Natural xanthines caffeine and theobromine rescue myotonic dystrophy-like features in cell and animal models of DM1

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Abstract: Muscle mass wasting is one of the most debilitating symptoms of myotonic dystrophy type 1 (DM1), ultimately leading to immobility, respiratory defects, dysarthria, dysphagia and death in advanced stages of the disease. Malignant heart arrhythmias constitute an additional medical concern. Defined compositions of the natural xanthines caffeine and theobromine, now commercialized as the food supplement MYODM, were found to rescue DM1-like features in DM1 models.

Method: We used the INSR:lucspliceosensor flies to screen for natural compounds that can rescue this MBNL1-dependent splicing. We further checked MBNL1 and MBNL2 protein expression in the nucleus of DM1 transdifferentiated myoblasts. *In vivo* preclinical studies enclose a *Drosophila* DM1 models expressing either 480 CTG repeats in muscle or 250 CTG repeats in heart.

Results, conclusions and funding:

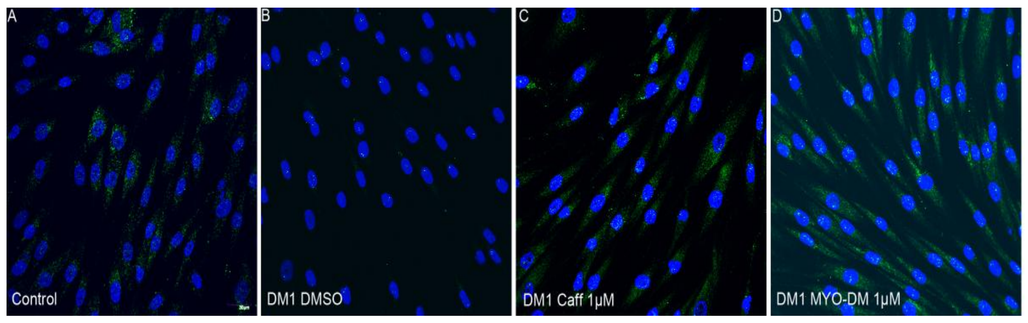

Fig 1: MYODM significantly increases free MBNL1 in DM1 myoblasts.

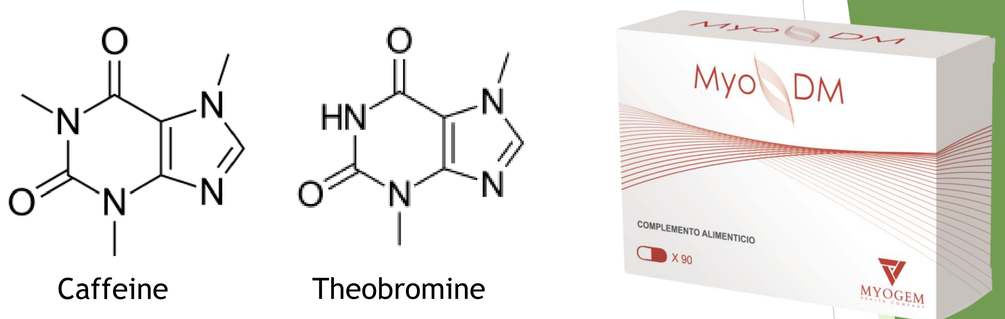

Fig 2: Caffeine and Theobromine, the natural methylxanthines in MYODM

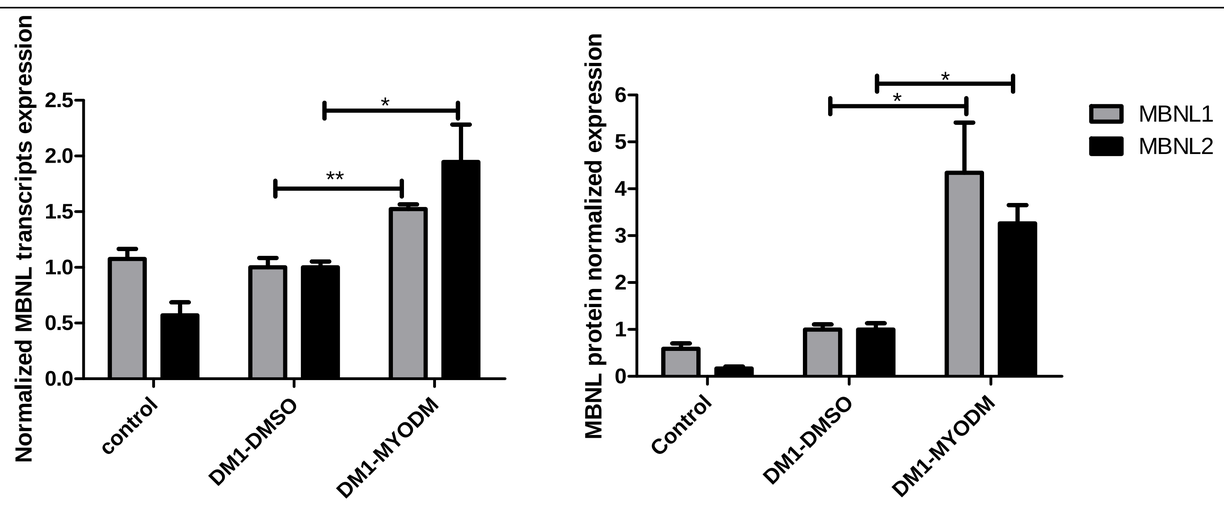

Fig 3: MYODM (the formulated composition containing *Theobroma cacao* supplemented with caffeine, at caffeine/theobromine 1/1.85, w/w) increased MBNL1 and MBNL2 expression level in human DM1 cells.
(Left) Bar graphs showing the mean ± SEM of the expression levels of MBNL1 (grey) and MBNL2 (black) transcripts. GAPDH was used as endogenous control.
(Right) Bar graphs showing the mean ± SEM of the expression levels of MBNL1 (grey) and MBNL2 (black) proteins. B-Actin was used as endogenous control. In both graphs, results were normalized to the DM1-DMSO, which were given the value of 1. *p<0.05, ** p<0.01, ***p<0.001 in Student's t test.

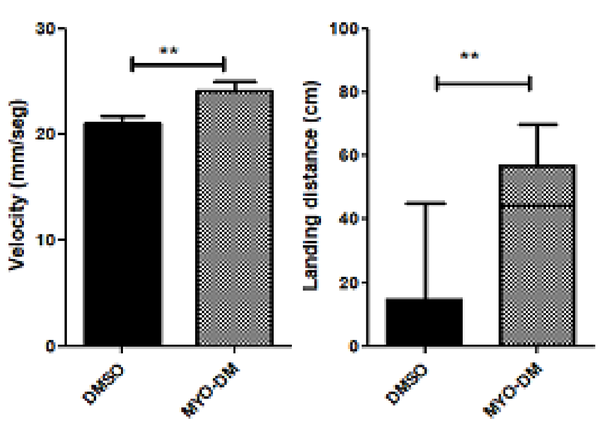

Fig. 4. Histogram showing the climbing speed (left) as the mean speed ± *SEM* in mm/s, and the landing distance (right) as the average landing height data ± *SEM* in cm.
Treatment with MYODM increased the climbing velocity and flying distance of model flies expressing expanded repeats compared to model flies fed with DMSO as solvent.

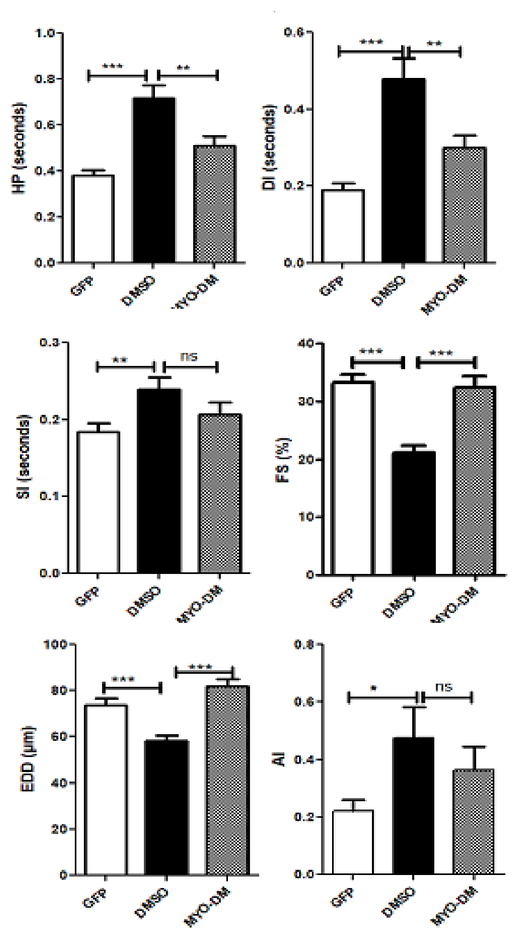

Fig 6: Characteristic cardiac dysfunction of flies expressing long CUG repeats, compared to control flies expressing GFP reporter (GFP), include increased Heart period mean (HP), due to increase of Diastolic and Sistolic Intervals (DI and SI), reduced percentage of Fractional Shortening (FS%), due to a reduction of End Diastolic Diameter (EDD), and increased Arrhythmicity Index (AI).

Treatment of adult flies with 0.75% MYODM in the food during 7 days (MYODM samples) rescued DI, %FS and EDD compared to DM1 model flies fed with the MYODM solvent DMSO (DM1).

The bars on the graph show mean values and their standard errors. *p< 0.05, **p<0.01, ***p< 0.001. Student t-test.

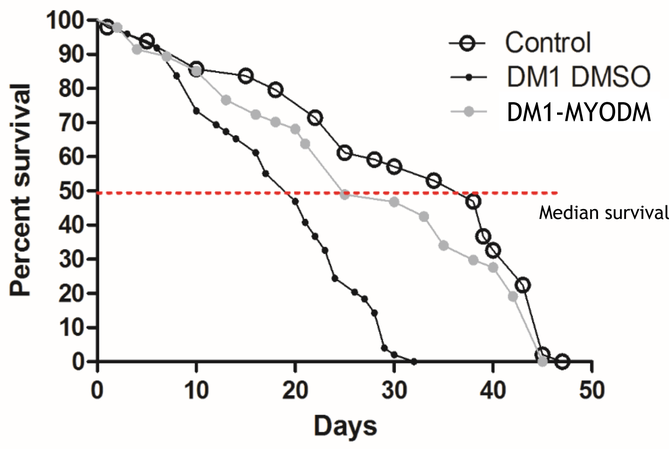

Fig 5: Average percentage of alive flies versus age (in days). The GMH5-Gal4 driver was used to induce the expression of the inocuos reporter GFP (control) or the 250 CUG repeats (DM1) to Drosophila cardiomyocytes.

MYODM induces a significant rescue of lifespan and median survival of DM1 model flies ($p < 0.0001$, log-rank test).

Results:
MYODM containing caffeine and theobromine promoted increase MBNL1 protein expression in the nucleus and cytoplasm of DM1 transdifferentiated myoblasts, and rescued DM1-like features in *Drosophila* models of disease.
Administered to *Drosophila* model flies, MYODM rescued impaired climbing and flight ability, and strongly improved survival and heart dysfunction phenotypes.

Manuscript under preparation

Conclusions: Our data support a therapeutic effect of caffeine and theobromine formulated as the food supplement MYODM on CUG-related phenotypes, which has been validated in different models of the disease. These effects stem, at least partially, from enhancement of MBNL1 expression levels.

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