Atherosclerosis 228 (2013) 98–109

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Inhibition of sphingolipid synthesis improves dyslipidemia in the diet-induced hamster model of insulin resistance: Evidence for the role of sphingosine and sphinganine in hepatic VLDL-apoB100 overproduction

Mark J. Dekker [a], Chris Baker [a], Mark Naples [a], Josh Samsoondar [a], Rianna Zhang [a], Wei Qiu [a], Jennifer Sacco [a], Khosrow Adeli [a,b,c,*]

[a] Molecular Structure and Function, Research Institute, The Hospital for Sick Children, University of Toronto, Toronto, Canada
[b] The Department of Biochemistry, University of Toronto, Toronto, Canada
[c] The Banting and Best Diabetes Centre, University of Toronto, Toronto, Canada

ARTICLE INFO

Article history:
Received 11 August 2012
Received in revised form
17 January 2013
Accepted 20 January 2013
Available online 10 February 2013

Keywords:
Ceramides
Sphingolipids
Hamster
VLDL
Apolipoprotein B
Insulin resistance

ABSTRACT

Sphingolipids have emerged as important bioactive lipid species involved in the pathogenesis of type 2 diabetes and cardiovascular disease. However, little is known of the regulatory role of sphingolipids in dyslipidemia of insulin-resistant states. We employed hamster models of dyslipidemia and insulin resistance to investigate the role of sphingolipids in hepatic VLDL overproduction, induction of insulin resistance, and inflammation. Hamsters were fed either a control chow diet, a high fructose diet, or a diet high in fat, fructose and cholesterol (FFC diet). They were then treated for 2 weeks with vehicle or 0.3 mg/kg myriocin, a potent inhibitor of *de novo* sphingolipid synthesis. Both fructose and FFC feeding induced significant increases in hepatic sphinganine, which was normalized to chow-fed levels with myriocin ($P < 0.05$); myriocin also lowered hepatic ceramide content ($P < 0.05$). Plasma TG and cholesterol as well as VLDL-TG and -apoB100 were similarly reduced with myriocin treatment in all hamsters, regardless of diet. Myriocin treatment also led to improved insulin sensitivity and reduced hepatic SREBP-1c mRNA, though it did not appear to ameliorate the activation of hepatic inflammatory pathways. Importantly, direct treatment of primary hamster hepatocytes *ex vivo* with C2 ceramide or sphingosine led to an increased secretion of newly synthesized apoB100. Taken together, these data suggest that a) hepatic VLDL-apoB100 overproduction may be stimulated by ceramides and sphingosine and b) inhibition of sphingolipid synthesis can reduce circulating VLDL in hamsters and improve circulating lipids—an effect that is possibly due to improved insulin signaling and reduced lipogenesis but is independent of changes in inflammation.

1. Introduction

Increased circulating triglycerides (TG) is a risk factor for cardiovascular disease and, along with increased waist circumference, elevated blood pressure, and insulin resistance, is a component of the 'metabolic syndrome' [30]. The TG content of insulin-sensitive tissues like liver and muscle is inversely correlated with insulin sensitivity in obesity [14,40]. However, a growing body of evidence suggests that it is the accumulation of bioactive lipid products like sphingolipids and not TG *per se* that potentiate insulin resistance [6,19,24,29,35,51]. Particular attention has been focused on ceramides: sphingolipids derived from saturated fatty acids that are involved in both lipid metabolism and the regulation of apoptosis [20]. Diets high in saturated fat [24] and/or fructose-containing sugars—for example, fructose [2,42] or sucrose [46]—can elicit an insulin-resistant phenotype. Increased dietary saturated fat content (including palmitate) elevates tissue sphingolipid concentrations and subsequently induces insulin resistance via the inhibition of AKT or IRS1 phosphorylation [20]. In addition to ceramides, sphingolipids such as glycosphingolipids [6], sphingosine, and sphinganine [31] have been implicated in impaired insulin signaling. Moreover, increased tissue ceramide is associated with inflammation [20]. The role of inflammation in insulin resistance is well established [21] and we have previously shown that infusion

* Corresponding author. The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. Tel.: +1 416 813 8682; fax: +1 416 813 6257.
E-mail addresses: k.adeli@utoronto.ca, khosrow.adeli@sickkids.ca (K. Adeli).

0021-9150/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.atherosclerosis.2013.01.041

of TNF-α can directly induce both hepatic and intestinal insulin resistance in the Syrian Golden hamster [36,37]. This may represent a mechanistic link between the accumulation of hepatic lipid intermediates and inflammatory mediators, factors that have been shown independently to exert profound effects on hepatic insulin sensitivity and lipoprotein production.

The enzyme serine palmitoyl transferase (SPT) catalyzes the first step of sphingolipid biosynthesis and is potently inhibited by the drug myriocin [28]. Several recent publications have demonstrated that reduced sphingolipid synthesis with myriocin treatment improves insulin sensitivity [19], hepatic steatosis [12,19,51], and atherosclerosis [12,17,32–34,38]. Although several publications indicate that myriocin improves dyslipidemia [12,17,32–34], other studies do not support these findings [13,17]. Two particular methodological approaches may contribute to this discrepancy. First, it appears that myriocin may decrease the intestinal absorption of dietary lipid [34]. This may explain why oral administration has been shown to improve plasma TG and cholesterol in mice [12,32–34], while intraperitoneal (IP) injection does not seem to improve dyslipidemia [13,17]. Second, IP injection of myriocin improved dyslipidemia in ZDF rats [19] but not in apoE knockout mice [17]. Although it appears that myriocin has beneficial effects on plasma lipids and atherosclerosis, the exact *in vivo* effects of sphingolipid modulation on hepatic lipoprotein production—including apoB100 containing VLDL—are unclear.

In the present study, we utilized the well-characterized Syrian Golden hamster model of diet-induced dyslipidemia and insulin resistance. Two diets were tested: the first was high in fructose and the second was high in fat, fructose, and cholesterol (FFC). We have previously demonstrated that hamsters fed a diet high in fructose exhibit mild insulin resistance, hepatic steatosis, and dyslipidemia [44,45], while hamsters fed FFC demonstrate profound insulin resistance, steatosis, and dyslipidemia [4,5]. We hypothesized that myriocin would improve circulating TG in hamsters by reducing hepatic production of apoB100-containing VLDL particles.

2. Methods

2.1. In vivo dietary treatment

All *in vivo* protocols were approved by the Institutional Animal Care and Use Committee at the Hospital for Sick Children. In two separate *in vivo* feeding experiments, ~110 g male Syrian Golden hamsters (*Mesocricetus auratus*) were assigned to groups based on plasma TG and cholesterol levels. These values were determined following collection of baseline blood samples to ensure that levels were similar in each group. Blood samples were collected from fasted (5 h), anesthetized (isoflurane) hamsters via the retro-orbital sinus. Hamsters were fed either the standard chow diet, a diet enriched in fructose (60%), or a diet containing 30% fat, 40% fructose, and 0.25% cholesterol (all purchased from Dyets, Bethlehem, PA). Hamsters received either vehicle (PBS) or myriocin (0.3 mg/kg every 48 h, Cayman Chemicals) by intraperitoneal injection. Food intake and body weight were monitored throughout the feeding protocol. Following two weeks of feeding, hamsters were sacrificed, blood samples were taken via cardiac puncture, and tissues were collected and snap frozen in liquid nitrogen for later analysis.

2.2. Lipid and sphingolipid analyses

Blood was collected in heparin-coated tubes and was kept on ice until being centrifuged (10 min, 6000 RPM) to isolate plasma (Beckman Allegra, Fullerton, CA). An aliquot of plasma was analyzed for TG, cholesterol, alanine transaminase (ALT), and aspartate transaminase (AST) using a clinical autoanalyzer (VITROS 950; Ortho Chemical Diagnostics, Rochester, NY). Hepatic lipids were extracted using the methanol/chloroform extraction method as detailed by Folch et al. [9]. Total tissue TG and cholesterol were measured from the extracted lipids using the appropriate commercially available endpoint colorimetric assay (Randox Triglyceride and Cholesterol, Crumlin, UK).

To measure hepatic sphingolipids, samples were analyzed by Liquid Chromatography/Mass Spectrometry/Mass Spectrometry (LC/MS/MS) at The Centre for the Study of Complex Childhood Diseases and The Analytical Facility for Bioactive Molecules at the Hospital for Sick Children, Toronto, Ontario. Biological extracts were injected onto a reverse phase C18 HPLC Column to separate and resolve the various target sphingolipids. The sphingolipids were eluted from the column and analyzed on a triple quadrupole mass spectrometer (Sciex API 4000; AB Sciex, Foster City, CA). The data acquisition was performed in a targeted MRM (MS/MS) mode using sphingolipid-specific precursor ion to product ion (or parent to daughter ion) mass transitions resulting in highly sensitive and specific quantitative data. The resulting data was processed using Applied Biosystems (Forest Analyst, CA) 1.4.2 software.

2.3. Assessment of glucose tolerance by IPGTT

Glucose tolerance was assessed by an intraperitoneal glucose tolerance test (IPGTT) in chow-, fructose-, and FFC-fed hamsters treated with myriocin. Following a 5-hour fast, blood samples were collected from the saphenous vein using a hand-held glucometer (TrueTouch) and baseline glucose was assessed. Glucose (3 g/kg) was introduced via the IP injection of a 30% glucose solution. Blood glucose was measured at 15, 30, 60, and 90 min following injection.

2.4. Immunoblot analysis

Standard western blotting techniques were applied to analyze target proteins as previously described [5]. Briefly, a portion of tissue was weighed and homogenized in ice-cold solubilization buffer

Table 1
List of primers used for qRT-PCR.

		Product size (bp)	Ref. or accession number
SREBP-1c	5′-GCGGACGCAGTCTGGG-3′ 5′-ATGAGCTGGAGCATGTCTTCAAA-3′	95	[41]
SCD-1	5′-ATTACTGGAGTGAAGCTTTCGTG-3′ 5′-GATTCAATGTTCTTGTCGTAGGG-3′	139	EU003989
FAS	5′-GCAGTCTTGAGTAGCTTTGTGCT-3′ 5′-GGGAGCTGTCCAGATTAATACCT-3′	139	AF356086 [a]
apoE	5′-ACACAGGAACTGACGGTACTGAT-3′ 5′-GTTCCTCCAGCTCCTTTTTGTAT-3	73	NM009696 [a]
PPARα	5′-CCCTAGAACTGGATGACAGTGAC-3′ 5′-CCGATCTCCACAGCAAATTATAG-3′	65	AJ555631
TNF	5′-CTGGTTTACTCCCAGGTTCTCTT-3′ 5′-TTATCCTCGTAGGACACAGCAAT-3′	98	AF046215
SOCS3	5′-ATCTTTGTCGGAAGACTGTCAAC-3′ 5′-GGTCACTTTCTCATAGGAGTCCA-3′	53	NM053565 [a]
PAI-1	5′-TCGGAGTAAAAGTGTTTCAGCAT-3′ 5′-ATCCCATAGCATCTTGGATCTG-3	147	NM012620 [a]
Il-6	5′-AAACTAGTGTGCTGTGCCTTAGC-3′ 5′-TAGATCCCCAATGACAGGATATG-3′	81	AB028635
SPT1	5′-GGAAGGAATCCTCATACTTTGGA-3″ 5′-AATCAGTTCTTCCTTTTCCTTGG-3′	103	NM001108406 [a]
18S	5′-TAAGTCCCTGCCCTTTGTACACA-3′ 5′-GATCCGAGGGCCTcACTAAAC-3′	71	K03432

Primer pairs for qRT-PCR were designed using Primer3 software and sequence information obtained from NCBI.

[a] No hamster sequence available. Primers were designed to highly conserved regions determined by multiple sequence alignments performed on closely related species.