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Overexpression of sphingosine kinase 1 in liver reduces triglyceride content in mice fed a low but not high-fat diet

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ABSTRACT

Hepatic insulin resistance is a major risk factor for the development of type 2 diabetes and is associated with the accumulation of lipids, including diacylglycerol (DAG), triacylglycerols (TAG) and ceramide. There is evidence that enzymes involved in ceramide or sphingolipid metabolism may have a role in regulating concentrations of glycerolipids such as DAG and TAG. Here we have investigated the role of sphingosine kinase (SphK) in regulating hepatic lipid levels. We show that mice on a high-fat high-sucrose diet (HFHS) displayed glucose intolerance, elevated liver TAG and DAG, and a reduction in total hepatic SphK activity. Reduced SphK activity correlated with downregulation of *SphK1*, but not *SphK2* expression, and was not associated with altered ceramide levels. The role of SphK1 was further investigated by overexpressing this isoform in the liver of mice *in vivo*. On a low-fat diet (LFD) mice overexpressing liver SphK1, displayed reduced hepatic TAG synthesis and total TAG levels, but with no change to DAG or ceramide. These mice also exhibited no change in gluconeogenesis, glycogenolysis or glucose tolerance. Similarly, overexpression of SphK1 had no effect on the pattern of endogenous glucose production determined during a glucose tolerance test. Under HFHS conditions, normalization of liver SphK activity to levels observed in LFD controls did not alter hepatic TAG concentrations. Furthermore, DAG, ceramide and glucose tolerance were also unaffected. In conclusion, our data suggest that SphK1 plays an important role in regulating TAG metabolism under LFD conditions.

1. Introduction

Insulin resistance is an escalating global health problem that is associated with a number of diseases, including cardiovascular disease and type 2 diabetes [1]. A major risk factor for the development of type 2 diabetes is the presence of hepatic insulin resistance [2]. Defects in hepatic insulin action develop rapidly in response to nutrient excess in both animals and humans and occur prior to the development of peripheral insulin resistance [3–9], indicating that defects in hepatic glucose metabolism are a primary event in the development of impaired glucose handling. There is evidence demonstrating an association between abnormalities in hepatic lipid metabolism and the development of insulin resistance and glucose intolerance [9–13]. Increases in hepatic triacylglycerol (TAG) content have been shown to correlate with defects in liver glucose metabolism [14]. However, rather than being causative, it is believed that TAG may simply be an inert intracellular lipid storage depot, and that accumulation of other bioactive lipid species, such as diacylglycerol (DAG) and/or the sphingolipid ceramide, may be mechanistically linked to the induction of hepatic insulin resistance [13,15,16].

The mechanism(s) underlying the regulation of increased hepatic lipid deposition in response to energy excess are not clear, but have been linked to elevated circulating free fatty acids (FFA), increased delivery of chylomicron remnants and/or an impaired ability to oxidize fatty acids [17]. Alternatively, it has recently been hypothesized that hepatic lipid accumulation may arise as a result of alterations in sphingolipid metabolism [18]. Indeed, an interaction between sphingolipid and glycerolipid metabolism has recently been demonstrated [19]. Mice lacking sphingosine 1-phosphate (S1P) lyase, the enzyme that controls the final degradative step in sphingolipid metabolism, had substantially elevated ceramide levels in liver, while also exhibiting increased hepatic DAG and TAG [19]. These data suggest a functional link between sphingolipid and glycerolipid metabolic pathways, which may be relevant to the pathogenesis of glucose intolerance and type 2 diabetes [19].

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Another important enzyme in sphingolipid metabolism is sphingosine kinase (SphK) which phosphorylates sphingosine to S1P. S1P is an important signaling lipid and intermediate in the catabolism of sphingolipids to ethanolamine-phosphate and hexadecanal [20]. SphK therefore plays an important role in regulating sphingolipid levels [20]. SphK exists in two isoforms, SphK1 and SphK2. Although SphK1 overexpression has been shown to cause a reduction in ceramide [21–23], the role of SphK1 in regulating the metabolic pathways of other lipids is not well studied. In principal, SphK1 could impact on glycerolipid levels by affecting ceramide production and the subsequent generation of sphingomyelin, a reaction that produces DAG, the immediate precursor for TAG synthesis [19]. In this study we have investigated the role of SphK in regulating hepatic lipid levels. Specifically, we investigated whether the increase in hepatic TAG levels seen during high-fat high-sucrose diet (HFHS) feeding are associated with changes in SphK activity. In addition, we investigated whether increased SphK1 expression leads to a reduction in hepatic lipid content and improved glucose homeostasis. We hypothesized that targeted activation of hepatic SphK would not only cause a reduction in liver ceramide but would also lower DAG and TAG content, which would ameliorate defects in glucose metabolism induced by a HFHS.

2. Methods

2.1. Animals

All experiments were approved by the Monash University Animal Research Platform Animal Ethics Committee and were in accordance with the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation. Mice were maintained at 22 ± 1 °C on a 12 h light/dark cycle, with free access to food and water.

2.2. Effect of HFHS feeding on hepatic SphK

Eight week old male C57BL/6 mice were maintained on standard low-fat control diet (LFD; 9% energy as fat, Barastoc Rat & Mouse, Ridley AgriProducts, Melbourne, Australia) or high-fat high-sucrose diet (HFHS; 42% energy from fat, 20% by weight from sucrose, Specialty Feeds SF4-001, Glen Forrest, WA, Australia) for 8 weeks. One week prior to the end of the dietary intervention, an oral glucose tolerance test (OGTT, described below) was performed on mice following a 5 h fast. One week later, at the end of the dietary intervention, mice were humanely killed following a 5 h fast (7:00 am food withdrawal) and livers snap frozen in liquid nitrogen for subsequent biochemical analysis to determine the effect of HFHS feeding on hepatic SphK gene expression and enzyme activity.

2.3. AAV mediated overexpression of hepatic SphK1

An adeno-associated viral (AAV) vector plasmid containing a cDNA construct encoding mouse SphK1 with a cytomegalovirus promoter packaged into a pseudotype 8 capsid which displays strong liver tropism [24,25] was purchased from Vector BioLabs (PA, USA), accession GENBANK: BC037710. Systemic delivery of the recombinant AAV8:SphK1 vector was achieved by administering the vector via a tail vein injection into 4 week old LFD-fed male C57Bl/6 mice. Two doses of AAV8:SphK1 were used: 2×10^{11} and 1×10^{12} vector genomes. An empty vector (AAV8:null) was administered to control animals.

2.4. Stable isotope labeled oral glucose tolerance test (OGTT)

To determine whether glucose homeostasis was altered by overexpression of SphK1, a stable isotope labeled OGTT was performed. This allows for the simultaneous assessment of the glucose disposal and endogenous glucose production (EGP) components under dynamic conditions which closely reflect the postprandial state [26–33]. Following a 5 h fast, AAV8:SphK1 and AAV8:null mice (four weeks post AAV injection) received a 50 mg oral gavage of stable isotope labeled glucose (25 mg of 2-^{2}H glucose (M1) and 25 mg 6,6-^{2}H glucose (M2), Cambridge Isotope Laboratories, MA). Blood glucose was measured with a glucose meter (Accu-Check, Roche, NSW, Australia) prior to and at 15, 30, 60, 90 and 120 min following gavage. Blood (~10 μL) was collected from the tail tip prior to and at 15, 30, 60 and 120 min following glucose gavage. Samples were spun in a centrifuge (4 °C, 8000 rpm) and plasma was used to perform mass isotopomer analysis of plasma glucose via gas chromatography–mass spectrometry (GC–MS) [31]. The principle of this technique is based on the fact that the measured (absolute) blood glucose concentration can be separated into its constituent components, that is, that derived from the labeled oral glucose load (M1 and M2 isotopomers) and that derived from endogenous sources such as the liver and kidneys (the unlabeled endogenous M0 glucose isotopomer). Thus, the labeled load component represents glucose disposal while the unlabeled represents glucose derived from endogenous sources [26–30]. By calculating the difference between the 6,6-^{2}H glucose (M2) area under the curve (AUC) from that of the 2-^{2}H glucose (M1) AUC, futile glucose cycling can be measured during the OGTT [31,34–37]. This provides an index of the efficiency of hepatic glucose uptake throughout the OGTT by quantifying the activities of hepatic glucokinase relative to that of glucose-6 phosphatase (glucose phosphorylation potential). During fasting, futile glucose cycling is a function of glucokinase while following glucose or meal administration the flux is reversed (net glucose uptake), and glucose cycling is a function of glucose 6-phosphatase [34–37]. Samples were deproteinized by adding 50 μL of ice cold methanol to 2 μL of plasma. After thorough mixing the sample was centrifuged (4 °C, 16,000 rpm, 10 min) and the supernatant was dried and methoximated by addition of 20 μL methoxyamine HCl in pyridine (20 mg/mL, Supelco, Sigma-Aldrich, St. Louis, USA). After incubation at 90 °C for 1 h methoximated glucose was converted to the TMS derivative by addition of 20 μL N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (Thermo Fisher Scientific, Waltham, USA). Samples were analyzed using a 7890A gas chromatography (GC) system and a 5975C mass selective detector (MSD) (Agilent Technologies, Santa Clara, USA) in positive chemical ionization (PCI) mode using methane as a reagent gas and helium as a carrier. The GC was equipped with a DB5 capillary column with a 10 m inert duraguard (J&W Scientific, 30 m, 250 μm inner diameter, 0.25 μm film thickness). The injector insert and GC–MS transfer line temperatures were 270 and 250 °C, respectively. The oven temperature gradient was set to: 70 °C (1 min); 70 °C to 295 °C at 12.5 °C/min; 295 °C to 320 °C at 25 °C/min; 320 °C for 2 min. Sample (1 μL) was injected with the inlet set to split mode (split ratio 10 mL/min). The isotopomers of glucose TMS derivatives were identified and quantitated by selected ion monitoring (SIM) for ions m/z 554 to 562 corresponding to M0 to M8 of the fragment $[M + 1–16]^{+}$ of methyloxime penta-TMS glucose (m/z 569). The mass isotopomer abundances were determined using Mass Hunter Workstation (Agilent Technologies, Santa Clara, USA). After correction for natural abundance (background enrichment), the true fractional abundance (mole % excess) [38] of the M0 (endogenous glucose), M1 (2-^{2}H glucose) and M2 (6,6-^{2}H glucose) isotopomers were multiplied by the blood glucose concentration at each corresponding time point of the OGTT to calculate the absolute concentrations of load (labeled) and endogenous (unlabeled) glucose [31]. Load glucose was calculated by multiplying the M2 glucose concentration by a factor of 2, to account for the 50% concentration of the M2 glucose in the administered oral glucose load [31]. Futile glucose cycling was calculated by subtracting the M1 AUC from that of the M2 AUC.

2.5. Determination of the sources of EGP

To determine whether hepatic overexpression of SphK1 had an impact on gluconeogenesis or glycogenolysis, we employed the $^{2}H_2O$