even for the growth and establishment of hepatology at the respective national levels.

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How reproducible are rat steatosis models using high-fat diets?

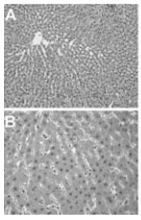
To the Editor:

Non-alcoholic fatty liver disease (NAFLD) is a disease on the increase in the Western world with vast clinical implications. Patients with NAFLD are susceptible to develop NASH and are at risk of complications when undergoing a liver resection, particularly in living donor liver transplantation procedures [1]. In this setting moderate and severe steatosis are considered exclusion criteria. Consequently, clinically representative animal models yielding an adequate steatosis degree are imperative to conduct mechanistic and interventional investigations. Presently, most models are based on genetic or dietary adaptations. With respect to the former, ob/ob mice lacking the leptin gene and Zucker fa/fa rats lacking the leptin receptor exhibit hyperphagia resulting in obesity, insulin resistance, and steatosis. However, the drawback of these models includes the dissimilar etiology of steatosis induction *vs.* its clinical counterpart (i.e., genes *vs.* diet). Moreover, experimental studies show that liver regeneration in these models appears to be impaired not due to steatosis *per se,* but due to the leptin deficiency. A clinically representative model for experimental steatosis would thus be of great value.

In 2004 Lieber et al. [2] introduced a model using a liquid high-fat diet for the induction of NASH in Sprague–Dawley rats, referred to as the Lieber–Decarli (LDC) diet. Within 3 weeks the animals purportedly developed panlobular steatosis with inflammatory foci, in association with insulin resistance and increased hepatic TNF-α levels and lipid peroxidation products, i.e., clinical features of NASH. In a recent study published in this Journal, Lieber et al. [3] used medium chain triglycerides to prevent LDC diet-induced NASH. In this letter we would like to address several irreproducibility issues regarding the LDC diet and its solid high-fat equivalent.

We have attempted to set up the LDC model in Wistar rats following the authors' exact methodology [2]. However, after a 3-week LDC diet none of the livers ($n = 5$) showed any histological evidence of steatosis (Fig. 1A). Our findings were recently corroborated by Akin et al. [4] in Sprague–Dawley rats, where only 20% developed 1–2% microvesicular steatosis after a 3-week LDC diet (Fig. 1B). In both studies, animals had gained significantly less weight (98 and 76 g, respectively) after 3 weeks than the animals in the original study (173 g). Alternatively, we attempted to induce steatosis with a solid high-fat diet in accordance with Svegliati-Baroni et al. [5]. A 3-, 5-, and 7-week diet resulted in a mean ± SD histological steatosis degree of 10 ± 5%, 5 ± 3%, and 10 ± 9%, respectively, in Wistar rats ($n = 5$/group) and 14 ± 11%, 2 ± 1%, and 0%, respectively, in Sprague–Dawley rats ($n = 5$/group).

Next, the reproducibility of the LDC diet was evaluated against other studies referring to the LDC diet. Using the ISI Web of Science database (accessed 01/15/2008), 53 articles citing the original work were retrieved: 38 experimental studies, 9 reviews, 5 clinical studies, and 1 editorial paper. Of the 38 experimental studies, 6 articles (Fig. 1C) reported the use of the LDC diet, of which 2 actually followed its original experimental 3-week diet protocol; both by Lieber et al. [3,6]. In another study by Lieber et al. [7] the diet was employed during 3 and 6 weeks. Interestingly, histo-



C Ref	LDC diet	Duration [weeks]	Steatosis degree [%]
Lieber et al. (6)	Yes	3	N.Q.
Lieber et al. (7)	Yes	3 and 6	N.Q.
Yalniz et al. (8)	No	4	5-33
Akin et al. (4)	Yes	3	<5
Lieber et al. (3)	Yes	3	N.Q.
Wang et al. (9)	Yes	6	5-33

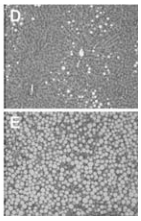


Fig. 1. Histology from our experiments and from Akin et al. (A and B, respectively). No steatosis was found after 3 weeks LDC diet in both experiments. A literature search using ISI web of science revealed 6 studies (C) reporting the use of the LDC diet for steatosis induction. Histological examples of mild (D) and severe (E) steatosis after a 1- and 5-week methionine–choline deficient diet. N.Q.=not quantified.

logical steatosis degrees were not reported in any of the studies by Lieber et al. Yalniz et al. [8] used a solid high-fat diet with the same fat composition for 4 weeks, resulting in mild steatosis, whereas Wang et al. [9] employed the LDC diet for 6 weeks and observed mild steatosis.

Thus, albeit frequently cited, the original LDC protocol for NASH induction is in fact sparsely employed. Instead, the LDC diet is either completely modified to achieve a greater degree of steatosis or applied for longer time periods to induce mild steatosis. These findings make the utility of liquid and solid high-fat diets for NASH-related studies highly questionable. Furthermore, interventional studies [3] in this model are to be interpreted with due caution. It is well known that mild steatosis (0–33%), as was observed in the studies listed in Fig. 1C, have no major clinical implications in contrast to moderate (33–66%) or severe (>66%) steatosis. Consequently, it is paramount to use experimental models that produce high degrees of histological steatosis in order to adequately test therapeutic and nutritional regimes or surgical interventions. Unfortunately, no reports exist achieving clinically relevant high degrees of steatosis with liquid or solid high-fat diets.

In the final analysis, a reproducible and effective means is needed to induce clinically relevant NASH. A suitable method comprises the methionine–choline-deficient (MCD) diet. This diet metabolically deprives the liver from crucial components required for the synthesis of very low density lipoprotein, which is responsible for triglyceride export from the liver [1]. In contrast to the LDC diet, the MCD diet is highly efficient, resulting in mild steatosis after 1–2 weeks (Fig. 1D), moderate steatosis after 3 weeks, and severe steatosis after 5 weeks (Fig. 1E). This model is also associated with inflammatory foci as well as increased TNF-α and lipid peroxidation levels. Most importantly, this model has been used by many different research groups, all reporting the same degrees of histological steatosis, and has therefore proven to be highly reproducible.

In conclusion, the difficulties with reproducing high-fat steatosis models is underexposed in literature and should be taken into account when performing experimental NAFLD studies.

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