THE EXTRACELLULAR NADH METABOLISATION ASSAY - ENMA - A NEW MARKER FOR CELLULAR ENERGY

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OBJECTIVE:

The suitability of the new extracellular NADH metabolisation assay (ENMA) as a marker of accurate cellular energy levels during physical exercise was tested. Blood was collected from highly conditioned athletes at various time points before and after exercise. NADH consumption (ENMA) was compared to ATP/ADP ratios.

METHODS:

Extracellular NADH metabolisation assay (ENMA)

500 µl of EDTA-blood (analysed between one and four hours after collection) are diluted with 1xPBS (8 g/l NaCl; 0.2 g/l KH_2PO_4; 1.15 g/l Na_2HPO_4; adjusted to pH 8,0 with 6M KOH) in a Centrisart I filter tube with a cut off range of 20.000 MW (Sartorius). 50 µl of NADH solution (8 mg NADH/ml 1xPBS) are added to start the reaction. This mixture is incubated for two hours at 37°C. The reaction is stopped by centrifugation for 10 minutes. The filtrate which contains NADH not consumed by the reaction is collected and analyzed by HPLC. Two standards with 2 ml 1xPBS including a) 25 µl or b) 50 µl of NADH solution (described above) are incubated under the same conditions and used to quantify the metabolized NADH amount.

HPLC determination of NADH, ADP and ATP

The determination is in principle according to the method of Formato et al. [1] and described in detail in Nadlinger et al. [2]. In brief: A Shimadzu LC10A System was used with a Lichrospher RP18, 5 µm 250x4 mm (Merck) column. NADH was measured at 340 nm with a diodearray detector (Shimadzu SPD-M10A).
The result of the assay was calculated as mg of NADH not metabolized in 10 ml blood compared to the 50 µl NADH standard (8 mg NADH, 0 mg NADH metabolized).
For measuremaent of ADP and ATP we used an alkaline extraction procedure according to Stocchi et. al [3]. The adenine nucleotides were measured at 254 nm with HPLC. The ratio of ATP/ADP is expressed as µM ATP divided by µM ADP.

Practical application of the ENMA

The assay was used in a study, which included 14 highly conditioned endurance athletes who had a significant higher aerobic performance compared to normal healthy individuals. In these athletes the extracellular NADH metabolisation in blood cells was determined on the morning, directly after a warm up, immediately after a maximum aerobic performance test on a treadmill and the next day.

RESULTS:

Directly after warm up, all athletes show a higher NADH metabolisation compared to baseline ($p<0.01$). The increase of NADH metabolisation is varying in an individual manner and reaches equivalent values after maximum aerobic performance. At this time point the concentration of NADH not metabolised by blood cells declines dramatically ($p<0.01$). The residual NADH not consumed by the blood cells decreases to 7% of the baseline value after aerobic performance. The day after aerobic performance it returns to 38% of the baseline, which is in the range of the mean values observed during the post warm up phase (see figure 1).
The ATP/ADP ratio shows a similar profile. In contrast to NADH consumption the variation of the values after warm up was not significant. Compared to baseline the ATP/ADP ratio falls to significant levels of the maximum aerobic performance and returns almost to baseline on the next day (see figure 2).

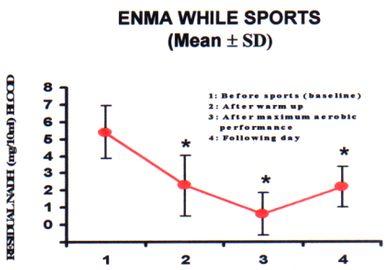

Fig.1: Influence of maximum aerobic performance on ENMA
After 2 hours incubation at 37°C the amount of NADH (total: 8mg) not metabolized by whole EDTA blood was determined at 340nm using a HPLC system. Compared to baseline the warm up leads to a significant increase of NADH metabolisation. After 30 minutes of maximum aerobic performence on a treadmill the ENMA (NADH consumption) was elevated dramatically. An increased metabolisation compared to the baseline was also found on the following day.
★ Significant compared to baseline.

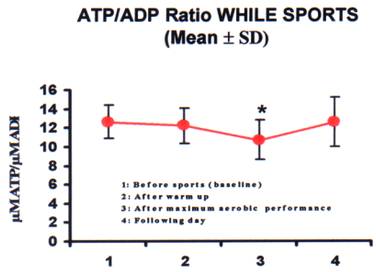

Fig.2: Influence of maximum aerobic performance on the ATP/ADP-ratio
ATP and ADP concentrations were measured at 254nm with HPLC system after an alkaline extraction procedure. The ATP/ADP ratio declines significantly after 30 minutes of maximum aerobic performance on a treadmill. On the following day ATP/ADP ratio returned to the baseline level.

CONCLUSIONS:

Starting with a relatively low extracellular NADH metabolisation before physical activity, athletes show a steady increase in the turn over rate of NADH already during a physical warm up period. In some cases – according to the individual exertion during this period – the increase was substantial. Moreover, NADH metabolisation was still above baseline upon the following day. The observed NADH profile correlates with the measured intracellular ATP/ADP ratio, which is an established indicator for the cellular energy status. But in contrast to the ATP/ADP assay the ENMA shows a significantly broader analytical range under all test conditions (i.e. warm up-, regeneration periods).
Due to its easy and versatile handling and the possibility to perform ENMA on a simple photometer wide spread applications like monitoring the training efficiency and especially the quality of the warm up of athletes, the fitness of senior citizens or the recovery from disease as well as an objective testing of energy enhancing nutritional supplements may be envisioned.

REFERENCES:

[1] M. Formato, B. Masala, G. De Luca, The levels of adenine nucleotides and pyridine coenzymes in red blood cells from the newborn, determined simultaneously by HPLC, Clin. Chim. Acta 189 (1990) 131-138.
[2] K. Nadlinger, W. Westerthaler, D. Storga-Tomic, J. G.D.Birkmayer, Extracellular metabolisation of NADH by blood cells correlates with intracellular ATP levels, Biochim. Biophys. Acta, 2002 (in press)
[3] V. Stocchi, L. Cucchiarini, M. Magnani, L. Chiarantini, P. Palma, G. Crescentini, Simultaneous extraction and reverse-phase high-performance liquid chromatographic determination of adenine and pyridine nucleotides in human red blood cells Anal. Biochem. 146 (1985) 118-124.

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