

## Effect of oral D-tagatose on liver volume and hepatic glycogen accumulation in healthy male volunteers

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### Introduction

D-tagatose, a stereo-isomer of D-fructose, is a novel sugar substitute that is absorbed only by 20-25% during intestinal passage. The absorbed portion is metabolized via the same pathway as fructose while the rest is fermented by the intestinal microbiota. Standard toxicity tests with high levels of D-tagatose showed a reversible enlargement of the liver in rats without increase of liver enzymes (1 and refs therein). The present study tests the hypotheses that partial substitution of dietary sucrose by D-tagatose for 28 days increases the volume of human liver and the concentration of liver glycogen.

### Volunteers and Methods

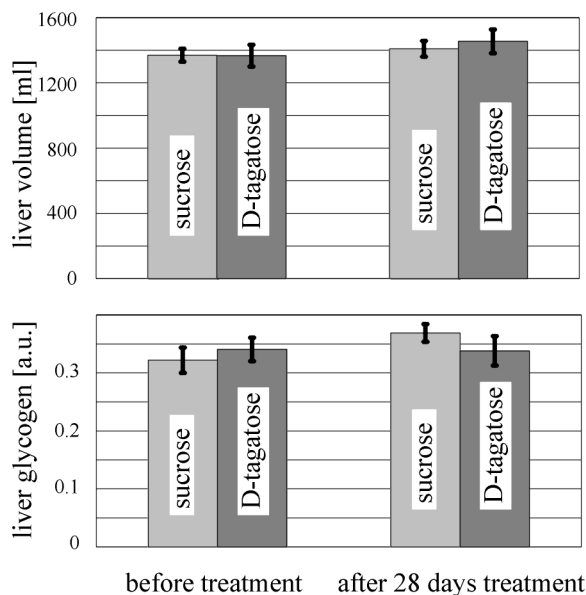
**Subjects:** 12 healthy, male volunteers (21-30 years, 59-93 kg, body mass index  $\leq 25$  kg/m<sup>2</sup>) were studied in a double-blind crossover study with supervised ingestion of D-tagatose (3 x 15 g daily, MD Foods a.m.b.a, Denmark) and placebo (sucrose, 3 x 15 g daily) for periods of 28 days each, separated by a "wash-out" period of 28 days. Three MR examinations per period (morning day 1, afternoon day 1, and morning day 29) were performed. Dinner before the examination day was standardized. After the first MR examination of day 1, the volunteers received a standardized breakfast (190 g toast and 15 g D-Tagatose or sucrose) and were examined 5 hours later again. MR-examinations were accompanied by a routine medical examination with a questionnaire and the determination of hematological and clinico-chemical parameters (including 7h-profiles of glucose, insulin, glucagon, and uric acid). The study was approved by the Ethics Committee of the local government.

**MRI and MRS-examination:** MR images and <sup>1</sup>H decoupled natural abundance <sup>13</sup>C MR spectra were recorded on a standard 1.5 Tesla SIGNA system (GE, Milwaukee WI) equipped with a partially homebuilt <sup>1</sup>H-decoupler channel (s.m.i.s., Surrey UK). MR images for liver volume determination were acquired by the body coil, while a double-tuned flexible surface coil (Medical Advance, Milwaukee WI) was used for <sup>13</sup>C signal excitation and reception (square surface coil of 11.3 cm x 11.3 cm), and for a localizer <sup>1</sup>H-imaging and <sup>1</sup>H-decoupling (Helmholtz-type coil, flexible, approx. 16 cm). In order to position volunteers and surface coil reproducibly within a few millimeters, a thorough positioning procedure on a special cast was implemented, using the right lower edge of the 12<sup>th</sup> thoracic vertebra as landmark. Liver volume was determined by the point counting method (2) on three imaging series in coronal, sagittal, and axial slice orientation during three subsequent, end-expiratory breath-holds (fast-SPGR, flip-angle 60 degrees, TE 1.5 ms, TR 110 ms, matrix 512x192, slice thickness 8 mm). Liver glycogen was measured by a 2.7ms sech/tanh adiabatic half passage excitation pulse (3) with CW decoupling and NOE build-up (TR 165 ms, approx. 2048 responses). Coil loading was corrected by an external acetone phantom. Since the position of the liver volume relative to the surface coil was reproduced in all sessions within a few millimeters, the sensitive volume of the surface coil and the excitation profile of the pulse-sequences are expected to vary less than any potential correction by a spatial algorithm or phantom replacement. Therefore, glycogen concentrations are given in arbitrary units [au] that are quantitatively comparable within a volunteer over all six MR examinations.

### Results

Liver volumes and glycogen concentrations measured in the morning examinations before and after the 28-day treatment periods are shown in the Figure, representing the average and the standard error over 12 volunteers. Initial liver volumes and glycogen concentrations did not differ significantly between the treatments ( $p = 0.902$  and  $p = 0.479$ , respectively). Independent of the sequence of treatments (D-tagatose > sucrose vs. sucrose > D-tagatose), liver volumes were significantly increasing with time (start 1.period 1297 ml, end 1.period 1378 ml, start 2.period 1440 ml, end 2.period 1485 ml,  $p < 0.001$ ). In parallel, liver volumes tended to increase during both 28-day treatment periods (mean increase during D-tagatose 88 ml vs. 39 ml during sucrose), however, without reaching significance between the two treatments.

This has been shown by ANOVA tests of the changes in liver volume between MR examinations in the morning sessions at the beginning and the end of the treatments that revealed no effects of treatment ( $p = 0.209$ ), period ( $p = 0.347$ ), or subject ( $p = 0.201$ ). There were also no effects of treatment ( $p = 0.209$ ), period ( $p = 0.335$ ), or subject ( $p = 0.787$ ) on liver glycogen concentration changes. Following the standardized meal with 15 g D-tagatose, no short-term effects of treatment ( $p = 0.804$ ), period ( $p = 0.347$ ), or subject ( $p = 0.097$ ) on liver volume changes were detected. In addition, no short-term effects of treatment ( $p = 0.670$ ), period ( $p = 0.448$ ) or subject ( $p = 0.302$ ) on liver glycogen concentration changes were measured.



### Discussion

In contrast to earlier tests in animals with higher doses of D-tagatose, the present study did not support the hypotheses that D-tagatose (45 g/d for a period of 28 days) would change liver volumes or glycogen concentrations in healthy male volunteers as compared to sucrose. With a CV of 2.9%, changes of 3.7% in liver volume should be detectable in 12 volunteers (significance 0.05, power 80%). Accurate repositioning of volunteers and surface coil within a few millimeters allowed for a direct intra-individual comparison of the corrected glycogen signals between different sessions. The absence of short-term changes is explained by the long, 5h-delay after the standard breakfast. A steady increase of liver volumes over the study, independent of the D-tagatose or placebo intake, is not fully understood. Seasonal effects (November to February) or the more regular food intake under supervision may play a role. It is important to recognize, however, that this long-term effect, which is not dependent on treatment, has been detected and compensated by the placebo-controlled crossover design of the study. Without this intra-individual control, the effect could have been attributed erroneously to the treatments.

### References

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