The Effect of 3 Weeks of a Black- and Redcurrant Extract on the Urine Metabolome in Cyclist Athletes – A Randomized Placebo-Controlled Pilot Study

Poster by Asiros A/S, Copenhagen, Denmark

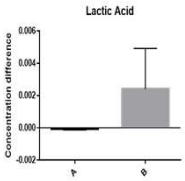
Abstract

MitoActive™ is an extract of black- and redcurrants high in polyphenols and particularly high in the patented active compound Ribetril™ A, a polyphenol-alkaloid present in certain cultivars of black- and redcurrant. The extracts in MitoActive™ have been demonstrated to enhance mitochondrial amount and Ribetril™ A significantly increases basal respiration and spare respiratory capacity in muscle cells *in vitro*. The effect of daily supplementation of MitoActive™ on the urine-metabolome in cycle athletes was investigated in a randomized, placebo-controlled, double-blind pilot study. Healthy male cyclist athletes with a high fitness level and an intensive training schedule were randomized to two groups of either 30 ml MitoActive™ beverage or placebo beverage daily for 3 weeks. The urine metabolome before and after three weeks of supplementation was analyzed and metabolites with levels that differed at least 10 times between the two groups were identified. Urine lactic acid increased in the placebo group but remained stable in the intervention group and urine pyruvate decreased in the placebo group but remained stable in the intervention group. The results may correlate to previous findings of improved mitochondrial capacity in muscle cells exerted by MitoActive™.

Introduction

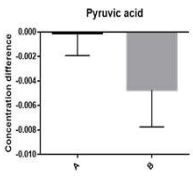
Blackcurrant powder supplementation has been associated with lower lactate accumulation at aerobic capacity[1] and faster clearance at recovery[2,3].
MitoActive™ is an extract based on black- and redcurrants particularly high in the newly discovered bioactive compound Ribetril™ A. Ribetril™ A is a polyphenol alkaloid present only in selected cultivars of blackcurrants (*Ribes Nigrum*) and redcurrants (*Ribes Rubrum*). In a previous series of *in vitro* experiments, extracts of black- and redcurrants cultivars particularly high in Ribetril™ A increased mitochondrial amount and spare respiratory capacity in muscle cells and pure Ribetril™ A increased mitochondrial spare respiratory capacity as well as basal respiration in muscle cells[4], effects that are closely associated with maintenance of aerobic metabolism during maximal muscle stress.

Figure 1.



Mean concentration change in urine lactic acid (mM), post vs pre treatment in the intervention group (A) and the placebo group (B).

Figure 2.



Mean concentration change in urine pyruvic acid (mM), post vs pre treatment in the intervention group (A) and the placebo group (B).

Materials and Methods

Study design
The study was designed as a randomized, placebo-controlled, double-blind trial performed in collaboration with Copenhagen University and funded by Asiros A/S. Only subjects with a fitness level (VO2max) at or above 55ml·kg-min, age 18-45 years, a minimum of 5 years' cycle training experience and a weekly training program with at least three intensive bike training sessions, no smoking and no serious diseases were included. The subjects were randomized to either an intervention group supplemented with 30 ml of MitoActive™ containing 840 mg total polyphenols including Ribetril A for 3 weeks or a placebo group supplemented with 30 ml of a taste and color matched placebo beverage without polyphenols. The subjects delivered morning urine on the day before initializing a 3 week daily supplementation period and again after 3 weeks with at least 12 hours between their last training pass and urine delivery. The urine thus reflected resting levels of the urine metabolome. 31 subjects completed the study according to protocol.

Urine-metabolomics
The urine metabolome reflects the metabolic processes in the body. Analysis of the urine metabolome with Gas Chromatography-Mass Spectrometry (GC-MS) is one of the most applied analytical tools in metabolomics due to its high separation power and capacity for reliable identification of hundreds of metabolites. Chemical derivatization was performed with methyl chloroformate to enable GC-MS analyzes of nonvolatile compounds. To reduce urine concentration effects data were normalized to the sum of all variables in the individual samples. The large amount of raw GC-MS data was processed by software developed by MS-Omics and collaborators, using the powerful PARAFAC2 model to extract more compounds and cleaner MS spectra than most other GC-MS software.

Statistics
A 10-fold difference was set as a cut-off level for identification of compounds that were analyzed for differences between groups. For metabolomics data analysis the statistical program Metaboanalyst 3.0 was applied. Taking the variation of metabolism between humans into account, a pareto scaling algorithm was applied to the dataset before further statistical analysis. A two-tailed Student's t-test was employed to test for numerical differences. An unpaired parametric t-test was employed to test for normalized percentage changes.

Results

Principal component analyses (PCA) of the urine metabolome identified 5 components that complied with the 10-fold difference cut-off level. Of these, lactic acid and pyruvic acid are well known metabolites associated with energy metabolism whereas the remaining three were unidentified. As seen in figure 1 and 2, the urine concentration of lactic acid was increased in the placebo group while it was relatively stable in the intervention group and the urine concentration of pyruvic acid was decreased considerably in the placebo group while it was relatively stable in the intervention group. The numerical changes were not significantly different, however, the normalized percentage change displayed statistical significance; $P \leq 0.0001$ for lactic acid and $P = 0.0041$ for pyruvic acid.

Discussion

Lactate production is normally present at a low rate and is central to the regeneration of NAD+ to sustain glycolysis and an important substrate for oxidation and conversion into energy in the mitochondria[5,6,7].
A small but identifiable amount of lactate is present in the urine at all times[8]. Stable levels were observed of otherwise increased lactic acid and decreased pyruvic acid in the resting urine metabolome in cyclist athletes at the end of a three weeks' supplementation with MitoActive™ containing high amounts of red-and blackcurrant polyphenols including the bioactive polyphenol-alkaloid Ribetril™ A. Endurance athletes have significantly higher resting blood lactate than either sedentary subjects or strength athletes, reflecting higher basal glycolytic activity in the endurance athletes[9] . The intervention period of this study concurred with the beginning of the athletic cycling season characterized by increasing endurance and sprint capacity. The increase in resting urine lactate in the placebo group may reflect an increasing endurance capacity accompanied by an increase in basal glycolytic activity as seen in endurance athletes. Conversely, the lack of an increase in resting urine lactate in the intervention group may have been elicited by the supplementation of black- and redcurrant extracts with Ribetril™ A previously demonstrated to increase mitochondrial amount, basal respiration and spare respiratory capacity in muscle cells[4] . This notion is supported by previous reports of lower lactate accumulation and faster clearance at recovery associated with blackcurrant powder supplementation[1-3].
Not surprisingly, as lactate is generated from pyruvate, a rise in lactate is accompanied by a fall in resting pyruvate, as observed in the placebo group.
The ability of Ribetril™ A to inhibit phosphodiesterase 5[10] may facilitate increased NO-cGMP-mediated vasodilation, resulting in increased lactate removal and optimized substrate and O2 delivery, thereby contributing to the stabilized levels of lactate and pyruvate found in the group supplemented with MitoActive™. This notion is supported by the findings that blackcurrant juice concentrate activates eNOS[11], and anthocyanins including blackcurrant anthocyanins elicit vasodilation through activation of the NO-cGMP signaling pathway[12].
Although stabilization in the intervention group of otherwise considerable changes in metabolites associated with energy metabolism were observed in this pilot study, the urine metabolome varies significantly on an individual level and detection of significant changes may require considerably larger data sets to ensure sufficient statistical power.

Conclusions

The indicated enhancing effects on energy metabolism found in this pilot study may be associated with the previously demonstrated enhancing effects of Ribetril™ A on mitochondrial amount and capacity in muscle cells. Large variations in the individual urine metabolome call for larger studies to further look into these associations, however the present study indicates that the urine metabolome may be an interesting new tool to investigate effects on energy metabolism of food supplements.

References

1. Willems et al. J Int Soc Sports Nutr. 2014; 11(Suppl 1):P2.
2. Willems et al. J Int Soc Sports Nutr. 2014; 11(Suppl 1):P14.
3. Perkins et al. Int J Sport Nutr Exerc Metab. 2015 Oct;25(5):487-93.
4. Weidner et al 2015, Conference Poster, Resveratrol Regional Meeting, at Dijon 2015, France.
5. Kane D. Front Neurosci. 2014;8:366.
6. Graham TE. Physiologist. 1984 Aug; 27(4):299-303.
7. Facey et al, Am J Sports Sci Med 2013; 1(3):42-46.
8. Human Metabolome Database, http://www.hmdb.ca/
9. Ahlgrim et al. Conference Paper 43. Deutscher Sportärztekongress, At Berlin, October 2012, Volume 63:7-8.
10. Bioactive alkaloid compositions and their medical uses. US patent No US 9,023,816 B2.
11. Edirisinghe et al. J Agric Food Chem. 2011 Aug 24;59(16):8616-24.
12. Zhu et al. Clin Chem. 2011 Nov;57(11):1524-33.