ENADAlert ENHANCE INTRACELLULAR ENERGY LEVELS EVEN IN TRAINED ATHLETES

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Objective:

The aim of the study was to test the hypothesis if supplemented NADH is able to enhance the available cellular energy prior and during physical exercise as well as enhance after exertion on highly conditioned athletes.

Methods:

Test subjects
14 highly conditioned endurance athletes, who had a significant higher aerobic performance compared to normal healthy individuals (VO_{2max} > 55mL/kg/min), were included in a double blind cross over placebo (Nicotinamide) controlled trail. The test intervals were 4 weeks ingestion of 30mg NADH (ENADAlert) or Nicotinamide daily, interrupted by a 6 weeks wash out period. On the test days no NADH/placebo intake was allowed.

GROUP 1

PLACEBO	Washout	ENADAlert
4 weeks	6 weeks	4 weeks
Baseline		ENADAlert

GROUP 2

ENADAlert	Washout	PLACEBO
4 weeks	6 weeks	4 weeks
ENADAlert		Baseline

Extracellular NADH metabolization assay (ENMA)

500 µl of EDTA-blood (used 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 1 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 topped by centrifuging the tube for 10 minutes. The filtrate which contains the not consumed NADH 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) incubated under the same conditions are used to quantify the metabolized NADH amount.

Determination of NADH with HPL

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 Shimadtzu LC 10A 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 by 10 ml blood compared to the 50 µl NADH standard (8mg NADH, 0mg NADH metabolized).

Results:

Both groups show a lower NADH metabolization after administration of 30mg ENADAlert compared to the administrated placebo. This effect is found for both groups at all analyzed times (before exercise, after warm up, after maximum aerobic performance and on the next day).

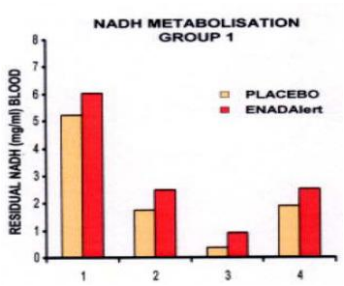

Fig. 1: Effect of ENADAlert (NADH) intake on group 1

The values of extracellular NADH metabolization after four weeks placebo (baseline) and a following ENADAlert intake of 30mg daily (interrupted by a six week wash out period) are compared at different time points of physical excertion.
1: Prior excertion
2: After a warm up period
3: After 30 minutes maximum aerobic performance on a treadmill
4: Next day
At each point, there was less extracellular NADH metabolization after ENADAlert intake than after the placebo interval.

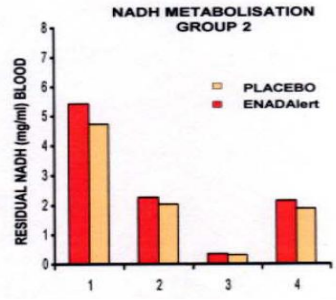

Fig. 2: Effect of ENADAlert (NADH) intake on group 2

The values of extracellular NADH metabolization after four weeks ENADAlert (30mg NADH daily) and a following placebo intake (baseline) interrupted by a six week wash out period are compared at different time points of physical excertion.
1: Prior excertion
2: After a warm up period
3: After 30 minutes maximum aerobic performance on a treadmill
4: Next day
At each point, there was less extracellular NADH metabolization after ENADAlert intake than after the placebo interval.

Conclusions:

Although there was no NADH (ENADAlert) administrated on the day of testing, NADH metabolization was less pronounced after the ENADAlert intervals (higher residual NADH values in the ENMA) than after the placebo intervals. Considering that the ENMA correlates with the intracellular ATP/ADP ratio, we conclude that the intake of NADH leads to a higher amount of available cellular energy.

REFERENCES:

[1] M. Formato, B. Masala, G. DeLuca, 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 metabolization of NADH by blood cells correlates with intracellular ATP levels, Biochem. et Biophys Acta, 2002 (in press).

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