in local aerobic capacity with repeated hard exercise are compensated by an increased capillarisation (Table 2). Capillary density was positively correlated to the time spent in the very high intensity zone, including competition, in the subsequent training phase (Table 4). This observation hints, that the lack of an essential performance enhancement over the two years of training is due to a reduced time allocated in high intensity zones of training. This view raises the suggestion that a polarized training scheme, incorporating high intensity bicycle exercise during the preparation phase, could be beneficial to improve aerobic scope in the studied cyclist during competition.

In this study, tenascin-C protein content was positively correlated with the time (and energy) spent in the high intensity zone of exercise in the training phase prior to the biopsy sampling. This suggests, that energy expenditure during muscle work and expression of this regeneration-associated extracellular matrix protein (11) are related. The notion of a relationship between regeneration-associated factors and energy expenditure was also indicated by positive and negative correlations between myonuclear number and the energy expended in the very high intensity zone of the preceding and proceeding training phases (Table 4). This finding mirrors previous observation showing that expression of tenascin-C in skeletal muscle is enhanced by mechanical stimuli (4, 11, 12). The observed positive relationship between tenascin-C protein content and energy expenditure during high intensity exercise indicates, that the impact of mechanical factors during endurance type exercise is reflected by tenascin-C expression in skeletal muscle.

The considerable reduction in the content of mitochondrial protein after the competition phase highlights a negative influence of competition on mitochondria. Such a relationship was previously suggested from an investigation into the effect of strenuous endurance exercise (21). Interestingly, the reduction in average mitochondrial protein content after the competition phase occurred coincidental with an increase in myonuclear number (Table 3) and was followed by increased content of myogenin and tenascin-C in the subsequent preparation phase (Fig. 5B/D). To our knowledge, these findings are the first to highlight the regulation of myogenic processes by endurance training in competitive sportsmen (Table 4). This view is supported by the negative correlation between mitochondrial protein content and satellite cell number (Table 4). Our findings are in line with newer considerations on the role of myogenic processes for muscle adaptation and repair (3); raising the questions, whether a memory effect of exercise, as reported previously in the rat (1), may explain our observation. In this regard, the identified up-regulation of the transcription factor myogenin and tenascin-C after the preparation phase of the second season is of specific interest (compare Fig. 3, 5D), because myogenin controls transcript expression for genes being involved in mitochondrial metabolism (16) and is controlled by tenascin-C expression (11). Overall our present novel observations on the linear relationship between the duration of intense exercise and the studied myogenic markers (tenascin-C and myonuclei), provides arguments of how to maximize regenerative reactions in muscle and suggests this can be steered through the classical monitoring of the time spent in different intensity zones during bicycle training (and competition).

Limitations

A number of factors may confound the interpretation of data originating from the investigation of human subjects over extended periods of time and outside the context of a confined laboratory. Towards this end, care was taken to record the volume and intensity of exercise and standardize the sampling of muscle biopsies, to control possible sources of error. In this regard we note that morphometric assessment of muscle fibers was not carried out for the second season, due to insufficient amounts of biological material for the preparation of high quality cross sections. A major point was, that the principal investigators did not have influence on the training load of the subjects and that training load was not systematically recorded during the competitive phase of the second season due to the reality-driven circumstances that were dictated by the race calendar and personal motivation.

Conclusion and Perspective

Our study of elite junior cyclists shows that mitochondrial protein content in the main knee extensor muscle, vastus lateralis, show seasonal alterations that are anticyclical to changes in muscle capillarisation and myonuclear number. The inverse relationship between average mitochondrial protein content and satellite cell number implies, that the capacity to replenish mitochondrial protein and myonuclei became exhaust in the studied elite athletes during the competition phase. Correlations between myonuclear number, and tenascin-C, with the volume of high intense exercise in the preceding training phase suggest, that regenerative processes in exercised muscle are graded to metabolic or mechanical load during physical activity. Thus, the present novel observations motivate investigations into how concepts of muscle plasticity can be applied, to refine training of parameters that set aerobic capacity at the local level in the competitive endurance athlete.

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Conflict of Interest
The authors have no conflict of interest.

Abbreviations

ANOVA – analysis of variance
ATP5A1 – ATP synthase subunit alpha
ATPase – adenosine-triphosphatase
BMI – body mass index
COX4I1 – cytochrome c oxidase subunit 4 isoform 1
DAB – 3,3`-diaminobenzidine tetrahydrochloride
EE – Energy expenditure
MET – metabolic equivalent of task
MyHC – myosin heavy chain
myoD – myoblast determination protein 1
NDUFA9 – NADH dehydrogenase ubiquinone 1 alpha subcomplex subunit 9
Pax7 – paired box transcription factor 7
PPO – peak power output of aerobic exercise
RNA – ribonuclei acid
SDHA – succinate dehyrogenase A
SE – standard error
UQCRC1 – ubiquinol-cytochrome c reductase complex subunit 1
UCP3 – uncoupling protein 3
UCP3L – large isoform of uncoupling protein 3
VO_{2max} – maximal oxygen uptake
VO_{2peak} – peak oxygen uptake
Relative VO_{2peak} – body mass related peak oxygen uptake
VOL – Volume of training in min in a respective intensity zone

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ERWEITERTES ABSTRACT

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Expressionsveränderungen metaboler und myogener Faktoren während zwei Wettkampfsaisons in Junioren-Radrennfahrern

Expression of Metabolic and Myogenic Factors during two Competitive Seasons in Elite Junior Cyclists

Einleitung und Fragestellung

Ausdauerathleten absolvieren ihre Vorbereitungs- und Wettkampfphase bei unterschiedlich hohen Trainingsumfängen und -intensitäten. Ziel der hier vorliegenden Studie war es zu klären, inwiefern muskuläre Prozesse durch die Trainingsphase beeinflusst werden, und ob diese auf das Trainingsvolumen und den Energieverbrauch zurückzuführen sind.

Design der Studie und Methoden

Zehn männliche Junioren Radrennfahrer (Alter: 17,3 Jahre; peak Sauerstoffaufnahme: 64,9ml/min/kg) wurden über zwei Wettkampfsaisons begleitet. Neben der Messung der aeroben Leistungsfähigkeit mittels Spiroergometrie, wurden die Muskelfaserzusammensetzung, sowie Marker der mitochondrialen Atmungskette und der Myogenese in Biopsien des Kniestrecker Musculus vastus lateralis nach der Vorbereitungs- und Wettkampf-phase über zwei Saisons hinweg bestimmt (Abb. 1).

Ergebnisse und Diskussion

Die peak Sauerstoffaufnahme und das Verhältnis Kapillare/Muskelfaser im untersuchten Kniestrecker waren nach der ersten Wettkampfphase um 8%, respektive 21%, erhöht. Der durchschnittliche Muskelgehalt mitochondrialer Proteine verringerte sich jeweils nach der Wettkampfphase antizyklisch zur gemessenen peak Sauerstoffaufnahme (Abb. 1) zur Kapillarisierung, sowie zu der Zahl der Muskelzellkerne. Der Gehalt der myogenen Regulatoren Tenascin-C (+34%), und Myogenin (+166%), war transient nach der zweiten Vorbereitungsphase erhöht und korrelierte mit dem Trainingsvolumen und dem Energieverbrauch bei höchster Intensität in der vorangehenden Trainingsphase (r=0.65).

Was ist neu und relevant?

Die identifizierten molekularen Veränderungen im Muskelgewebe während einer Wettkampfsaison ergänzen das althergebrachte Konzept zur Trainingssteuerung über Volumen und Intensität. Insbesondere unterstreichen die Messungen, dass die Fähigkeit zur mitochondrialen Biogenese bereits am Ende der Wettkampfphase erschöpft ist. In Folge einer Saison mit hoher Trainingsintensität und -volumen tritt der Muskel anschließend in ein Regenerationsprogramm ein.

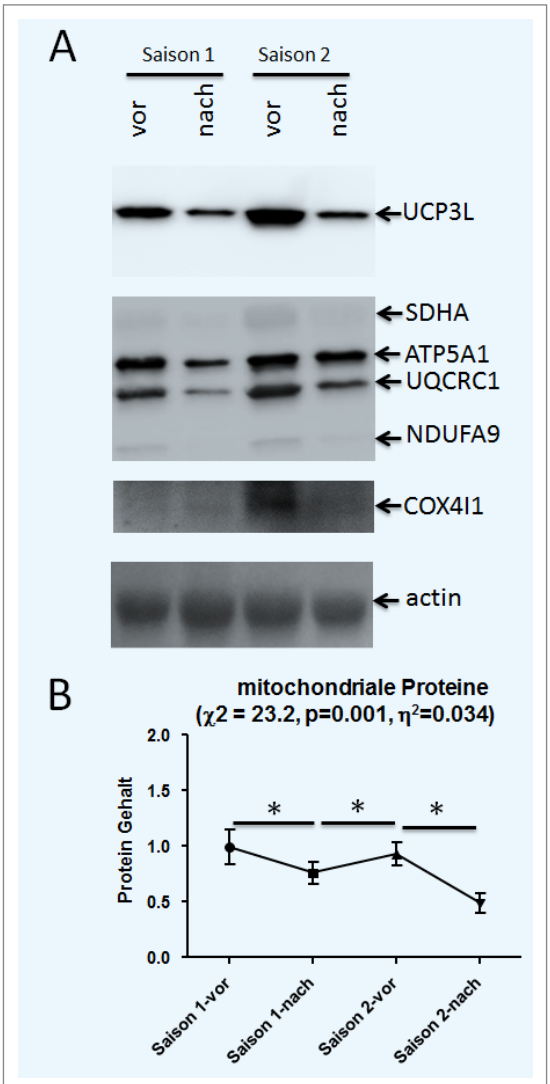

Abbildung 1

Gehalt mitochondrialer Proteine im Kniestrecker-Muskel eines Junioren Radrennfahrers. A, B) Exemplarische Detektion mittels Immunoblot (A) und Liniengrafik (B) des Gehaltes ausgewählter Atmungsketten Proteine vor und nach der jeweiligen Wettkampfsaison. Die Positionen der detektierten Proteine sind mit Pfeilen markiert.