DIABETES

Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes

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A potentially useful approach for drug discovery is to connect gene expression profiles of disease-affected tissues ("disease signatures") to drug signatures, but it remains to be shown whether it can be used to identify clinically relevant treatment options. We analyzed coexpression networks and genetic data to identify a disease signature for type 2 diabetes in liver tissue. By interrogating a library of 3800 drug signatures, we identified sulforaphane as a compound that may reverse the disease signature. Sulforaphane suppressed glucose production from hepatic cells by nuclear translocation of nuclear factor erythroid 2–related factor 2 (NRF2) and decreased expression of key enzymes in gluconeogenesis. Moreover, sulforaphane reversed the disease signature in the livers from diabetic animals and attenuated exaggerated glucose production and glucose intolerance by a magnitude similar to that of metformin. Finally, sulforaphane, provided as concentrated broccoli sprout extract, reduced fasting blood glucose and glycated hemoglobin (HbA1c) in obese patients with dysregulated type 2 diabetes.

INTRODUCTION

A large number of genetic variants and tissue gene expression profiles ("disease signatures") have been associated with complex polygenic diseases over the last decade (*1*). However, these data have not been maximally used to identify new therapies. One potentially interesting approach is to use genetic and gene expression data to interrogate libraries of drug signatures (*2*). A drug signature denotes differentially expressed genes between untreated and treated samples and takes into account that most compounds have multiple gene effects on expression beyond the primary target.

A few previous studies have used gene set enrichment analysis to connect disease signatures with drug signatures and identified candidate drugs for cancer, neurological, and gastrointestinal disorders, as suggested by subsequent effect studies in cell lines and animal models (*3*–*6*). However, it remains to be shown whether such signature connections can be used to identify compounds with pathophysiological relevance for humans. The usefulness of this approach for drug discovery may have been hampered by the fact that expression signatures from disease-affected tissues largely represent secondary changes. Moreover, the rank order of a gene in a signature based on expression fold change does not necessarily reflect the pathophysiological importance of the gene.

To overcome these limitations, we generated disease signatures based on disease-relevant tissue networks and human genetic data. Network models have been proposed as a useful framework for studying complex data (*7*). We and others have shown that highly connected network genes ("hub genes") are more likely to be involved in disease processes (*8*, *9*). Here, we hypothesized that disease signatures reflecting the network topology and not merely expression fold change could be used to identify compounds that reverse aberrantly expressed key drivers of disease (overexpressed genes should be downregulated in the drug signature and vice versa). To test this strategy, we analyzed diabetes-associated gene networks in liver tissue. We aimed to identify compounds to treat exaggerated hepatic glucose production in patients with type 2 diabetes (T2D), which is a clinically severe problem (*10*). T2D is affecting a growing number of the population, with more than 300 million people worldwide afflicted by the disease and even more having prediabetes (*11*). Metformin is currently the first-line therapy and reduces hepatic glucose production via adenosine monophosphate (AMP) kinase-dependent and kinase-independent mechanisms (*12*–*14*). However, 15% of all T2D patients cannot take metformin because of reduced kidney glomerular filtration rate and hence increased risk of lactic acidosis (*15*). Moreover, up to 30% of patients treated with metformin develop nausea, bloating, abdominal pain, or diarrhea, and 5 to 10% of the patients are therefore unable to continue with metformin (*16*). Finding additional treatment options to reduce exaggerated hepatic glucose production is therefore a high priority.

RESULTS

Network-based disease signatures were connected to drug signatures

We first analyzed global gene expression data in liver tissue from an F2 cross between C57BL/6J *ApoE*$^{-/-}$ and C3H/HeJ *ApoE*$^{-/-}$ mice with a total of 334 mice (169 female and 165 male). This cross, termed the B × H cross, recapitulates a range of phenotypes associated with the metabolic syndrome, including dyslipidemia, increased body weight, and hyperglycemia (*17*, *18*). We analyzed the topological overlap of the gene expression data and identified groups of coexpressed genes ("modules"), comprising a total of 1720 genes, which were associated with hyperglycemia.

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We then used four different criteria to select those of the 1720 genes that were most likely to be involved in diabetes pathophysiology. First, we analyzed connectivity (k_{in}) as a measure of gene coexpression to identify highly connected network hubs (*7*). Second, we used Bayesian modeling to identify key regulators of the coexpression networks (*19*). Third, we used genetic risk variants associated with T2D to inform the generation of the disease signature by constructing a protein-protein interaction network with a total of 319 genes centered on those that are in close proximity of known T2D risk variants (*20*). Fourth, we used data on single-nucleotide polymorphisms associated with gene expression traits (eSNPs) in human liver samples to identify genes with eSNPs that were also risk variants for T2D, because such genes have been suggested to cause metabolic disease (*21*). The rationale for using these four criteria was to incorporate both network topology and genetic information to focus on those of the 1720 genes that were likely to have the highest pathophysiological impact based on previous reports on features that are important for metabolic disease networks (*8*, *17*, *19*, *21*).

A leave-one-out approach with linear modeling was used to impute weights for each criterion. The linear modeling was iterated for each of the four criteria variables. Solving the resultant equation system provided a coefficient for each variable, which was used as a weight to reflect the relative importance of the variable. Finally, we computed a score for each of the 1720 genes based on the four criteria and used it as a filter to generate a 50-gene disease signature for T2D in liver tissue (table S1).

We then compiled a library of 3852 drug signatures from publically available data sets at the Gene Expression Omnibus (GEO) or the European Bioinformatics Institute based on experiments with compound treatments of cell lines or primary tissues and analyzed by Affymetrix, Agilent, or Illumina chips (see table S2 for a list of the compounds). The hepatic diabetes signature and each of the drug signatures were analyzed using an enrichment metric based on a Kolmogorov-Smirnov (KS) test. The average KS enrichment metric for the 10 top-ranked drugs was significantly higher when using the filtered 50-gene disease signature compared with using 50 genes randomly selected out of the 1720 genes or using all 1720 genes (>2-fold higher KS scores; $P < 0.001$; the top-ranked drugs differed depending on which disease signature was used), suggesting that the disease signature filtered by the four criteria generates more robust data. The drug signature that exhibited the highest overlap with the 50-gene hepatic diabetes signature was derived from studies of sulforaphane (SFN)–treated human hepatocytes (GEO accession number GSE20479) (*22*). A complete rank list of all compounds is provided in table S3.

SFN reduces glucose production in hepatoma cells

Several other methods could potentially be used to compare disease and drug signatures, and it is critical that bioinformatics predictions are validated both in vitro and in vivo. We therefore investigated the compound with the highest overlap, SFN, in greater detail. SFN is a naturally occurring isothiocyanate found in cruciferous vegetables such as broccoli. It activates nuclear factor erythroid 2–related factor 2 (NRF2) by modifying the conformation of Kelch-like ECH-associated protein 1 (KEAP1) cytoplasmic chaperone, thus releasing NRF2 for translocation to the nucleus and transcriptional activation of genes with the antioxidant response element (ARE) in their promoters (*23*). Although SFN uptake into cells leads to an initial burst of reactive oxygen species, it then rapidly activates the KEAP1-NRF2-ARE system to induce antioxidant enzymes and increase cellular glutathione for an overall antioxidative effect (*24*). As an inducer of endogenous antioxidants, SFN has been extensively studied for its protective effects in different experimental models associated with oxidative stress and chemoprotection (*25*), inflammatory disorders (*26*), and fatty liver disease (*27*, *28*). To date, SFN has not been implicated for the treatment of exaggerated hepatic glucose production in T2D.

We first studied the effect of SFN on glucose production using H4IIE cells, a rat hepatoma cell line. Preincubation with SFN at 0.5 to 10 μM for 24 hours resulted in a dose-dependent decrease of glucose production during a subsequent 5-hour incubation in glucose-free buffer supplemented with gluconeogenetic substrates (41% decrease at 3 μM; $P = 0.0009$; Fig. 1A). SFN at doses up to 3 μM did not induce apoptosis (fig. S1A). Metformin also decreased glucose production in a dose-dependent manner (Fig. 1B). Addition of insulin to the buffer reduced glucose production by 40% in control cells. The combined effects of SFN and insulin on glucose production were only additive (Fig. 1A). In contrast, we observed synergistic effects of metformin and insulin on glucose production ($P = 0.005$ for analysis of additive versus synergistic effects at 250 μM metformin; Fig. 1B and Materials and Methods).

We also pretreated H4IIE cells with high concentrations of palmitate (250 μM) to mimic diabetogenic conditions. Palmitate pretreatment increased glucose production by 34%, in line with previous observations (*29*, *30*). Concomitant treatment with SFN not only prevented the exaggerated glucose production but also reduced overall glucose production by 45% ($P = 0.0011$; Fig. 1C).

The effect of SFN on glucose production is mediated by the transcription factor NRF2

We then investigated the effect of SFN on nuclear translocation of NRF2, which has been demonstrated as a major mechanism of action for SFN in other cell types (*31*). We observed a clear dose-dependent effect of SFN on nuclear translocation of NRF2 in the H4IIE cells (Fig. 1D). Silencing of *Nrf2* by small interfering RNA (siRNA) (81 ± 3% knockdown) increased glucose production 2.3-fold ($P = 0.0009$) and attenuated the relative effect of SFN on glucose production ($P = 0.007$; Fig. 1E). This suggests that a large part of SFN-mediated reduction of glucose production is mediated via NRF2, although we do not exclude that other mechanisms may also be involved.

SFN has no effect on insulin signaling and mitochondrial oxygen consumption

Because insulin is a key regulator of hepatic glucose production in the fed state, we next examined the effect of SFN on key enzymes in the insulin signaling cascade. However, in H411E cells, insulin-mediated phosphorylation of insulin receptor substrate 1 (IRS1/Tyr^{608}) and AKT serine/threonine kinase 1 (AKT1/Ser^{473}) was unaffected by SFN. Moreover, we observed no effect of SFN on insulin signaling after palmitate pretreatment (fig. S1B). The observations are in agreement with the data on glucose production (Fig. 1A), which suggest that the combined effects of SFN and insulin on glucose production are nonsynergistic and merely additive. These results show that the effect of SFN on glucose production is not mainly exerted via altered insulin signaling.

Because NRF2 affects the activity of complex 1 in the mitochondrial respiratory chain via limitation of substrates (*32*), we also investigated whether SFN affected mitochondrial function using the Seahorse XF24 instrument to measure the mitochondrial oxygen consumption rate (OCR) in H4IIE cells in response to gluconeogenetic substrates (L-lactate, pyruvate, and L-glutamine). However, SFN (3 μM) did not affect mitochondrial OCR under these experimental conditions (fig. S1C).

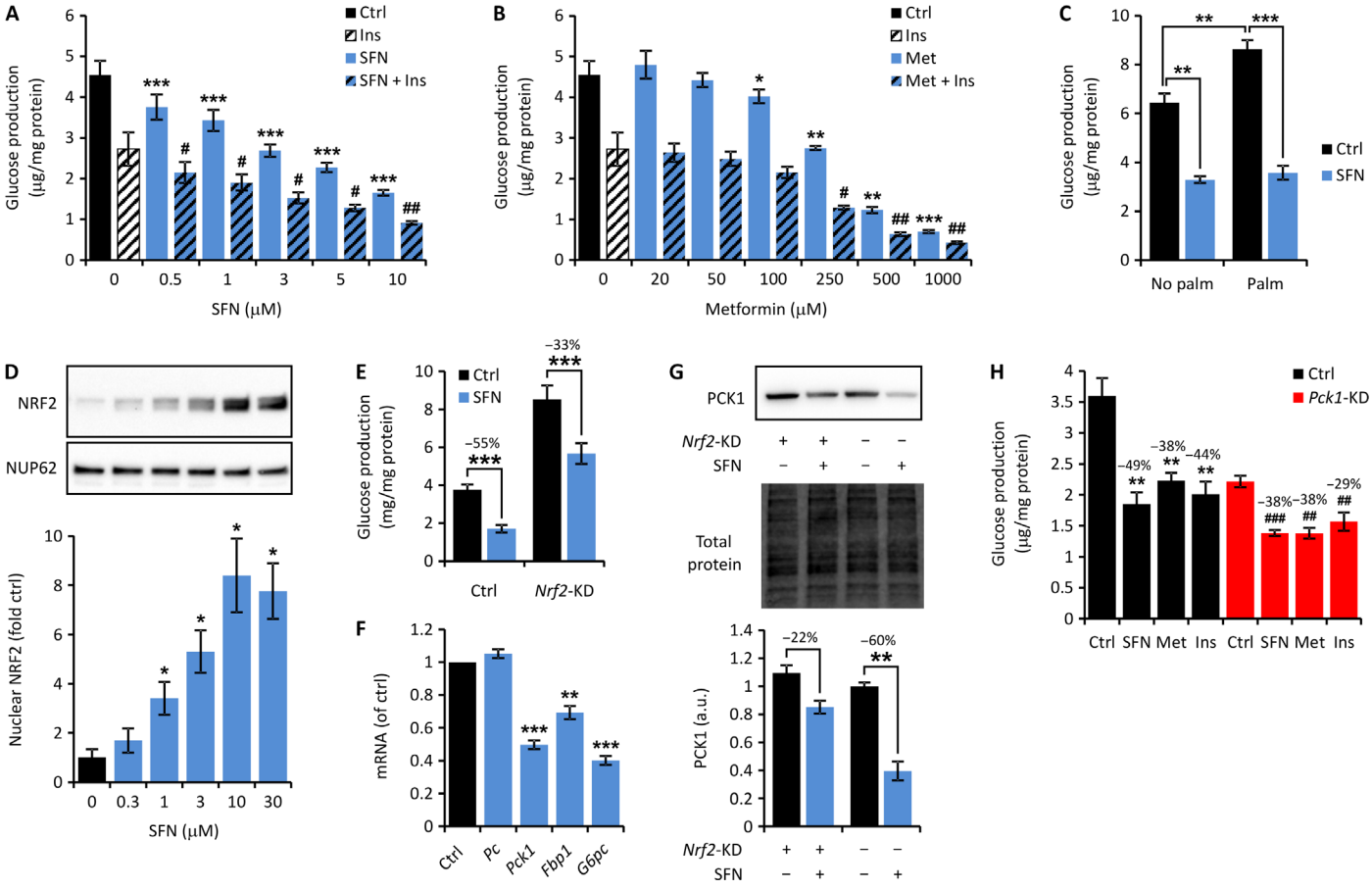

Fig. 1. Effects of SFN on glucose production in H4IIE hepatoma cells. Glucose production (GP) was assessed during a 5-hour incubation in glucose-free buffer with pyruvate, L-lactate, and L-glutamine (GP-buffer). (**A**) GP in the presence or absence of 10 nM insulin (INS) in the GP-buffer assessed after 24-hour preincubation with or without SFN as indicated ($n = 5$). * denotes control (ctrl) cells versus SFN-treated cells; # denotes insulin-treated cells in the absence versus presence of SFN. (**B**) As in (A), with or without metformin (met) preincubation for 24 hours instead of SFN ($n = 5$). (**C**) GP assessed after 16 hours of pretreatment with 250 μM palmitate (palm) followed by 24 hours with or without 3 μM SFN ($n = 4$). (**D**) Representative immunoblot and summary statistics showing nuclear translocation of NRF2 protein after 1 hour of incubation with SFN at the doses indicated. Nucleoporin 62 (NUP62) was used as loading control ($n = 3$). (**E**) GP after knockdown of *Nrf2* (*Nrf2*-KD) or treatment with a negative control siRNA followed by 24-hour preincubation with or without 3 μM SFN ($n = 5$). (**F**) mRNA expression of genes involved in gluconeogenesis after treatment with 3 μM SFN for 24 hours. Statistical analysis was performed using $\log_2$-transformed data ($n = 4$ to 6). (**G**) Representative immunoblot and summary statistics of PCK1 protein expression after knockdown of *Nrf2* (*Nrf2*-KD) or treatment with a negative control siRNA followed by 24-hour preincubation with or without 3 μM SFN ($n = 4$). a.u., arbitrary units. (**H**) GP after knockdown of *Pck1* (*Pck1*-KD) or treatment with a negative control siRNA. Cells were then treated with or without 3 μM SFN or 250 μM metformin for 24 hours as indicated ($n = 5$). * denotes control versus SFN, metformin, or insulin in cells treated with a negative control siRNA; # denotes control versus SFN, metformin, or insulin in *Pck1*-KD cells. Data are means ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$.

SFN reduces the expression of genes involved in glucose production

To further explore the mechanism by which SFN affects glucose production, we analyzed the expression of genes involved in gluconeogenesis, a major determinant of hepatic glucose production. Of the four key enzymes involved in gluconeogenesis—pyruvate carboxylase (PC; $P = 0.2$), phosphoenolpyruvate carboxykinase 1 (PCK1; also known as PEPCK-C; $P = 0.004$), fructose-1,6-bisphosphatase 1 (FBP1; $P = 0.0007$), and glucose-6-phosphatase, catalytic subunit (G6PC; $P = 0.002$)—all except PC were significantly down-regulated by SFN, as assessed by expression microarrays of H4IIE cells (table S4). Quantitative reverse transcription polymerase chain reaction of H4IIE cells treated with 3 μM SFN for 24 hours confirmed these findings, with *Pck1* and *G6pc* being the gluconeogenesis genes most strongly down-regulated by SFN (Fig. 1F). PCK1 is of special interest because it catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, the rate-limiting step in gluconeogenesis. SFN reduced PCK1 protein by 60% in H4IIE cells ($P = 0.0011$; Fig. 1G). We also analyzed the effects of SFN on PCK1 protein after *Nrf2* knockdown and observed a mere 22% reduction of PCK1, which is a significant attenuation compared with the 60% reduction in control cells ($P = 0.0082$ for the comparison of SFN effects in *Nrf2*-KD versus control cells; Fig. 1G). This suggests that PCK1 down-regulation by SFN is largely mediated via NRF2.

We next silenced *Pck1* with siRNA (*Pck1*-KD) in H4IIE cells (69 ± 2% knockdown), which resulted in a 38% reduction of glucose production. The inhibitory effect of SFN on glucose production was attenuated by 23% (49% reduction of glucose production by SFN in control cells compared to 38% reduction in *Pck1*-KD cells; $P = 0.025$; Fig. 1H). In contrast, the effect of metformin (250 μM) was unaffected by *Pck1*-KD (38% reduction in both cases), showing that metformin-induced

suppression of glucose production is independent of PCK1. We also observed that the effect of insulin was significantly reduced (33% reduction of effect size; $P = 0.037$) in *Pck1*-KD cells, confirming previous observations that PCK1 is regulated by insulin (*33*). Together, these data suggest that a major mechanism for SFN-mediated reduction of glucose production is down-regulation of key gluconeogenetic enzymes via NRF2. The mechanism of action of SFN is therefore different from that of metformin, which acts via AMP-activated protein kinase, by lowering cyclic AMP and inhibiting mitochondrial glycerophosphate dehydrogenase (*12*–*14*). As with metformin, which has multiple modes of action, we do not exclude that additional mechanisms may contribute to the effects of SFN on glucose production.

SFN reduces glucose production in mouse hepatocytes

We also verified that SFN affects glucose production in primary mouse hepatocytes. Hepatocytes were incubated with SFN in the presence of palmitate for 24 hours before the experiments to mimic a diabetogenic milieu. Palmitate incubation increased glucose production 2.2-fold. SFN induced a 45% reduction of glucose production in palmitate-exposed hepatocytes ($P = 0.042$; Fig. 2A) and restored glucose production to levels observed in the absence of palmitate. These data corroborate the observations in H4IIE cells and show that SFN treatment suppresses exaggerated glucose production triggered by a diabetogenic milieu in vitro.

SFN prevents the development of glucose intolerance in diet-challenged rats

After these experiments, we aimed to investigate the effect of SFN in different animal models in vivo. We first assessed the ability of SFN to prevent the development of glucose intolerance. Male Wistar rats received a diet with 45% fat content [high-fat diet (HFD)] and were concomitantly treated with SFN [2.5 mg/kg, intraperitoneally (ip), three times per week] or vehicle over 15 weeks. In vehicle-treated animals, fasting blood glucose, which reflects hepatic glucose production, increased by 16% during the 15-week period on HFD ($P = 0.0014$). By contrast, there was no impairment of fasting glucose in SFN-treated rats (Fig. 2B). Over the entire period, fasting blood glucose was significantly lower in SFN-treated compared to nontreated rats (on average 7.5% lower; $P = 2 \times 10^{-5}$; Fig. 2B). At the end of the 15-week period, there was also a significant difference in insulin sensitivity between the SFN-treated group and the nontreated group as measured by an intraperitoneal insulin tolerance test (IPITT) [$P = 0.032$ for area under the curve (AUC); Fig. 2C].

To further explore the ability of SFN to prevent glucose intolerance under different dietary conditions, we changed diets after 15 weeks on 45% HFD. Half of the rats received instead a diet with even higher fat content (60%), and half received a diet with 60% fructose content [high-fructose diet (HFrD)] with continued treatment with SFN or vehicle. SFN improved glucose tolerance, assessed by an intraperitoneal glucose tolerance test (IPGTT) after 5 weeks on 60% HFrD ($AUC_{30–120}$ $P = 0.025$, Fig. 2D; Fig. 2E for IPITT). In rats on 60% HFD, SFN did not affect glucose tolerance (Fig. 2F) but changed insulin sensitivity as assessed by an IPITT (AUC $P = 0.0038$; Fig. 2G). These findings show that SFN was able to prevent the development of diet-induced glucose intolerance induced by a 45% HFD or a 60% HFrD, although the 60% HFD was too severe a stressor to fully prevent glucose intolerance by SFN.

We also extracted liver tissue from rats on 60% HFrD treated with SFN (2.5 mg/kg) three times a week for 27 weeks and analyzed global gene expression by microarray. We analyzed the effect of SFN on the 50-gene hepatic disease signature and observed that a significant fraction of the signature was reversed in SFN-treated rats compared with vehicle-treated animals ($P < 0.0001$, Fisher's exact test; fig. S2).

SFN improves glucose tolerance in rats on HFD or HFrD

On the basis of the observation that SFN prevents diet-induced glucose intolerance, we next wanted to test whether SFN could be used to treat rats that had already developed glucose intolerance. Male Wistar rats were therefore put on a 60% HFD for 11 months and then received SFN (5 mg/kg, ip) daily for 14 days. Treatment with SFN resulted in improved glucose tolerance, as assessed by an oral glucose tolerance test (OGTT) ($P = 0.049$ for $AUC_{30–120}$; Fig. 2H).

We next compared the effects of SFN and metformin on glucose tolerance and hepatic glucose production in vivo. Male Wistar rats that had been fed a 60% HFrD for 6 months were treated with either SFN (10 mg/kg, ip) or metformin [300 mg/kg, per os (po)] for 9 to 12 days. Glucose tolerance was significantly improved by both SFN and metformin during an OGTT ($AUC_{30–120}$ $P = 0.046$ for SFN-treated versus untreated rats and $P = 0.019$ for metformin-treated versus untreated rats; Fig. 2I). On the basis of our observations in H4IIE cells and primary hepatocytes, we hypothesized that SFN would reduce hepatic glucose production. Hepatic glucose production was assessed by an intraperitoneal pyruvate tolerance test (IPPTT), which showed that SFN significantly reduced blood glucose at 30 and 120 min and reduced $AUC_{30–120}$ by 20% ($P = 0.049$, one-sided *t* test; Fig. 2J). Metformin significantly reduced blood glucose at 60 min during the IPPTT and reduced $AUC_{30–120}$ by 25% ($P = 0.046$, one-sided *t* test). These results demonstrate that SFN improves glucose tolerance in rats by a magnitude similar to that of metformin.

SFN improves glucose tolerance in mice with diet-induced diabetes

To test the effect of SFN in a more severe model of diabetes, we used C57BL/6J mice, which develop overt diabetes when challenged with an HFD. After 10 weeks on a 60% HFD, male C57BL/6J mice were treated with SFN (10 mg/kg) daily for 4 weeks (same dose as used in the rat experiments). We also tested the effect of a considerably lower dose (0.5 mg/kg SFN daily). The high dose of SFN improved both fasting glucose ($P = 0.044$) and glucose tolerance ($AUC_{30–120}$ $P = 0.012$; Fig. 3A) as measured by an IPGTT. In this model, SFN did not affect insulin sensitivity (Fig. 3B). The low dose of SFN had no effect on glucose tolerance. Extracted liver from the mice treated with SFN at high dose had reduced triglyceride content relative to mice receiving the low dose ($P = 0.038$; Fig. 3C). In lean mice receiving a low-fat control diet, glucose tolerance was unaffected by SFN (Fig. 3A).

We next performed specific measurements of absolute gluconeogenetic rate in C57BL/6J mice on 60% HFD using mass spectrometry of glucose fragments after ingestion of deuterium water combined with hyperinsulinemic-euglycemic clamps. The mice were treated with SFN (10 mg/kg) or control vehicle daily for 4 weeks. After a 7- to 9-hour fast and a 2-hour infusion of [6,6-$^2H_2$]glucose, glucose production was almost entirely derived from gluconeogenesis (101 ± 2% for control and 97 ± 3% for SFN-treated mice). In the controls, we observed a clear correlation between body weight of the mice (range, 36 to 47 g) and gluconeogenetic rate ($R^2 = 0.81$; $P = 0.002$). This is in line with reports in humans showing that glucose production is exaggerated in obese compared to lean subjects (*34*–*36*). In SFN-treated mice, the correlation between weight and increased gluconeogenesis was completely abolished ($R^2 = 0.11$; $P = 0.5$), suggesting that SFN protects against increased gluconeogenesis in the overweight animals. There was no difference when

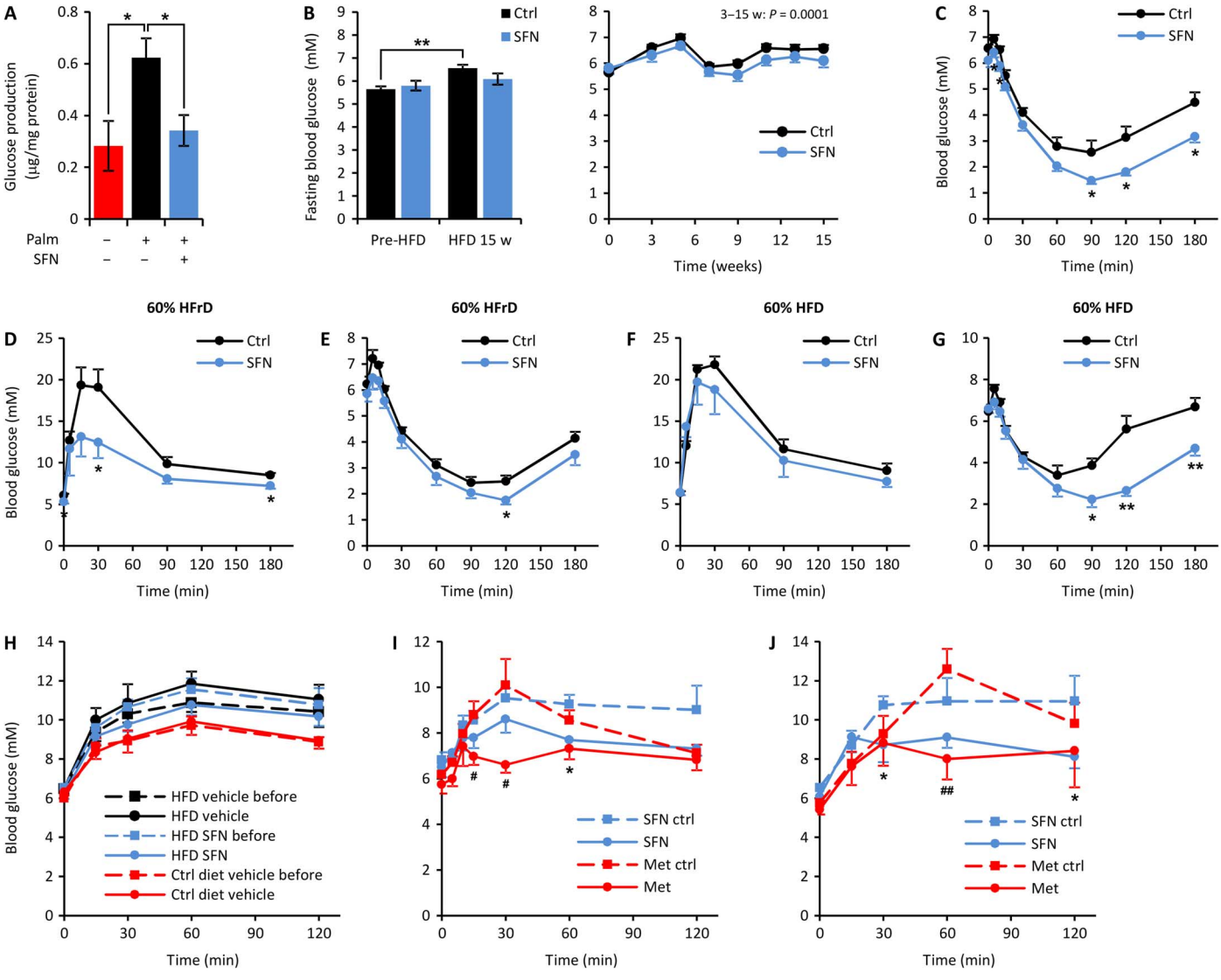

Fig. 2. Effects of SFN in mouse hepatocytes and in rat models of diet-induced glucose intolerance. (**A**) Glucose production from primary mouse hepatocytes during 45 min. Cells were preincubated in the presence or absence of 500 µM bovine serum albumin–bound palmitate followed by 24-hour incubation with or without 3 µM SFN as indicated ($n = 3$). (**B**) Left: Fasting blood glucose in male Wistar rats before and after 15 weeks of HFD feeding with or without concomitant SFN treatment [SFN (2.5 mg/kg) three times per week; $n = 9$ per group]. Right: Longitudinal measurements of fasting glucose during the 15-week period. *P* value for the blood glucose for the SFN-treated compared to control-treated rats during the 3- to 15-week period is shown. (**C**) IPITT after the 15-week period for the same rats as in (B). (**D** to **G**) Insulin resistance (IR) and glucose tolerance assessed in the same animals as in (B) after an additional 5 weeks on 60% HFrD or HFD with or without concomitant SFN treatment. IPGTT (D) and IPITT (E) data from rats on 60% HFrD ($n = 4$ to 5 per group), and IPGTT (F) and IPITT (G) data from rats on 60% HFD ($n = 4$ to 5 per group). (**H**) OGTT data from male Wistar rats fed a low-fat control diet (ctrl diet) or a 60% HFD for 11 months before and after 14 days of treatment with SFN (5 mg/kg per day) or vehicle ($n = 5$ HFD ctrl, $n = 6$ HFD SFN, and $n = 8$ ctrl diet). (**I**) OGTT in male Wistar rats with diet-induced glucose intolerance (fed 60% HFrD for 6 months) after 10 days of treatment with or without SFN (10 mg/kg per day, ip) or metformin (300 mg/kg, po) as indicated. * denotes SFN ($n = 6$) versus vehicle ($n = 7$); # denotes metformin ($n = 6$) versus the corresponding vehicle ($n = 8$). (**J**) IPPTT on the same rats as in (I) after 9 to 12 days of treatment with or without SFN or metformin as indicated. One-sided *t* test was used for statistical analysis. * denotes SFN ($n = 6$) versus vehicle ($n = 7$); # denotes metformin ($n = 6$) versus the corresponding vehicle ($n = 7$). Data are means ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$.

taking all animals into account, independent of weight. However, by using a post hoc analysis of the heaviest mice, we observed that SFN significantly reduced absolute gluconeogenesis rate compared to controls in this subset (6.5 mg/kg per minute in control and 5.6 mg/kg per minute in SFN-treated mice; $P = 0.035$; Fig. 3D).

During the clamp, we also measured total body insulin-stimulated glucose uptake (Rd clamp), which reflects insulin sensitivity. This was similar between the groups [ctrl (11.8 mg/kg) and SFN (12.8 mg/kg); Fig. 3E], which parallels our findings in H4IIE cells that SFN does not influence insulin signaling. Together, these experiments demonstrate that SFN improves glucose tolerance in animals with diet-induced diabetes via reduced gluconeogenetic rate.

The effect of SFN-containing broccoli sprout extracts was studied in T2D patients

Prompted by these findings in vitro and in vivo, we set out to investigate the effects of SFN on glucose control in T2D patients. SFN has been provided at high concentration as broccoli sprout extracts (BSEs) in

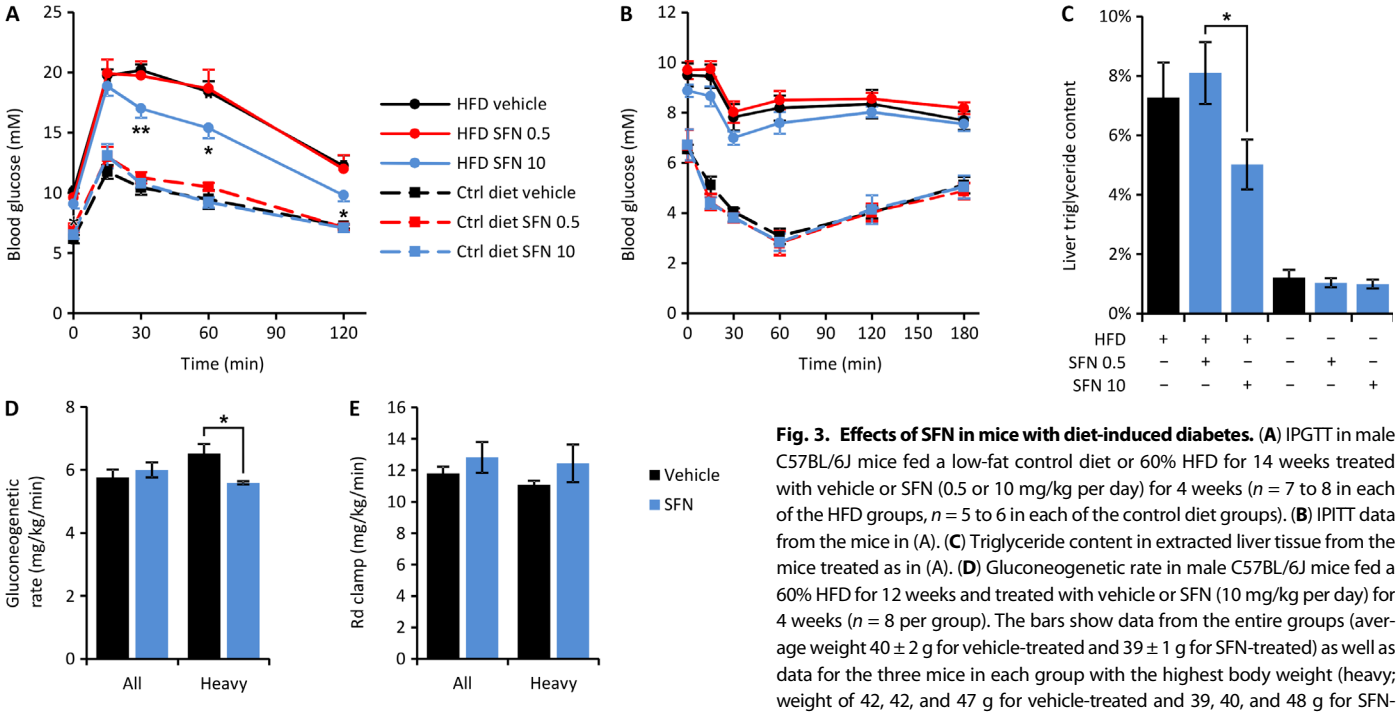

Fig. 3. Effects of SFN in mice with diet-induced diabetes. (**A**) IPGTT in male C57BL/6J mice fed a low-fat control diet or 60% HFD for 14 weeks treated with vehicle or SFN (0.5 or 10 mg/kg per day) for 4 weeks ($n = 7$ to 8 in each of the HFD groups, $n = 5$ to 6 in each of the control diet groups). (**B**) IPITT data from the mice in (A). (**C**) Triglyceride content in extracted liver tissue from the mice treated as in (A). (**D**) Gluconeogenetic rate in male C57BL/6J mice fed a 60% HFD for 12 weeks and treated with vehicle or SFN (10 mg/kg per day) for 4 weeks ($n = 8$ per group). The bars show data from the entire groups (average weight 40 ± 2 g for vehicle-treated and 39 ± 1 g for SFN-treated) as well as data for the three mice in each group with the highest body weight (heavy; weight of 42, 42, and 47 g for vehicle-treated and 39, 40, and 48 g for SFN-treated compared to an average weight of 38 ± 1 g for vehicle-treated and 37 ± 0.2 g for SFN-treated nonheavy mice). (**E**) Rate of disappearance of glucose (Rd) during clamp, reflecting whole-body insulin-stimulated glucose uptake, for the same mice as in (D). Data are means ± SEM. *$P < 0.05$; **$P < 0.01$.

several clinical studies for cancer, chronic obstructive pulmonary disease, autism, and inflammatory diseases (www.clinicaltrials.gov) [we used high-performance liquid chromatography (HPLC)–purified SFN at 99.5% in our cell and animal studies, but this has not been used for human studies so far].

Here, we used a dried powder of an aqueous extract of broccoli sprouts, which contains high concentrations of glucoraphanin, the inert glucosinolate precursor of SFN. Glucoraphanin is converted to SFN by the release of intrinsic sprout myrosinase during chewing and also by human enteric bacteria (*37*–*40*). After intake, the plasma concentration of SFN rises within 1 hour with a mean half-life of 1.77 ± 0.13 hours, but SFN exerts a sustained effect on gene expression (*41*). Renal tubular secretion is suggested to play a major role in the elimination (*37*). Safety studies using BSE corresponding to 50 to 400 μmol SFN daily have shown that BSE is well tolerated without clinically significant adverse effects (*42*–*45*).

We first tested the effect of BSE relative to HPLC-purified SFN on hepatic glucose production in H4IIE cells. The amount of SFN in BSE (SFN equivalents) was determined on the basis of the concentration of SFN obtained when glucoraphanin in BSE is hydrolyzed by adding exogenous myrosinase. We confirmed that BSE (at 3 μM SFN equivalents) was as effective as HPLC-purified SFN (3 μM) in suppressing glucose production ($P = 0.004$ for BSE and $P = 0.004$ for SFN relative to control; fig. S3A). No exogenous myrosinase was added during the experiment, demonstrating that the BSE contained sufficient amounts of myrosinase to effectively convert inert glucoraphanin to SFN.

Next, we wanted to ascertain that the effect of BSE on glucose production was caused by the SFN component. We therefore boiled the BSE, which inactivates myrosinase and prevents the conversion of glucoraphanin to SFN (*46*). After boiling, BSE had no effect on glucose production (fig. S3A). We also showed that the placebo (maltodextrin sprayed with copper-chlorophyllin) to be used in the clinical trial had no effect on glucose production. Moreover, we verified that BSE had a similar effect to that of SFN on glucose control in animals. Male C57BL/6J mice were made diabetic by a 60% HFD for 4 weeks and were then given BSE via gavage once daily at a dose corresponding to SFN (1.1 mg/kg) for 4 weeks. The BSE-treated mice had significantly lower fasting blood glucose compared to controls (9.6 versus 10.9 mM; $P = 0.009$) and improved glucose control during an IPGTT (fig. S3B).

After these verifications, we recruited 103 T2D patients for a randomized double-blind placebo-controlled study with BSE for 12 weeks. Patients with either well-regulated or dysregulated T2D (defined as having HbA1c above 50 mmol/mol) were recruited. All patients were of Scandinavian ethnicity and had been diagnosed with T2D less than 10 years ago. We hypothesized that BSE would improve fasting glucose (reflecting hepatic glucose production) and reduce HbA1c in patients with dysregulated T2D but have no effect in patients with well-regulated T2D, because well-regulated T2D patients exhibit impaired peripheral glucose uptake rather than exaggerated glucose production (*47*). The patients with dysregulated T2D were further divided into nonobese and obese [body mass index (BMI) > 30 kg/m^2], because hepatic glucose production has been shown to be more severely affected in obese compared to lean patients (*34*–*36*).

A total of 97 patients completed the study, of whom 60 had well-regulated and 37 dysregulated T2D. Of the patients with dysregulated T2D, 20 were nonobese and 17 were obese. All patients except three (well-regulated) had metformin treatment. The patients underwent initial blood sampling, including fasting glucose and HbA1c, and an OGTT, after which they received oral BSE or placebo once daily for 12 weeks. The BSE contained 150 μmol SFN per dose, which corresponds to one-third of the dose per body surface area compared with the animal experiments (using 10 mg/kg). This dose has been well

tolerated in clinical safety studies (*42*–*45*). A second OGTT and blood sampling were conducted at the end of the 12-week treatment period. The difference in fasting blood glucose (Δglucose) and HbA1c (ΔHbA1c) before and after treatment were determined for each patient.

BSEs improves fasting glucose and HbA1c in obese patients with dysregulated T2D

We observed a clear association between HbA1c levels at start of treatment and ΔHbA1c in response to BSE treatment (ΔHbA1c, 0.2 mmol/mol reduction per 1 mmol/mol higher HbA1c at start; $P = 0.004$, Fig. 4A), whereas there was no association in the placebo group ($P = 0.5$). There was also an association between BMI and ΔHbA1c in BSE-treated patients (ΔHbA1c, 0.4 mmol/mol reduction per 1 kg/m^2 higher BMI; $P = 0.015$ for the BSE group; not significant for the placebo group).

We then analyzed the patients with dysregulated T2D in detail, using intraindividual one-tailed comparisons before and after treatment. There was a significant change of fasting blood glucose (Δglucose) in BSE-treated compared with placebo-treated subjects ($P = 0.023$). Fasting plasma glucose was on average 9 ± 0.4 mM after placebo treatment and 8.3 ± 0.3 mM after BSE treatment. There was, however, no difference in ΔHbA1c between BSE and placebo in the entire group of patients with dysregulated T2D. In obese patients (BMI > 30 kg/m^2) with dysregulated T2D, who we hypothesized would benefit most from the treatment, both Δglucose ($P = 0.036$) and ΔHbA1c ($P = 0.034$) were significantly affected by BSE (Fig. 4B and Table 1). At the end of the 12-week period, HbA1c was 57 mmol/mol in placebo-treated and 53 mmol/mol in BSE-treated patients (ΔHbA1c, −4 mmol/mol; $P = 0.034$). There was a concomitant decrease of fasting blood glucose (8.9 mM with placebo and 8.2 mM with BSE; $P = 0.036$ for Δglucose; Fig. 4B) in these patients.

We also analyzed the serum concentration of SFN at the final visit (immediately before the OGTT) in the obese patients with dysregulated T2D using HPLC. SFN serum concentration ranged from 0.6 to 1.8 nmol/ml in BSE-treated patients (the concentration was 0.01 nmol/ml in placebo-treated subjects). The variation may be attributed to individual differences in bioavailability, body weight, and distribution volume. There was a clear association between serum SFN concentration and change in fasting blood glucose (Δglucose) in the BSE-treated patients ($P = 0.002$, corrected for body weight; Fig. 4C). The serum concentrations of SFN in BSE-treated patients (0.6 to 1.8 μM) were in the same range as the concentrations that reduced glucose production in vitro in H4IIE cells (Fig. 1A).

Previous studies using ^{13}C magnetic resonance spectroscopy have demonstrated that elevated fasting glucose production in T2D patients could be entirely attributed to increased gluconeogenesis. Our data from H4IIE cells show that SFN affects glucose production by reducing the expression of genes involved in gluconeogenesis rather than affecting insulin sensitivity. Moreover, SFN reduced gluconeogenetic rate in

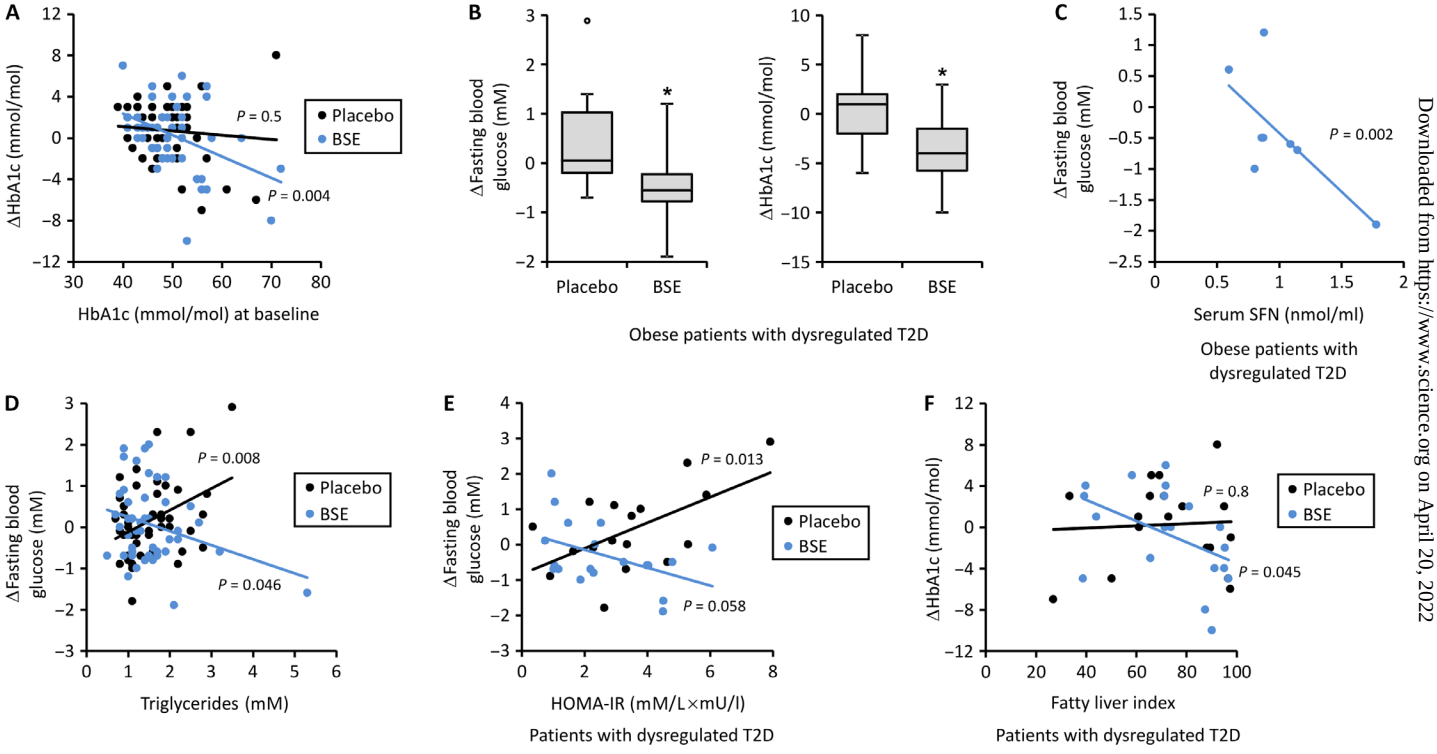

Fig. 4. Effects of highly concentrated SFN provided as BSE in T2D patients. (**A**) Association between HbA1c at the start of the study (baseline) and treatment-induced change in HbA1c (ΔHbA1c) after 12 weeks in all patients ($n = 50$ placebo and $n = 47$ BSE). (**B**) Box plots showing median, upper and lower quartiles, and maximum and minimum values of treatment-induced change in fasting blood glucose and HbA1c in obese patients with dysregulated T2D ($n = 9$ placebo and $n = 8$ BSE). Circle denotes outlier. (**C**) Association between serum concentration of SFN (after 12 weeks of treatment) and treatment-induced change in fasting blood glucose in obese patients with dysregulated T2D ($n = 8$ BSE). (**D**) Association between treatment-induced change in fasting blood glucose and plasma triglyceride concentrations at the start of the study in all patients ($n = 50$ placebo and $n = 47$ BSE). (**E**) Association between treatment-induced change in fasting blood glucose and HOMA-IR at the start of the study in patients with dysregulated T2D ($n = 18$ placebo and $n = 19$ BSE). (**F**) Association between treatment-induced change in HbA1c and fatty liver index at the start of the study in patients with dysregulated T2D ($n = 18$ placebo and $n = 19$ BSE). *$P < 0.05$.

Table 1. Effects of 12 weeks of treatment with BSE on clinical variables in T2D patients. Data are means ± SD for patients with well-regulated (HbA1c ≤ 50 mmol/mol) and with dysregulated T2D (HbA1c > 50 mmol/mol) who are nonobese (BMI ≤ 30 kg/m^2) or obese (BMI > 30 kg/m^2). Data measured before treatment start (baseline) and after 12 weeks on placebo or BSE.

Treatment	Phenotype	Time	Well-regulated T2D		Dysregulated T2D	
			Nonobese (*n* = 28)	Obese (*n* = 31)	Nonobese (*n* = 21)	Obese (*n* = 17)
Placebo	HbA1c (mmol/mol)	Baseline	46.4 ± 2.8	45.6 ± 3.4	54.6 ± 3.2	56.3 ± 7.5
		12 weeks	47.5 ± 4.1	46.3 ± 3.4	54.9 ± 4.4	56.6 ± 9.2
	Fasting P-glucose (mM)	Baseline	7.51 ± 0.91	7.26 ± 0.86	8.84 ± 0.84	8.33 ± 1.03
		12 weeks	7.55 ± 0.84	7.29 ± 0.87	9.08 ± 1.31	8.91 ± 1.75
	P-glucose 120 min (mM)	Baseline	13.77 ± 3.16	11.55 ± 2.58	17.00 ± 3.14	15.81 ± 2.50
		12 weeks	13.27 ± 3.58	11.22 ± 2.02	17.10 ± 3.82	15.28 ± 3.42
	BMI (kg/m^2)	Baseline	27.6 ± 1.9	33.3 ± 2.4	28.0 ± 1.3	33.1 ± 2.0
		12 weeks	27.8 ± 1.9	33.4 ± 2.5	28.1 ± 1.5	33.0 ± 2.1
	HOMA-IR (mM × mU/liter)	Baseline	1.75 ± 0.78	2.73 ± 1.50	2.36 ± 1.52	4.32 ± 1.80
		12 weeks	1.83 ± 0.86	3.04 ± 2.10	2.52 ± 1.70	5.11 ± 3.30
	ISI	Baseline	3.39 ± 1.59	2.47 ± 1.90	3.48 ± 2.69	1.60 ± 0.73
		12 weeks	3.70 ± 2.04	2.55 ± 2.08	3.41 ± 2.39	1.67 ± 0.79
	Fatty liver index	Baseline	60.2 ± 18.1	83.1 ± 13.9	58.0 ± 18.7	87.9 ± 10.8
		12 weeks	58.0 ± 17.7	81.2 ± 16.6	57.5 ± 22.2	87.9 ± 10.9
	P-triglycerides (mM)	Baseline	1.49 ± 0.65	1.39 ± 0.56	1.50 ± 0.49	1.88 ± 0.89
		12 weeks	1.40 ± 0.67	1.26 ± 0.42	1.40 ± 0.55	1.94 ± 0.89
BSE	HbA1c (mmol/mol)	Baseline	45.7 ± 3.2	46.1 ± 3.0	55.7 ± 6.0	57.1 ± 6.6
		12 weeks	46.9 ± 3.5	46.7 ± 2.7	57.3 ± 5.2	53.4 ± 6.8
	Fasting P-glucose (mM)	Baseline	7.49 ± 1.16	7.34 ± 0.94	8.61 ± 1.41	8.58 ± 1.60
		12 weeks	7.91 ± 1.68	7.60 ± 1.36	8.39 ± 1.24	8.15 ± 1.26
	P-glucose 120 min (mM)	Baseline	13.99 ± 4.22	13.33 ± 3.84	15.52 ± 3.26	15.64 ± 3.63
		12 weeks	13.83 ± 4.27	13.36 ± 3.17	16.51 ± 3.09	15.41 ± 3.61
	BMI (kg/m^2)	Baseline	28.1 ± 1.4	32.9 ± 1.8	27.8 ± 1.3	33.2 ± 2.1
		12 weeks	28.2 ± 1.4	33.4 ± 2.3	27.7 ± 1.3	33.1 ± 2.3
	HOMA-IR (mM × mU/liter)	Baseline	1.76 ± 0.76	2.46 ± 1.42	2.32 ± 1.77	3.02 ± 1.34
		12 weeks	2.44 ± 0.88	3.08 ± 1.50	2.20 ± 1.62	3.05 ± 0.87
	ISI	Baseline	2.78 ± 0.89	2.00 ± 0.84	4.08 ± 2.09	2.32 ± 1.03
		12 weeks	2.40 ± 1.00	1.92 ± 0.76	3.92 ± 1.98	2.28 ± 1.05
	Fatty liver index	Baseline	60.6 ± 18.7	83.2 ± 10.4	61.5 ± 18.8	87.7 ± 9.8
		12 weeks	62.9 ± 18.4	85.6 ± 10.3	62.4 ± 18.2	87.2 ± 7.6
	P-triglycerides (mM)	Baseline	1.42 ± 0.64	1.35 ± 0.40	1.87 ± 1.33	1.50 ± 0.53
		12 weeks	1.46 ± 0.65	1.42 ± 0.42	2.10 ± 1.23	1.40 ± 0.48

heavy C57BL/6J mice with diet-induced diabetes but had no effect on total body insulin-stimulated glucose uptake. The patient data are also compatible with a direct effect on the rate of gluconeogenesis, because BSE reduced fasting glucose and decreased HbA1c without any concomitant effect on hepatic IR, measured as homeostatic model assessment (HOMA)–IR, insulin sensitivity index (ISI), or glucose concentration at

2 hours of the OGTT (Table 1). There was a clear association between the reduction of fasting blood glucose (Δglucose) and the decrease in HbA1c (ΔHbA1c) in patients with dysregulated T2D ($P = 0.019$). This association was also significant after correction for HOMA-IR, ISI, and 2-hour glucose ($P = 0.0003$), suggesting that the change in HbA1c was mainly caused by reduced fasting blood glucose. We also estimated hepatic fat content using a validated fatty liver index (*48*) but observed no effect of BSE on this metric (Table 1). BSE did not change body weight, BMI, liver parameters, cholesterol concentration, plasma triglycerides, or blood hemoglobin concentration. BSE had no effect in patients with well-regulated T2D. It remains possible, however, that higher doses of SFN could also affect IR, in addition to the pronounced direct effect on the expression of gluconeogenesis genes.

BSE is most effective in obese patients with dysregulated T2D

Glucose production is exaggerated in dysregulated T2D, which is reflected in the higher fasting blood glucose among the patients with dysregulated T2D in our study (8.6 ± 0.2 in patients with dysregulated T2D versus 7.5 ± 0.2 mM in patients with well-regulated T2D; $P = 0.0001$). Consequently, BSE reduced fasting glucose in patients with dysregulated T2D but not in patients with well-regulated T2D ($P = 0.023$). We also observed an association between BMI and BSE-induced change in HbA1c ($P = 0.017$), and HbA1c was significantly reduced after BSE treatment in obese patients with dysregulated T2D ($P = 0.034$; Fig. 4B). BSE was more effective in lowering fasting blood glucose in patients with elevated plasma triglyceride concentrations ($P = 0.046$ for the association between plasma triglycerides at study start and Δglucose; an inverted association was observed in placebo-treated patients; $P = 0.008$; Fig. 4D). It is also notable that BSE was more effective in lowering fasting blood glucose in patients with high HOMA-IR ($P = 0.058$ for the association between HOMA-IR and Δglucose; Fig. 4E), and the BSE-induced reduction of HbA1c correlated with high fatty liver index ($P = 0.045$; Fig. 4F).

No severe adverse effects of BSE were reported

Most patients tolerated the BSE well. Eight patients receiving BSE and seven patients receiving placebo reported gastrointestinal side effects such as loose stools and flatulence, typically present during the first few days of the treatment period, after which these symptoms disappeared. Ten BSE-treated and five placebo-treated patients reported mild respiratory infections, and there were also a few other reported adverse events, including orthopedic ailments, most likely unrelated to the study compound (table S5). Of the 103 patients, 6 (5 with BSE and 1 with placebo) discontinued the study because of nausea (2 patients), headache (1 patient), glucose above 15 mM (one of the exclusion criteria; 1 patient), hospital visit for suspected ileus (later successfully treated; 1 patient), and depression (1 patient on placebo) (table S6).

DISCUSSION

Together, our data show that SFN reduces glucose production, partly via NRF2 translocation and decreased expression of key gluconeogenetic enzymes, and that highly concentrated SFN administered as BSE improves fasting glucose and HbA1c in obese patients with dysregulated T2D. BSE was well tolerated, and SFN reduced glucose production by mechanisms different from that of metformin. SFN also protects against diabetic complications such as neuropathy, renal failure, and atherosclerosis in animal models because of its antioxidative effects (*49*–*52*).

Our data suggest that BSE has a direct effect on gluconeogenesis rather than hepatic insulin sensitivity, but the degree of IR may still influence the efficacy of BSE via altered constitutive NRF2 activity. It has been shown that insulin signaling activates NRF2 via phosphatidylinositide 3-kinase (*53*). Moreover, studies in cardiomyocytes have shown that NRF2 is activated at the early stages of T2D to protect against increased reactive oxygen species but is reduced at later stages of the disease (*54*). This is further supported by observations of reduced NRF2 expression in animals with IR (*55*, *56*) and hepatic steatosis (*27*, *28*).

It is not surprising that BSE was most effective in obese patients with dysregulated T2D. First, our animal experiments showed an effect of SFN on glucose control in metabolically dysregulated animals on a HFD but not in metabolically well-regulated animals on a low-fat diet. Second, gluconeogenetic rate was correlated with body weight in mice with diet-induced diabetes, and SFN reduced gluconeogenetic rate specifically in the heaviest mice. Third, hepatic glucose production is often exaggerated in patients with high HbA1c, whereas patients with low HbA1c primarily have an impairment of peripheral glucose uptake (*47*). Fourth, it has been shown that hepatic glucose production is increased particularly in obese T2D patients, potentially via elevated free fatty acids (*34*–*36*).

There is abnormal regulation of hepatic glucose production early in the development of T2D, but it is typically compensated for by increased insulin secretion (*57*). SFN has been shown to protect from pancreatic β cell damage in animals (*58*). We observed no changes in insulin secretion, measured as HOMA-B and insulinogenic index, and BSE did not affect fasting glucose or HbA1c in well-regulated T2D patients. However, we observed that SFN prevented the development of hyperglycemia in diet-challenged rats, and it would be of interest to longitudinally study the long-term effects of BSE on glucose production and insulin secretion capacity in prediabetic individuals.

Glitazones and metformin were not ranked particularly high in the drug comparisons, suggesting that they do not affect the hepatic gene coexpression network that was associated with hyperglycemia in this case but exert their effects via other pathways. It is not entirely surprising because these drugs have different mechanisms of action from that of SFN.

It has been demonstrated that 1% [DCCT (Diabetes Control and Complications Trial) units] decrease of HbA1c corresponds to 37% reduced risk of microvascular complications (*59*). BSE treatment reduced HbA1c from 57.1 mmol/mol (or 7.38%) to 53.4 mmol/mol (or 7.04%) in obese patients with dysregulated T2D. The patients thereby reached the 7% treatment goal recommended by the American Diabetes Association (*60*), which is likely to represent a clinically meaningful effect.

Although the effect of BSE on glucose production was abolished in vitro when the conversion of glucoraphanin to SFN was prevented, we cannot fully determine that SFN explains the effect of BSE given to patients. High doses of BSE cannot yet be recommended to patients as a drug treatment but would require further studies, including data on which groups of patients would potentially benefit most from it. Finally, the findings provide support for using disease signatures based on coexpression networks to interrogate drug signatures, thereby using the large public repositories of gene expression data, as one of many strategies for repurposing compounds of immediate clinical relevance.

MATERIALS AND METHODS

Study design

We first used published gene expression data (*18*) to construct a 50-gene hepatic disease signature and identified SFN as a potential treatment for

excessive hepatic glucose production. We then studied the effects of SFN on the hepatoma cell line H4IIE, primary mouse hepatocytes, and diabetic animal models (Wistar rats and C57BL/6JBomTac and C57BL/6J mice fed a diet with high-fat or high-fructose content). The numbers of independent tests/animals used for each experiment are indicated in the figure legends. Finally, we investigated the effect of SFN-containing BSE in T2D patients in a controlled randomized study with two parallel arms. The predefined inclusion and exclusion criteria for the clinical study are described in the Supplementary Materials and Methods. T2D patients of Scandinavian ethnicity were recruited from the All New Diabetics In Scania (ANDIS) cohort and attended a screening visit, followed by an OGTT 2 weeks later. Placebo or BSE powder was thereafter provided in a double-blind manner as dry mixtures in sealed portion size bags of similar shape and size. Randomization was done using a computer-based block randomization algorithm. After a 12-week treatment period, the patients returned for a final visit including an OGTT. All data analyses were performed with blinded assessment of outcomes. Only patients who had taken >75% of their compound during the total study period and >80% during the last month were included in the analyses. The clinical study was conducted at Skåne University Hospital, Sweden. The study was approved by the Regional Ethics Committee in Lund, and all patients expressed written informed consent. ClinicalTrials.gov Identifier is NCT02801448. For details, see the Supplementary Materials and Methods.

Statistics

Experimental in vitro and animal data were analyzed using Student's *t* test. Additive versus synergistic effects of SFN, metformin, and insulin on glucose production were determined by comparing expected and observed data. Because insulin caused 40% reduction and metformin at 250 μM caused 40% reduction, we would expect 64% reduction if the effects were only additive, whereas the observed reduction was 70% ($P = 0.005$ for expected versus observed effects as shown in Fig. 1B). For the clinical study, the primary effect variables were HbA1c and fasting glucose after versus before the treatment (ΔHbA1c and Δglucose). All analyses were done using one-tailed Student's *t* test between placebo and BSE arms. We also used two-tailed linear regression to compare the relationship between metabolic variables and ΔHbA1c and Δglucose as described in the text.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. Effect of SFN on apoptosis, insulin signaling, and OCR in H4IIE cells.
Fig. S2. Effect of SFN on the 50-gene hepatic disease signature.
Fig. S3. Effect of BSE on H4IIE cells and on mice fed an HFD.
Table S1. The 50-gene liver disease signature for T2D.
Table S2. List of compounds used in the analysis (see separate Excel file).
Table S3. Rank order of compounds (see separate Excel file).
Table S4. Expression of enzymes involved in gluconeogenesis.
Table S5. Adverse effects in patients treated with BSE or placebo for 12 weeks.
Table S6. Characteristics of patients discontinuing the study.
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Sulforaphane reduces hepatic glucose production and improves glucose control in patients with type 2 diabetes

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Another reason to eat your broccoli

Type 2 diabetes is becoming increasingly common worldwide, and not all patients can be successfully treated with the existing drugs. Axelsson *et al*. analyzed the pattern of gene expression associated with type 2 diabetes and compared it to the gene signatures for thousands of drug candidates to find compounds that could counteract the effects of diabetes. The leading candidate from this analysis was sulforaphane, a natural compound found in broccoli and other vegetables. The authors showed that sulforaphane inhibits glucose production in cultured cells and improves glucose tolerance in rodents on high-fat or high-fructose diets. Moreover, in a clinical trial, sulforaphane-containing broccoli sprout extract was well tolerated and improved fasting glucose in human patients with obesity and dysregulated type 2 diabetes.

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