Dmbt1 does not affect a Western style diet-induced liver damage in mice

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In the last three decades the prevalence of non-alcoholic fatty liver disease has markedly increased. Results from epidemiologic studies indicate that not only a general overnutrition but rather a diet rich in sugar, fat and cholesterol (= Western style diet) maybe a risk factor for the development of non-alcoholic fatty liver disease. Concerning liver diseases, it is known that Deleted in malignant brain tumors 1 is amongst others related to liver injury and repair. In addition Deleted in malignant brain tumors 1 seems to play a role in regard to the maintenance of the intestinal homeostasis and the regulation of food intake. Starting from this background the aim of the present study was to investigate if Dmbt1 plays a role in Western style diet-induced non-alcoholic steatohepatitis in mice. Dmbt1−/− and Dmbt1+/+ mice were fed a Western style diet or control diet ad libitum for 12 weeks. Both Western style diet fed groups gained significant more weight than the controls and developed a mild non-alcoholic steatohepatitis. The presence/absence of functional Deleted in malignant brain tumors 1 had no effect on parameters like food intake, weight gain, fasting glucose, and liver damage. These results suggest that Deleted in malignant brain tumors 1 plays a minor part on the development of a diet-induced liver damage in mice.

Key Words: NAFLD, NASH, deleted in malignant brain tumors 1, lipid peroxidation, Western style diet

Non-alcoholic fatty liver disease (NAFLD) is by now recognized as the one of the most common liver diseases in Western countries and comprises a spectrum of hepatic steatotic disorders in the absence of regular alcohol consumption. The spectrum of the disease ranges from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Lately it has become more obvious that steatosis, long thought to be a relatively benign state of injury, is a state of liver disease, in which the liver is more vulnerable to injury from various causes. Results from epidemiological studies indicate that not only a general overnutrition but more so a diet rich in sugar, fat and cholesterol (= Western style diet) may be a risk factor for the development of non-alcoholic fatty liver disease. Concerning liver diseases it is known that DMBT1 is related to liver cancer, injury, and repair mechanisms. In addition, analysis of the expression patterns of DMBT1/Dmbt1 in murine and human organs (e.g., in the gut) suggests that DMBT1/Dmbt1 seems to play a role in the maintenance of intestinal homeostasis. Furthermore, data from animal models indicate that there is a diet-dependent up or down regulation of the Dmbt1/dmbt1 expression in the liver and parts of the gut. Starting from this background the purpose of the present study was to investigate if Dmbt1 affects a Western style diet-induced liver damage in Dmbt1 knockout mice.

Materials and Methods

Animals and treatments. Eight-week-old C57BL/6-Dmbt1 wildtype (Dmbt1+/+) and C57BL/6-Dmbt1 knockout (Dmbt1−/−) mice (n = 4–6 per group) were housed in a specific pathogen-free barrier facility in individually ventilated cages. C57BL/6-Dmbt1 knockout mice were generated as shown before by Renner et al. Animals were either fed a control diet (C; Control Diet to TDE8137, ssniff Spezialitäten GmbH, Germany) or Western style diet (WSD; TDE88137 modified-Western Type Diet and CD88137 modified, ssniff Spezialitäten GmbH, Germany) ad libitum for 12 weeks. All procedures were approved by the local IACUC (Institutional Animal Care and Use Committee). Consumption of chow and body weight was assessed once a week. After 10 weeks, mice were fasted for 6 h and blood was taken from the tail vein to determine the fasting glucose. Animals were anesthetized with 80 mg ketamine and 6 mg xylazine/kg body weight intra-peritoneally, and blood was drawn from the portal vein. Portions of liver tissue were frozen immediately in liquid nitrogen and stored at −80°C, while others were fixed in neutral-buffered formalin.

Liver histology. Paraffin sections of liver (5 μm) were stained with haematoxylin and eosin to assess the liver histology. Using a system incorporated in a microscope (Axio Vert 200M, Zeiss, Jena, Germany) representative photomicrographs were captured at a 200× magnification. Sections were scored using the “NAFLD Activity Score (NAS)” as described by Kleiner et al.

Blood parameter. Fasting glucose was determined by using a glucometer (Bayer Vital, Leverkusen, Germany) and plasma alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activity were determined using a commercially available kit (Beckman Coulter, Krefeld, Germany).

Immunohistochemical staining for 4-hydroxynonenal protein adducts. Paraffin-embedded liver sections (5 μm)

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were used for the detection of 4-hydroxynonenal (4-HNE) protein adducts (1:1100, 30 min, AG Scientific, San Diego, California) using polyclonal antibodies. Tissue sections were then incubated with a peroxidase-linked secondary antibody and diaminobenzidine (DAKO, Hamburg, Germany). To determine the concentration of 4-HNE protein adducts in liver sections staining was assessed densitometrically in 8 randomly selected fields (200x magnification) using a microscope (Axio Vert 200M, Zeiss, Jena, Germany).

Statistical analyses. Data were expressed as mean ± SEM (standard error of the mean). Analysis of variances (one-way ANOVA) with the consequent post-hoc test of Tukey was applied for the determination of significance levels (GraphPad Software, La Jolla, California). A $p<0.05$ was considered to be significant.

Results

Effect of Dmbt1 knockout on food intake, absolute weight gain and fasting glucose. As shown in Table 1 there were no differences referring to the caloric intake, weight gain and fasting glucose in both control diet fed groups. In comparison to the respective controls, feeding a WSD led to a significant weight gain and fasting glucose levels in Dmbt1+/− and Dmbt1−/− mice (WSD+/+ vs C+/+; weight gain ~5.3 fold, $p<0.001$ and fasting glucose ~14 fold, $p<0.05$; WSD−/− vs C−/−; weight gain ~3.5 fold, $p<0.001$ and fasting glucose ~1.3 fold). WSD fed Dmbt1−/− mice did not differ significantly with regards to weight gain and fasting glucose. While WSD fed Dmbt1−/− mice seemed to have a higher caloric intake than the other groups; a similar effect was not found in Dmbt1+/− mice being fed a WSD. However, Dmbt1−/− mice spread the chow in the cages so it was quite difficult to determine the chow consumption.

Effect of Dmbt1 knockout on parameters of liver damage. Western style diet fed mice had a significant higher liver weight and liver to body weight ratio than its respective controls (WSD+/+ vs C+/+; liver weight ~2.0 fold, $p<0.01$ and liver body weight ratio ~1.3 fold, $p<0.05$; WSD−/− vs C−/−; liver weight ~2.0 fold, $p<0.001$ and liver body weight ratio ~1.3 fold, $p<0.01$). Whereas there were no differences referring liver weight and liver to body weight ratio in both control diet fed groups and also WSD fed mice did not differ among each other (see Table 2). The increased weight gain, liver weight and liver to body weight ratio in mice being fed a Western style diet was associated with a massive accumulation of fat and inflammation in the liver. Whereas Dmbt1−/− and Dmbt1+/− mice being fed a control diet did not show histological abnormalities (Fig. 1 A and B).

Discussion

Animal-based models resembling conditions of the early stages of NAFLD in humans (e.g., steatosis, steatohepatitis) have found to be useful tools to investigate both possible molecular mechanisms of the onset and progression of the disease and the evaluation of potential new therapeutic strategies. In line with these findings in humans with NAFLD including western dietary pattern and fast food (see for overview(9)), it has been shown by other groups(22-25) that the onset of NAFLD in mice fed a Western style diet is associated with obesity, steatosis, steatohepatitis, hepatic inflammation (e.g., increase of transaminases) and increased formation of reactive oxygen species.

Dmbt1 was identified based on frequent deletions and/or loss or reduction of expression,(9) which is probably due its increased susceptibility to genomic instability.(13,26) To this end, Dmbt1 was proposed as putative tumour suppressor gene for a variety of cancers like brain,(9,27) gastrointestinal,(12,28) breast(10,11,29,30) and lung cancer(31); albeit its role might be complex. Other studies showed that Dmbt1 is also involved in the development of epithelia and in functions of the immune system.(13,14) Collectively, these studies pointed to a potential role for Dmbt1 in various different diseases. As Dmbt1 is secreted to the surface of intestinal epithelial cells,(13,14) it is in immediate contact with environmental and nutritional factors, which raised the question whether it could also be related to NAFLD caused by Western

| Calorie intake (kcal/mouse/g/wk) | Dmbt1+/+ C | Dmbt1+/+ WSD | Dmbt1−/− C | Dmbt1−/− WSD |
|---------------------------------|------------|--------------|------------|--------------|
| 3.2 ± 0.1                       | 4.5 ± 0.5  | 3.1 ± 0.3    | 3.0 ± 0.2  |

Data are shown as mean ± SEM (n = 5–6); *p<0.05 compared with control diet (C+); $p<0.05$ compared with control diet (C−); $p<0.001$ compared with control diet (C−); $p<0.001$ compared with control diet (C−); *determined from week 2 to 12; the first week was an adaption week.

| Weight gain (g) | Dmbt1+/+ C | Dmbt1+/+ WSD | Dmbt1−/− C | Dmbt1−/− WSD |
|----------------|------------|--------------|------------|--------------|
| 2.9 ± 1.2      | 15.4 ± 1.9 | 4.9 ± 0.5    | 17.5 ± 1.0 |

**Table 2. Effect of a Dmbt1 deletion on liver weight and liver to body weight ratio**

| Liver weight (g) | Dmbt1+/+ C | Dmbt1+/+ WSD | Dmbt1−/− C | Dmbt1−/− WSD |
|-----------------|------------|--------------|------------|--------------|
| 0.9 ± 0.1       | 1.8 ± 0.2  | 1.2 ± 0.0    | 2.4 ± 0.1  |

| Liver to body weight ratio (%) | Dmbt1+/+ C | Dmbt1+/+ WSD | Dmbt1−/− C | Dmbt1−/− WSD |
|-------------------------------|------------|--------------|------------|--------------|
| 4.1 ± 0.3                     | 5.2 ± 0.4  | 4.8 ± 0.1    | 6.3 ± 0.2  |
The present study was designed to initially address this issue. The study was also warranted based on results from several in vivo studies suggesting that there is a diet-dependent up or down regulation of Dmbt1/dmbt1 in the liver and parts of the gut.\(^{18–20}\) Further, DMBT1 has been linked to liver injury and repair mechanisms concerning liver disease.\(^{16,17}\) Starting from this background, the present study investigated the influence of Dmbt1 on diet-induced liver disease in a mouse model. After feeding a Western style diet for 12 weeks ad libitum, Dmbt1\(^{+/+}\) and Dmbt1\(^{-/-}\), gained significant more weight and had higher fasting glucose levels than the control diet fed animals. No significant differences were observed between wildtype and knockout mice fed a Western style diet, likewise animals fed a control diet did not significantly differ. In comparison to the other groups it is conspicuous that Dmbt1\(^{+/+}\) mice fed a Western style diet had a higher calorie intake than the others. However, it was

**Fig. 1.** Effect of a Dmbt1 deletion on a Western style diet-induced liver damage in Dmbt1\(^{+/+}\) and Dmbt1\(^{-/-}\) mice. (A) Representative photomicrographs of H & E staining (200× and 400× magnification, respectively) and (B) results of the assessment of liver damage using the NAS (21). Data are shown as mean ± SEM, *p<0.001.

**Fig. 2.** Effect of a Dmbt1 deletion on ALT and AST levels after feeding a Western style diet in Dmbt1\(^{-/-}\) and Dmbt1\(^{+/+}\) mice. Activity levels of (A) alanine-aminotransferase (ALT) and (B) aspartate-aminotransferase (AST) in U/L determined in the portal plasma.
difficult to gather the consumption of the chow as the animals spread it into the cages; so, a false-negative-result cannot be excluded. In line with these findings also other studies showed that feeding a Western style diet is associated with an obvious weight gain and elevated fasting glucose levels.\(^{(32–34)}\) As shown by others feeding a Western style diet led to hepatic steatosis and inflammations;\(^{(35,36)}\) also the data of the present study show the correlation between feeding a Western style diet and the incidence of hepatic fat accumulation and inflammations. However, the presence/absence of \(Dmbt1\) did not affect Western style diet-induced liver damage and the activity of transaminases in the plasma. The Western style diet-induced liver injuries did not differ between \(Dmbt1^{+/+}\) and \(Dmbt1^{-/-}\) mice and also control diet fed \(Dmbt1^{+/+}\) and \(Dmbt1^{-/-}\) mice did not differ among each other. Results of several studies indicate that lipid peroxidation is a crucial factor for the development and pathogenesis of NAFLD (see for overview\(^{(37–39)}\)). Furthermore Kosinska \textit{et al.}\(^{(40)}\) also showed that a Western style diet is associated with hepatic lipid peroxidation. Data of the present study also showed a significant increase of hepatic lipid peroxidation after feeding a Western style diet. Here, \(Dmbt1\) did not significantly affect Western style diet-induced hepatic lipid peroxidation. Likewise control diet fed \(Dmbt1^{+/+}\) and \(Dmbt1^{-/-}\) mice did not differ.

### Conclusion

In summary, these data suggest that \(Dmbt1\) does not affect Western style diet-induced NASH. However, we cannot exclude that \(Dmbt1\) affects the pathogenesis of other metabolic diseases. Further studies with different diets (e.g., high fat diet) are needed to address the effects of \(Dmbt1\) on nutrition-related diseases.

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### Abbreviations

- ALT: alanine-aminotransferase
- AST: aspartate-aminotransferase
- C: control diet
- DMBT1: deleted in malignant brain tumors 1
- 4-HNE: 4-hydroxynonenal
- NAFLD: non-alcoholic fatty liver disease
- NASH: non-alcoholic steatohepatitis
- WSD: Western style diet

### Conflict of Interest

No potential conflicts of interest were disclosed.

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