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Effect of resveratrol on experimental non-alcoholic steatohepatitis

Sara Heebøll[a,b,*], Karen Louise Thomsen[a], Andrew Clouston[c], Elias Immanuel Sundelin[b,d], Yulia Radko[e], Lars Porskjær Christensen[e], Mehdi Ramezani-Moghadam[f], Martin Kreutzfeldt[a], Steen Bønløkke Pedersen[b,g], Niels Jessen[d], Lionel Hebbard[f], Jacob George[f], Henning Grønbæk[a,b]

[a] Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark
[b] Department of Clinical Medicine, Aarhus University, Denmark
[c] Centre for Liver Disease Research, University of Queensland, Brisbane, Australia
[d] Research Laboratory for Biochemical Pathology, Aarhus University Hospital, Denmark
[e] Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Denmark
[f] Storr Liver Centre, Westmead Millennium Institute, University of Sydney and Westmead Hospital, Australia
[g] Department of Endocrinology, Aarhus University Hospital, Denmark

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ABSTRACT

Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH) are increasing clinical problems for which effective treatments are required. The polyphenol resveratrol prevents the development of fatty liver disease in a number of experimental studies. We hypothesized that it could revert steatohepatitis, including hepatic inflammation and fibrosis, in an experimental NASH model.

To induce hepatic steatohepatitis, a 65% fat, 2% cholesterol and 0.5% cholate (HFC) diet was fed to rats for 1 or 16 weeks, prior to treatment. Subsequently, the diet was supplemented with resveratrol (approx. 100 mg/rat/day) to three intervention groups; week 2–4, 2–7 or 17–22. Treated animals were sacrificed at the end of each intervention period with appropriate control and HFC diet controls. Blood and liver were harvested for analysis.

When commenced early, resveratrol treatment partially mitigated transaminase elevations, hepatic enlargement and TNFα induced protein-3 protein expression, but generally resveratrol treatment had no effect on elevated hepatic triglyceride levels, histological steatohepatitis or fibrosis. We observed a slight reduction in Collagen1α1 mRNA expression and no reduction in the mRNA expression of other markers of fibrosis, inflammation or steatosis (TGFβ, TNFα, α2-MG, or SREBP-1c). Resveratrol metabolites were detected in serum, including *trans*-resveratrol-3-*O*-sulphate/*trans*-resveratrol-4′-*O*-sulphate (mean concentration 7.9 μg/ml).

Contrary to the findings in experimental steatosis, resveratrol treatment had no consistent therapeutic effect in alleviating manifest experimental steatohepatitis.

1. Introduction

Non-alcoholic liver disease (NAFLD) is the most common chronic liver disease and is associated with the obesity pandemic. Approximately 20–33% of adults in developed countries have NAFLD, while 2–10% have non-alcoholic steatohepatitis (NASH), the inflammatory form of the disease [1–3]. In NASH, steatosis is accompanied by intralobular inflammation with hepatocellular ballooning, which can lead to progressive fibrosis with an increased risk of cirrhosis and hepatocellular carcinoma [2]. Moreover, NASH is a risk factor for diabetes and ischemic heart disease, adding to the elevated mortality among these patients [4,5].

The medical management of NASH remains suboptimal [6] and therefore new therapies are required. To test new treatment approaches, reliable animal models of NASH are needed, but until

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HFC, high fat, high cholesterol and cholate; RSV, resveratrol; AMPK, AMP-activated protein kinase; SIRT1, silent information regulation-2 homolog-1; HFD, high-fat diet; TG, triglyceride; CD, control diet; HFC-R, HFC diet with added resveratrol; NASH-CRN, Non-alcoholic Steatohepatitis Clinical Research Network; NAS, NAFLD Activity Score; RT-PCR, quantitative real-time polymerase chain reaction; ALT, alanine aminotransferase; FFA, free fatty acid; α2-MG, α2-macroglobulin; TNFαIP3, TNFα induced protein 3; LC-MS/MS, liquid chromatography-tandem mass spectrometry; BW, body weight; Coll1α1, collagen 1α1; TGFβ, transforming growth factor β; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; SREBP-1c, sterol regulatory element-binding protein-1c.

* Corresponding author at: Department of Hepatology and Gastroenterology, Aarhus University Hospital, Nørrebrogade 44, 8000 Aarhus C, Denmark.
Tel.: +45 78463828.
E-mail address: saraheeb@rm.dk (S. Heebøll).

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recently, there have been few models that replicate the histopathology of the human disease with steatosis, inflammation, hepatocyte ballooning and liver fibrosis. A new rat model, in which steatohepatitis is induced by the consumption of a high fat, high cholesterol and cholate (HFC) diet, displays the typical histological changes of human NASH [7–9].

Several lines of experimental evidence indicate that *trans*-resveratrol (RSV, PubChemCID: 445154), could constitute a novel treatment option for NAFLD [10–17]. RSV is a polyphenol found in Japanese knotweed, and in minute amounts in the skin of grapes and other plants [18]. RSV is an activator of AMP-activated protein kinase (AMPK) and silent information regulation-2 homolog-1 (SIRT1), thereby mimicking caloric restriction and promoting anti-inflammatory and anti-oxidant effects [11]. These effects, documented in multiple *in vitro* and *vivo* reports, make RSV a celebrated candidate for the treatment of obesity-related complications like NAFLD [10,19]. In a recent report by our group, RSV added to a high-fat diet effectively prevented hepatic steatosis in rats [20], supporting other studies of experimental NAFLD [10,13,14,21]. These data suggest that RSV could be a novel nutraceutical for the treatment of NASH, but experimental data are limited as only few studies report NASH changes [12,22,23] and none report the full NASH spectrum. Also, translation of the positive *in vitro* and experimental results on various conditions, including NAFLD, has proved difficult [24–27]. This has rendered suspicion that RSV may be ineffective in clinically relevant conditions [28,29].

We hypothesized that RSV could improve both the early and advanced pathology of experimental steatohepatitis in the HFC model, including limiting the extent of hepatic inflammation and fibrosis.

2. Methods

2.1. Study design

In study 1, we investigated the effects of RSV on early NASH changes. Study 2 aimed to investigate the effects of RSV on the later stages of NASH. For both studies, we used female Wistar rats weighing 200–260 g at study start. The rats were kept on a regular 12-h light/dark cycle under controlled temperature conditions ($21 \pm 2\,^{\circ}C$). Animals were given free access to water and control diet (CD), HFC diet or a HFC diet supplemented with RSV (HFC-R). Diets were open source diets (CD, D09052201; HFC, D09052204 and HFC-R, D09052206) formulated by Research Diets (Inc., New Brunswick, USA) for study 1 and by Specialty Feeds (Glen Forest, WA, Australia) for study 2. The energy composition of the CD consisted of 10% fat (cocoa butter 4%, soybean oil 6%), 20% protein and 70% carbohydrate. In the HFC diet, 65% of the energy came from fat (cocoa butter 59% and soybean oil 6%), 20% from protein and 10% from carbohydrate and the diet contained 2% cholesterol and 0.5% cholate. RSV (>98% purity) was a gift from Evolva (Basel, Switzerland) and was mixed with the HFC diet to a concentration of 9 g/kg chow to provide an intended dose of 100 mg per rat daily.

Animals were randomly divided into groups according to the study design (Fig. 1). In study 1, the effect of RSV on NAFLD and NASH prevention was investigated in the groups HFC-R4 (RSV treatment week 2–4, both weeks included) and HFC-R7 (RSV treatment week 2–7), where RSV treatment began one week after commencing the HFC diet. In study 2, the effect of RSV on established NASH was investigated in HFC-R22 (RSV treatment week 17–22, both weeks included). In both studies, RSV-treated animals and their appropriate standard diet (CD; CD4, CD7, CD22) and HFC diet controls (HFC; HFC4, HFC7, HFC22) were sacrificed at the end of each intervention period. We also sacrificed CD and HFC at the end of week 1 and 16 to examine the level of NASH changes at RSV treatment start.

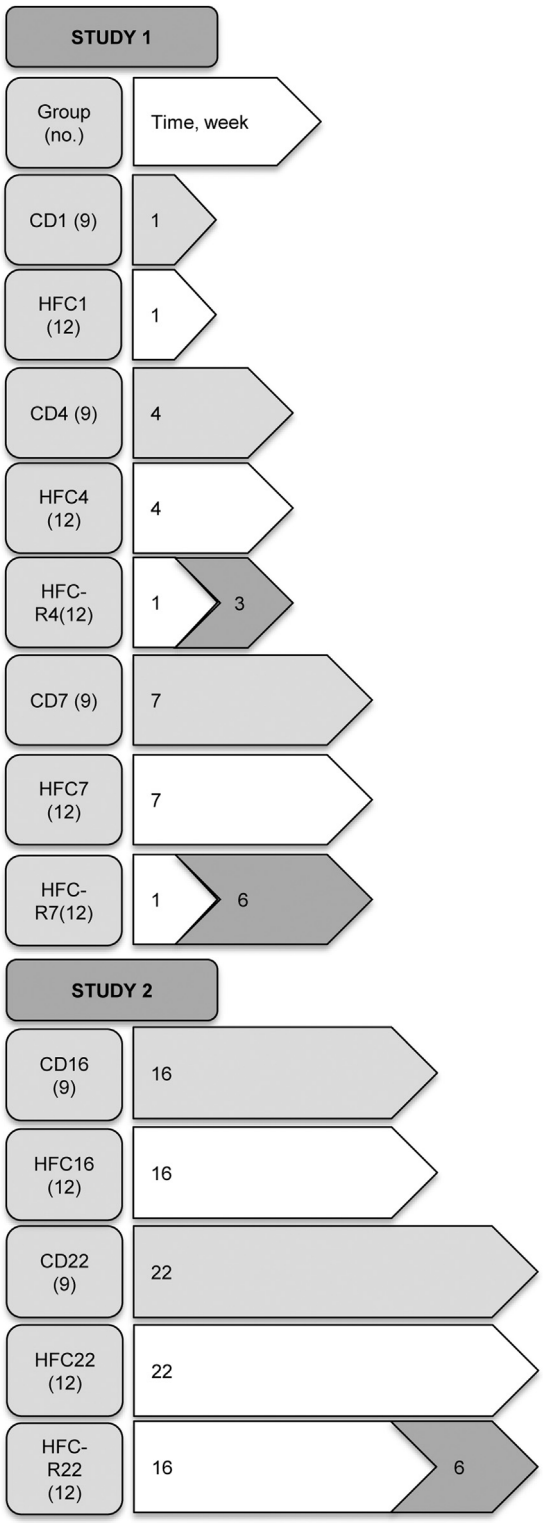

Fig. 1. Study design. Light gray arrows indicate periods where rats were treated with control diet (CD), white arrows periods with high-fat and -cholesterol diet (HFC) and dark gray arrows periods with HFC diet containing high-dose resveratrol (HFC-R, RSV).

Throughout the study, animals were weighed and food consumption was registered twice weekly. At experiment end, the un-fasted animals were anesthetized, plasma and serum isolated and the liver removed, weighed and processed for further analysis.