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Dietary docosahexaenoic acid reverses nonalcoholic steatohepatitis and fibrosis caused by conjugated linoleic acid supplementation in mice

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ABSTRACT

It has been shown that docosahexaenoic acid (DHA) prevents nonalcoholic fatty liver disease (NAFLD) and insulin resistance (IR) caused by conjugated linoleic acid (CLA), a *trans* fatty acid (TFA). Here, we evaluated whether DHA will reverse existing CLA-induced NAFLD and IR in mice. DHA-specific effects on existing NAFLD involved significant ($P < 0.005$) lowering of hepatic weight and triacylglycerol content and expression of genes involved in fatty acid synthesis, enhancing expression of genes involved in fatty acid oxidation, and increasing serum adiponectin levels. Also, immunohistochemistry showed lower expression of hepatic CD163 (inflammation) and smooth muscle α-actin (fibrosis). Compared to the CLA diet, mice fed DHA and control diets had significantly ($P < 0.05$) lower serum insulin and ALT activity, but only DHA had lower ($P = 0.05$) expression of genes involved in fibrosis. DHA supplementation for 4 weeks reversed already existing hepatic steatosis, inflammation, and fibrosis caused by CLA.

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Abbreviations: *Acca*, acetyl co-A carboxylase A; *Acox1*, acyl coenzyme A oxidase 1; AT, adipose tissue; ALT, alanine aminotransferase; ARS, Agricultural Research Service; CLA, conjugated linoleic acid; *Col1a1*, procollagen type I alpha 1; CON, control; *Cpt1a*, carnitine palmitoyltransferase 1a-liver; DHA, docosahexaenoic acid; ECM, extracellular matrix; EPA, eicosapentaenoic acid; HOMA-IR, homeostasis model assessment of insulin resistance; H&E, haematoxylin and eosin; HSC, hepatic stellate cells; IR, insulin resistance; IL-6, interleukin-6; IL-8, interleukin-8; ALA, alpha-linolenic acid; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PPARG, peroxisome proliferator-activated receptor gamma; *Scd1*, stearoyl-coenzyme A desaturase 1; SMA, smooth muscle α-actin; T2DM, type 2 diabetes mellitus; TFA, *trans* fatty acid; TG, triglycerides; *Timp1*, tissue inhibitors of metalloproteinase-1; TNF-α, tumour necrosis factor alpha; USDA, United States Department of Agriculture

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1. Introduction

Prevalence of obesity has remained high in the US since 2003; more than one third of adults (~78 million people) and nearly one in five youths aged 2–19 years old are obese (Ogden, Carroll, Kit, & Flegal, 2014). Obesity remains to be a common and costly health condition in the US, costing $147 billion in 2008 (Finkelstein, Trogdon, Cohen, & Dietz, 2009). The social and medical impacts of being overweight and obese have prompted consumers to seek over-the-counter management solutions for weight loss or gain prevention. This is substantiated by the US weight loss market segment which was over $61 billion in 2012 (Marketdata Enterprises, 2013). A specific ingredient claimed to reduce body weight and fat loss is a mixture of conjugated *trans* fatty acid (TFA) isomers, i.e. *trans*-10, *cis*-12 and *cis*-9, *trans*-11 conjugated linoleic acid (CLA), which are available in pill-form supplements, foods, and beverages (Larsen, Toubro, & Astrup, 2003). Chemically speaking CLA is a TFA, but it is exempt from the mandatory nutrition labelling of TFA in foods that went into effect in 2006 because the US Food and Drug Administration defines TFAs to include only non-conjugated forms, i.e. isolated *trans* double bonds (FDA, 2003). However, the Institute of Medicine of the National Academy of Sciences defines TFAs to include both conjugated and non-conjugated forms. CLA isomers in dietary supplements are conjugated TFA. Although CLA isomers hold GRAS (generally regarded as safe) status from the FDA, the European Food Safety Authority panel concludes that the safety of CLA consumption longer than 6 months has not been established under its proposed use (EFSA, 2012).

Consumption of conjugated TFA, such as CLA, for weight loss has been increasing, and global sales of the CLA ingredient alone are expected to reach $200 million by 2017 (PRWeb.com, 2011). Consumer interest in CLA exists due to the marketing of its claimed anticarcinogenic, antiadipogenic, antiatherosclerotic, and delipidating effects (Blankson et al., 2000; Ip, Masso-Welch, & Ip, 2003; Stachowska et al., 2012). However, the side effects of CLA, particularly due to the t10, c12 CLA isomer, include hepatic steatosis (lipid accumulation), inflammation, and insulin resistance (IR) in animals and humans (Fedor, Adkins, Mackey, & Kelley, 2012; Martinez, Kennedy, & McIntosh, 2011; Poirier, Shapiro, Kim, & Lazar, 2006; Riserus, Arner, Brismar, & Vessby, 2002). IR is a characteristic of T2DM and is associated with metabolic disorders. The resistance of adipose tissue (AT) to the action of insulin results in unsuppressed lipolysis, thereby releasing free fatty acids for liver uptake, re-esterification, and triacylglycerol (TG) storage. Consequently, this steatosis makes the liver susceptible to inflammation due to cellular events involving oxidative stress, lipotoxicity, mitochondrial dysfunction, pro-inflammatory cytokines, and hepatic stellate cell (HSC) activation, which promotes fibrogenesis (Browning & Horton, 2004; Malhi & Gores, 2008). Collectively, liver inflammation and injury present in steatotic liver result in nonalcoholic steatohepatitis (NASH), a progressive stage of nonalcoholic fatty liver disease (NAFLD) that leads to end stage liver disease.

NAFLD is regarded as the liver component of metabolic syndrome and is the most common form of chronic liver disease in developed countries, and it affects more than 30% of the US population (Browning et al., 2004). Its prevalence is even greater in high risk groups; NAFLD is present in 70% of adult T2DM patients and over 90% in those who are obese with associated IR, and 20% of NAFLD may develop to NASH, which requires medical attention (Henao-Mejia et al., 2012). More concerning is the rapid increase in NAFLD in the paediatric population worldwide over the last decade and its prevalence is increased to 85% in obese children (Welsh, Karpen, & Vos, 2013). Simple steatosis is considered to be a benign condition; however, NASH-associated end stage liver disease will be the main cause for liver transplants in the US in the next two decades. Between 26% and 37% of NASH patients develop fibrosis within 6 years, and 9% of these patients develop cirrhosis within 10 years; incidence of NASH-associated hepatocellular carcinoma is predicted to increase in the US in the coming decades (Starley, Calcagno, & Harrison, 2010).

Lifestyle intervention is the first line of defence to reduce hepatic steatosis, but reports on improvements in other histological aspects of the disease are lacking (Thoma, Day, & Trenell, 2012). Limited medicinal intervention is available for NASH in adults and none exists for children. Insulin sensitisers such as thiazolidinediones have been suggested as therapeutic candidates for improving steatosis and inflammation; however, its long-term safety has been questioned (Lincoff, Wolski, Nicholls, & Nissen, 2007). Nutritional interventions such as vitamin E and n-3 PUFAs offer evidence for their use but are not recommended for all populations, and interpretations of the results from human studies have been limited due to a small sample size, duration of treatment, and dosage administered (Chalasani et al., 2012).

Previous studies using fish oil, which contains a mixture of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), prevented the CLA-induced NAFLD and IR (Ide, 2005; Winzell, Pacini, & Ahren, 2006). To understand the role of EPA and DHA individually, we compared their efficacy in preventing CLA-induced IR and NAFLD (Vemuri, Kelley, Mackey, Rasooly, & Bartolini, 2007). We found that both EPA and DHA prevented the CLA-induced NAFLD while only DHA prevented the increase in IR, and it also partially prevented the decrease in circulating adiponectin. Our choice to use DHA in the current study was based on the results of our previous study (Vemuri et al., 2007). DHA possesses several metabolic and health promoting effects (Adkins & Kelley, 2010) and is present in a variety of natural and supplemented food products. The popularity of CLA as a weight-loss supplement comes from the marketing of its ability to reduce body fat mass without disclosure of its adverse effects, including those on NAFLD (Iwata et al., 2007; Ramos, Mascarenhas, Duarte, Vicente, & Casteleiro, 2009). The current study examined the capability of DHA to reverse existing CLA-induced (1) hepatic steatosis through the suppression of hepatic lipogenesis and activation of fatty acid oxidation, (2) hepatic inflammation and fibrosis by inhibiting tissue macrophage and HSC activation and serum alanine aminotransferase (ALT) activity, and (3) IR and low adiponectin levels by restoring AT mass.