# BASIC AND TRANSLATIONAL—ALIMENTARY TRACT

Loss of Sucrase-Isomaltase Function Increases Acetate Levels and Improves Metabolic Health in Greenlandic Cohorts

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SI Loss-of-function variant improves metabolic health
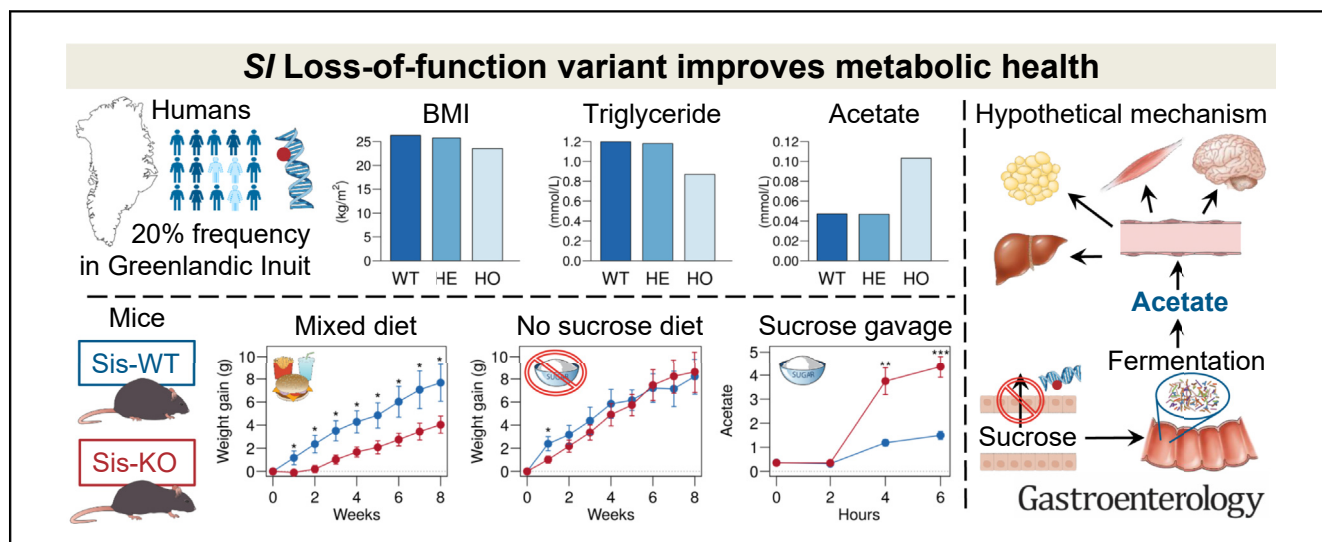
BACKGROUND & AIMS: The sucrase-isomaltase (*SI*) c.273_274delAG loss-of-function variant is common in Arctic populations and causes congenital sucrase-isomaltase deficiency, which is an inability to break down and absorb sucrose and isomaltose. Children with this condition experience gastrointestinal symptoms when dietary sucrose is introduced. We aimed to describe the health of adults with sucrase-isomaltase deficiency. **METHODS:** The association between c.273_274delAG and phenotypes related to metabolic health was assessed in 2 cohorts of Greenlandic adults (n = 4922 and n = 1629). A sucrase-isomaltase knockout (Sis-KO) mouse model was used to further elucidate the findings. **RESULTS:**

Homozygous carriers of the variant had a markedly healthier metabolic profile than the remaining population, including lower body mass index (β [standard error], –2.0 [0.5] kg/m^2; $P = 3.1 \times 10^{-5}$), body weight (–4.8 [1.4] kg; $P = 5.1 \times 10^{-4}$), fat percentage (–3.3% [1.0%]; $P = 3.7 \times 10^{-4}$), fasting triglyceride (–0.27 [0.07] mmol/L; $P = 2.3 \times 10^{-6}$), and remnant cholesterol (–0.11 [0.03] mmol/L; $P = 4.2 \times 10^{-5}$). Further analyses suggested that this was likely mediated partly by higher circulating levels of acetate observed in homozygous carriers (β [standard error], 0.056 [0.002] mmol/L; $P = 2.1 \times 10^{-26}$), and partly by reduced sucrose uptake, but not lower caloric intake. These findings were verified in Sis-KO mice, which, compared with wild-type mice, were leaner on a sucrose-containing diet, despite similar caloric intake, had significantly higher plasma acetate levels in response to a sucrose gavage, and had lower plasma glucose level in response to a sucrose-tolerance test.

CONCLUSIONS: These results suggest that sucrase-isomaltase constitutes a promising drug target for improvement of metabolic health, and that the health benefits are mediated by reduced dietary sucrose uptake and possibly also by higher levels of circulating acetate.

Keywords: Sucrase-Isomaltase; Genetics; Loss of Function; Metabolic Health; Drug Target.

To prevent or delay age-related conditions like type 2 diabetes and cardiovascular disease, it is vital to sustain metabolic health. Metabolic health is determined by genetic factors and health behavior, including dietary habits. Hence, understanding how different dietary components are metabolized and used may identify pathways important for sustaining or improving metabolic health. For most people, carbohydrates constitute the primary dietary component.[1,2] Carbohydrates are ingested mainly as starch and sugars and, in a Westernized diet, the most abundant dietary sugar is sucrose. The health effects of the increased carbohydrate and, in particular, sugar consumption, are heavily debated.[3,4]

When ingested, carbohydrates in the form of starch and sugar need to be broken down to monosaccharides in order to move across the intestinal epithelium and be taken up by the body. This carbohydrate digestion is initiated by α-amylases in the mouth and is finalized in the small intestine by the α-glucosidases, maltase-glucoamylase (MGAM), and sucrase-isomaltase (SI).[5–7] These digestive enzymes are among the targets of the anti-diabetic α-glucosidase inhibitor drugs acarbose, voglibose, and miglitol. These drugs target a combination of enzymes and are thought to reduce the degradation of starch and sugars, thereby reducing the amount of glucose absorbed by the blood. Acarbose mainly inhibits α-amylases and partly maltase and sucrase,[8–10] whereas miglitol and voglibose bind all 4 α-glucosidase subunits but have no or very limited affinity for α-amylases.[10–12]

Naturally occurring genetic variation that disrupts the function of MGAM and SI can help indicate the effect of specifically targeting these enzymes. Deficiency of both MGAM and SI has been linked to maldigestion and severe gastrointestinal symptoms in children.[13,14] Thus, congenital sucrase-isomaltase deficiency (CSID) is associated with a range of symptoms in children, including diarrhea, abdominal pain, and bloating,[15–17] yet gastrointestinal and metabolic-health status in adults has not been reported. CSID is rare in most parts of the world, except in Arctic populations, where the condition has an estimated prevalence of up to 10%.[15] Recently, the c.273-274delAG frameshift variant in the sucrase-isomaltase gene (*SI*) encoding SI was identified in a Canadian patient with CSID.[18] This variant is predicted to result in complete loss of SI function.[18] Hence, homozygous carriers of the variant represent human SI knockouts, which facilitate assessment of health-related implications of targeted SI inhibition. Importantly, the variant has an estimated allele frequency of 39% in the

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

In Arctic populations the sucrase-isomaltase c.273_274delAG loss-of-function variant causes congenital sucrase-isomaltase deficiency in children; however, the impact of the variant on metabolic health in adults is unknown.

NEW FINDINGS

Among Greenlandic adults, homozygous c.273_274delAG carriers had a markedly healthier metabolic profile than the remaining study population, likely mediated by higher circulating acetate levels and reduced sucrose uptake, but not lower caloric intake.

LIMITATIONS

We hypothesize that the healthier metabolic profile observed in homozygous c.273_274delAG carriers was mediated by acetate produced by gut bacteria; however, we lack data to firmly verify this hypothesis.

IMPACT

Our results suggest that sucrase-isomaltase constitutes a promising drug target for improvement of metabolic health and, in a broader perspective, add to the debate about the health effects of sugar consumption.

Greenlandic population.[19] Thus, it is possible to assess the effect of being a homozygous carrier of this variant in Greenlanders. We, therefore, aimed to thoroughly assess how *SI* knockout affected metabolic, gastrointestinal, and cardiovascular health in 6551 Greenlandic adults, by assessing 2 cohorts with complementary phenotypes. In addition, to gain further mechanistic insights, we monitored food intake, body weight, and body composition for 8 weeks in sucrase-isomaltase knockout (Sis-KO) mice on 2 different diet regimens.

Methods

Ethics Statement

All participants gave written informed consent, and the study was approved by the Scientific Ethics Committee in Greenland (cohort I: project 2011–13 [ref. no. 2011–056978], project 2013–13 [ref. no. 2013–090702], and project 2012-16/ 17 [ref. no. 2017-12997]; cohort II: project 2013–17), and was

conducted in accordance with the Declaration of Helsinki, second revision.

Study Populations

Cohort I comprised Greenlanders living in Greenland, from population surveys during 1999–2001 (B99, n = 1401) and 2005–2010 (IHIT, n = 3115), as well as Greenlanders living in Denmark, collected as part of the B99 survey (BBH, n = 547).[20,21] Cohort II was collected in 2013 as a population-based sample of Greenlanders (n = 1629).[22] Basic clinical data for cohort I and cohort II are presented in Supplementary Table 1.

Assays and Measurements

Cohort I. Anthropometrics, concentrations of fasting serum lipids, plasma apolipoproteins AI and B, as well as levels of fatty acids, were measured, and body mass index (BMI), fat percentage, lean mass, low-density lipoprotein, very-low-density lipoprotein, and remnant cholesterol calculated as described in detail previously.[23,24] All IHIT participants older than 18 years and B99 participants older than 35 years underwent an oral glucose tolerance test; serum insulin, plasma glucose, serum c-peptide, and hemoglobin A1c were measured; and homeostasis model assessment of insulin resistance was calculated.[24] Type 2 diabetes was defined based on the World Health Organization 1999 criteria,[25] and controls were defined as normal glucose tolerant based on the oral glucose tolerance test data.

Daily intake of macronutrients, selected types of carbohydrates and fat, as well as total energy, were calculated based on data from food frequency questionnaires and published food tables.[26,27] Information on the participants overall health and gastrointestinal health was obtained from questionnaires and analyzed with a case–control design. We classified cardiovascular disease events based on data from registries (Supplementary Tables 2 and 3).

Cohort II. Height and weight were measured and BMI was calculated. Samples for measurement of serum metabolites, characterized with a high-throughput nuclear magnetic resonance (NMR) metabolomics platform,[28,29] and plasma samples for measurement of alkaline phosphatase, albumin, aspartate aminotransferase, and bilirubin were collected at a clinical visit without prior fasting.

Genotyping

The *SI* c.273-274delAG variant was genotyped using the KASP Genotyping Assay (LGC Genomics) in 4922 and 1629 individuals from cohorts I and II, respectively. The genotyping call rate was 99.4% in both cohorts, and there were no mismatches in 357 individuals genotyped in duplicate in cohort I.

Association Analyses

Before analyzing, quantitative traits were transformed independently for men and women using a rank-based inverse normal transformation, and effect size estimates were reported in standard deviations (β_{SD}), as well as in non-transformed trait values (β). We applied a linear mixed model to take admixture and relatedness into account by including them as random effects. We estimated a genetic similarity matrix with GEMMA (version 0.95alpha)[30] from single nucleotide polymorphisms

with minor allele frequency of minimum 5% and missingness of maximum 1% from previously generated genome-wide genotype data from the Illumina Metabochip (Illumina, San Diego, CA) and Illumina OmniExpressExome chip (Illumina) for cohort I[23] and cohort II,[22] respectively. The estimated genetic similarity matrix was used as input for association testing. For quantitative traits, we included sex, age, and survey as covariates, and association tests were performed with GEMMA using a score test, whereas effect sizes and standard errors (SEs) were estimated using a restricted maximum likelihood approach. For dichotomous traits, association tests were performed with the GMMAT package[31] in R; odds ratios and *P* values were obtained from a logistic mixed model using the Wald test, including sex, age, and survey as covariates.

A full model, allowing for separate effects of being heterozygous and homozygous carriers of the c.273_274delAG variant, showed a strong effect on metabolic traits in homozygous carriers, but no effect in heterozygous carriers (Supplementary Table 4). Hence, we report results generated with a recessive model unless otherwise stated. For discovery analyses in cohort I and cohort II, *P* values $<7.2 \times 10^{-4}$ and 3.1×10^{-4}, respectively, corresponding to Bonferroni correction, were considered statistically significant. We verified that the linear mixed model was able to account for admixture by performing association analyses for BMI and triglycerides in cohort I, split according to Inuit ancestry proportion (Supplementary Figure 1). In addition, we performed a test for each of these traits against common variants on the Metabochip to ensure that the test statistics were not inflated (Supplementary Figure 2).

Analyses of Register-Based Cardiovascular Disease Data

We applied a Cox regression, adjusted for sex, birth year (as number of years since 1900), survey, and the top 10 principal components, to estimate the number of years lived until the first cardiovascular event, until getting censored, or until the conclusion of the study (December 31, 2016) with the R-package *survival* (https://cran.r-project.org/web/packages/survival/index.html). We allowed individuals to have their first event counted in each type of event analyzed. For information about selection analysis and estimation of allele frequencies in ancestral population components and in other populations, see the Supplementary Methods.

Sucrase-Isomaltase Knockout Mice

The mice experiments adhered to the Animal Research: Reporting of In Vivo Experiments guidelines, and were approved by the Animal Experiments Inspectorate. Heterozygous breeding pairs of C57BL/6NJ-Sisem1(IMPC)J mice were obtained from The Jackson Laboratory. Litters were weaned at 3-4 weeks and separated into new cages by sex. Unless specifically stated, all mice were kept in individually ventilated cages (Scanbur). Groups were matched by littermate. The facility was humidity controlled and temperature was 23°C; the light cycle was from 6:00 AM to 6:00 PM.

Diets

All diets were ordered from Research Diets Inc, and matched as much as possible for macronutrients and ingredient

composition. For the choice diet experiment, wild-type (Sis-WT, n = 9) and knockout (Sis-KO, n = 13) littermate mice between the ages of 8 and 29 weeks were separated according to sex. Male mice (n = 6 Sis-WT, n = 7 Sis-KO) were individually caged, and female mice (n = 3 Sis-WT, n = 6 Sis-KO) were group caged in individually ventilated cages. To ensure sucrose intake, the mice had ad libitum access to high-fat 12.6 kcal% sucrose diet (Research Diets, #D12331), low-fat 17 kcal% sucrose (17S) diet (Research Diets, #D12450H), and low-fat no-sucrose diet (Research Diets, #D12450K) for 8 weeks (Supplementary Tables 5 and 6). For the high-fat diet containing no sucrose (HFNS) experiment, Sis-WT (n = 6) and Sis-KO (n = 6) littermate mice between the ages of 5 and 11 weeks were placed into a mixture of group (Sis-WT n = 4, Sis-KO n = 4) and individual (Sis-WT n = 2, Sis-KO n = 2) caging according to how they arrived, due to lack of room to individually house all mice in the animal housing units (Supplementary Table 7). Mice had ad libitum access to HFNS diet (Research Diets, #D0806014B) for 8 weeks (Supplementary Table 6).

Sucrose Gavage, Tolerance Test, and Plasma Measurements

Mice were given an oral gavage of sucrose (3 g/kg body weight) after an overnight fast. For the sucrose tolerance test, blood was taken from the tail vein of Sis-WT (n = 7) and Sis-KO (n = 7) mice and blood glucose was determined by glucometer (Roche) at 0, 15, 30, 60, and 120 minutes. To quantify plasma acetate and conversion of sucrose to short-chain fatty acids, 75 μL of blood was collected from Sis-WT (n = 5) and Sis-KO (n = 7) by retro-orbital bleed. This was performed on 2 separate occasions due to the maximum sampling volume and recovery times for a mouse (ie, bleed 1 for 0- and 2-hour time points and bleed 2 for 4-hour and 6-hour time points).

Measurements

Food intake was calculated as weekly intake by means of weighing the amount of each diet given at the beginning of each week and at the same time 7 days later after a thorough search of the cages. An average per mouse was calculated for multi-caged mice.

Individual weights were measured at baseline and at the end of each week after placement of mice on diets. Fat and lean mass were measured using a Minispec LF90II low-frequency NMR system (Bruker) in the case of the HFNS experiment or an EchoMRI-500 for mice in the choice diet experiment. Mice were awake during the procedure and immobilized using a plunger system. The Minispec system was applied to measure total lean mass, fat mass, and free fluid. Body fat fraction was calculated as a percentage of total mass determined from the sum of fat mass, lean mass, and free fluid analyzed by the system in Microsoft Excel (Office 2009). Liver triglycerides were determined using the chemical assay (Randox #TR210) according to manufacturer's instructions. Plasma levels of acetate was measured by liquid chromatography–mass spectrometry (for additional information see Supplementary Methods).

Statistical Analysis

To test for differences in weight gain, fat percentage, and lean mass gain at each of the 8 weeks separately, we used a linear model adjusted for sex. Confidence intervals (CIs) were estimated using a profile likelihood approach. For the sucrose gavage experiments, sex was not included in the model, as all mice were female.

Results

Frequency of c.273_274delAG in Greenlanders and in Other Populations

In cohort I and cohort II, the frequency of the *SI* c.273_274delAG variant was 14.2% (95% CI, 13.5%–15.1%) and 14.1% (95% CI, 12.8%–15.3%), and the number of homozygous carriers was 99 and 34, respectively (Supplementary Table 1). The Greenlandic population is admixed, and we estimated the Inuit ancestry-specific allele frequency in cohort I to be 20.0% (95% CI, 19.0%–21.1%). We also estimated the frequency of the variant in populations from across the world, using publicly available datasets, and found it to be close to zero in non-Arctic populations, except in Siberians (Supplementary Table 8). Despite the higher frequency of the variant among Greenlanders, and in particular Inuit, we observed no signatures of selection at the locus (Supplementary Figure 3).

Anthropometric and Metabolic Traits

In cohort I, homozygous carriers of the c.273_274delAG variant had a healthier metabolic profile, and results from a full model showed that these effects were mainly recessive (Figure 1 and Supplementary Table 4). Specifically, with a recessive model we found that homozygous carriers had markedly lower BMI (β [SE], –2.0 [0.5] kg/m^2; P = 3.1 × 10^{-5}), smaller waist and hip circumference (–4.9 [1.3] cm; P = 1.8 × 10^{-4} and –3.3 [0.9] cm; P = 2.3 × 10^{-4}), and lower weight (–4.8 [1.4] kg; P = 5.1 × 10^{-4}). Homozygous carriers also had less body fat (subcutaneous adipose tissue, –0.70 [0.17] cm; P = 5.8 × 10^{-7}; subcutaneous adipose tissue to visceral adipose tissue ratio, –0.08 [0.03]; P = 3.8 × 10^{-6}; fat percentage, –3.3% [1.0%], P = 3.7 × 10^{-4}), and a healthier lipid profile (triglyceride, –0.27 [0.07] mmol/L; P = 2.3 × 10^{-6}; remnant cholesterol, –0.11 [0.03]; mmol/L; P = 4.2 × 10^{-5}; very-low-density lipoprotein cholesterol, –0.13 [0.04] mmol/L; P = 6.0 × 10^{-4}; Supplementary Table 9). We observed no association between the variant and risk of type 2 diabetes or traits related to glucose homeostasis (Supplementary Table 9).

In the smaller cohort II, we replicated the association with lower BMI and lower weight with comparable effect sizes, whereas the association with lower level of triglyceride was nonsignificant, however, with a comparable effect size (Supplementary Table 10). Moreover, from markers of liver health, we observed significantly lower levels of alkaline phosphatase among homozygous carriers (–15.41 [4.20] U/L; P = 9.8 × 10^{-6}) (Supplementary Table 10).

Additional Markers of Metabolic Health

To further understand the impact of the variant, we tested for associations with circulating metabolic markers measured by means of NMR spectroscopy, available for
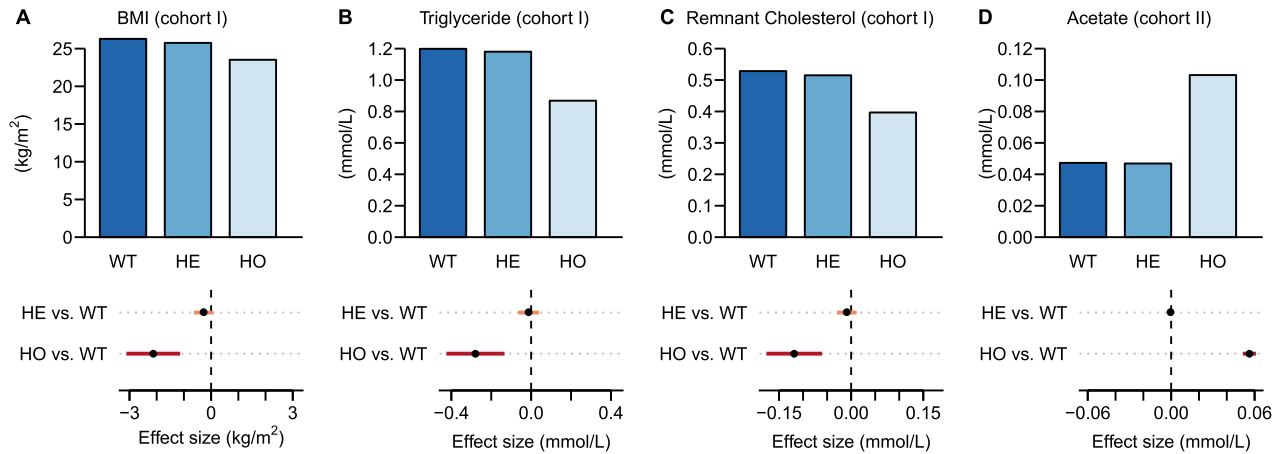
Figure 1. Effect of the *SI* c.273_274delAG variant on selected metabolic phenotypes according to a full model. Raw mean values stratified by genotype (*top*) and untransformed effect sizes with 95% CIs (*bottom*) for (*A*) BMI, (*B*) fasting serum triglyceride, (*C*) fasting serum remnant cholesterol, and (*D*) serum acetate.

cohort II. Interestingly, we observed markedly higher levels of circulating acetate in homozygous carriers (β [SE], 0.056 [0.002] mmol/L; $P = 2.1 \times 10^{-26}$; Figure 1 and Supplementary Table 11), but no significant associations with markers of glycolysis, ketone bodies, or amino acids, when adjusting for multiple testing (Supplementary Table 11).

With respect to lipoproteins, the variant had the strongest impact on high-density lipoprotein metabolism, with significantly higher concentrations of very large high-density lipoprotein particles (β_{SD} [SE], 0.621 [0.167] SD; $P = 2.1 \times 10^{-4}$) (Figure 2 and Supplementary Table 12), and
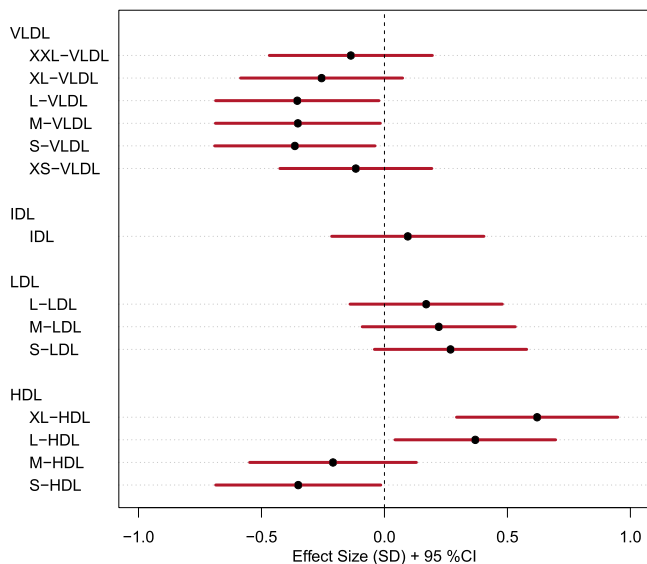

Figure 2. Effect of the c.273_274delAG variant on concentration of lipoprotein particles according to a recessive model. Effect estimates plotted as quantile transformed values and *error bars* as 95% CIs. HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; L, large; LDL, low-density lipoprotein; M, medium; S, small; VLDL, very-low-density lipoprotein; XL, very large; XS, very small; XXL, extremely large.

significantly higher content of free cholesterol, cholesterol esters, total cholesterol, and total lipids ($P < 2.5 \times 10^{-4}$ for all), as well as a nominally higher content of phospholipids ($P = 4.4 \times 10^{-4}$) in these particles (Supplementary Table 12).

From the NMR measurements in cohort II, we also assessed the fatty acid composition in serum. Relative to the total amount of fatty acids, we found significantly higher levels of polyunsaturated fatty acid (0.704 [0.172] SD; $P = 4.7 \times 10^{-5}$), total omega-6 fatty acids (0.883 [0.166] SD; $P = 1.2 \times 10^{-7}$), and linoleic acid (0.956 [0.163] SD; $P = 5.8 \times 10^{-9}$) in homozygous carriers, as well as lower levels of monounsaturated fatty acids (−0.822 [0.169] SD; $P = 1.2 \times 10^{-6}$) (Supplementary Table 13). For comparison, we assessed the fatty acid composition in erythrocyte membranes in cohort I and validated the association with higher levels of omega-6 fatty acids (0.253 [0.107] SD; $P = .018$) and linoleic acid (0.371 [0.102] SD; $P = 2.6 \times 10^{-4}$) in homozygous carriers. In addition, we observed significantly lower levels of oleic acid (−0.450 [0.125] SD; $P = 3.2 \times 10^{-4}$; Supplementary Table 14).

Gastrointestinal and Cardiovascular Health

In questionnaire-based data from cohort I, we observed no significant associations with either gastrointestinal symptoms or overall health perception (Table 1). With respect to cardiovascular disease events, queried from register-based data from cohort I, effect estimates indicated a lower risk of ischemic heart disease and heart failure in homozygous carriers; however, this risk reduction was statistically nonsignificant (Figure 3 and Supplementary Table 15).

Dietary Composition

In cohort I, the daily intake of added sugar, that is, sucrose, was significantly lower among homozygous carriers (β [SE]; −28.55 [7.92] g/d; $P = 2.8 \times 10^{-7}$), whereas we found no significant differences in intake of protein, fat,

Table 1. Association Between *SI* c.273_274delAG and Gastrointestinal and Overall Health According to a Recessive Model

Trait	With/without condition, n	OR (95% CI)	*P* value
Digestive problems	784/3058	1.58 (0.95–2.65)	.081
Stomach pain	770/3051	0.83 (0.46–1.49)	.530
Poor health	149/3762	0.52 (0.12–2.22)	.380

NOTE. Data were questionnaire-based and obtained from up to 3911 individuals from cohort I. Effect sizes were estimated as OR (95% CI).

Table 2. Association Between *SI* c.273_274delAG and Questionnaire-Based Diet Information According to a Recessive Model

Trait	β_{SD} (SE)	β (SE)	*P* value
Total energy (*kJ/d*)	–0.06 (0.13)	–146.69 (378.54)	.634
Macronutrients			
Carbohydrate (*g/d*)	–0.24 (0.12)	–19.77 (12.43)	.048
Protein (*g/d*)	0.07 (0.13)	2.92 (6.11)	.603
Fat (*g/d*)	0.08 (0.13)	2.66 (4.26)	.542
Fat components			
MUFA (*g/d*)	0.06 (0.13)	0.78 (2.38)	.624
PUFA (*g/d*)	0.07 (0.13)	0.54 (1.08)	.565
Saturated fat (*g/d*)	0.15 (0.13)	1.91 (1.53)	.232
Carbohydrates			
Added sugar (*g/d*)	–0.65 (0.13)	–28.55 (7.92)	2.8×10^{-7}
Fruit (*g/d*)	–0.02 (0.13)	–6.36 (21.07)	.874
Fiber (*g/d*)	0.10 (0.13)	1.39 (1.36)	.419
Whole grain (*g/d*)	0.22 (0.13)	24.00 (15.29)	.089
Refined grain (*g/d*)	0.05 (0.13)	3.00 (8.46)	.710

NOTE. Results were obtained with a linear mixed model for 2469 individuals from cohort I. Effect sizes are shown as quantile transformed (β_{SD}) or untransformed (β), and *P* values were calculated based on the quantile transformed values using the score test in GEMMA, including only individuals with a realistic energy intake.
MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

including the specific fat categories of monounsaturated fatty acids, polyunsaturated fatty acids, and saturated fat; or carbohydrates, including fiber, whole grain, refined grain, and fruit. In line with the nonsignificant differences for the majority of these dietary components, there was no difference in total daily energy intake (Table 2).

Analyses of Factors Potentially Mediating the Association Between c.273_274delAG and Metabolic Health

In cohort I, we tested whether the lower intake of added sugar among homozygous carriers of c.273_274delAG could explain their healthier metabolic phenotype, but the associations with anthropometric and metabolic traits remained when adjusting for intake of added sugar (Supplementary Table 16). Next, in cohort II we tested whether serum acetate levels might mediate the associations, and found that associations were attenuated for BMI (*P* = .077), weight (*P* = .160), and alkaline phosphatase (*P* = .018), when

adjusting for acetate level, indicating that serum acetate might mediate these associations (Supplementary Table 10).

Characterization of Sucrase-Isomaltase Knockout Mice

To further investigate our findings, we studied Sis-KO mice. Mimicking a Westernized human diet, Sis-KO and Sis-WT mice were fed a choice diet with ad libitum access to high-fat 12.6% sucrose diet, low-fat 17S diet, and low-fat no-sucrose diet for 8 weeks. There was no difference in the overall calorie intake between Sis-KO and Sis-WT mice (Figure 4*A*), but the choice of diet differed slightly, with the Sis-KO mice having a lower intake of sucrose in the form of the 17S diet, and a higher intake of the low-fat no-sucrose diet (Supplementary Figure 4). Although the caloric intakes were similar, Sis-KO mice had significantly lower weight gain (week 8, β = –3.06 g; *P* = .029), and lower body fat percentage (week 8, β = –10.2%; *P* = .0013), but similar lean mass gain (week 8, β = 0.62 g; *P* = .252) compared with Sis-WT mice (Figure 4*B–D* and Supplementary Table 17). To investigate the effect of sucrose in the diet, we repeated the experiment where Sis-KO and Sis-WT mice had ad libitum access to an HFNS diet for 8 weeks. Again, the total calorie intake over 8 weeks was similar in Sis-KO and Sis-WT mice (Figure 4*E*), but without the sucrose in the diet, we observed no differences in weight gain, fat fraction, or lean mass gain between Sis-KO and Sis-WT mice (Figure 4*F–G* and Supplementary Table 18). Before
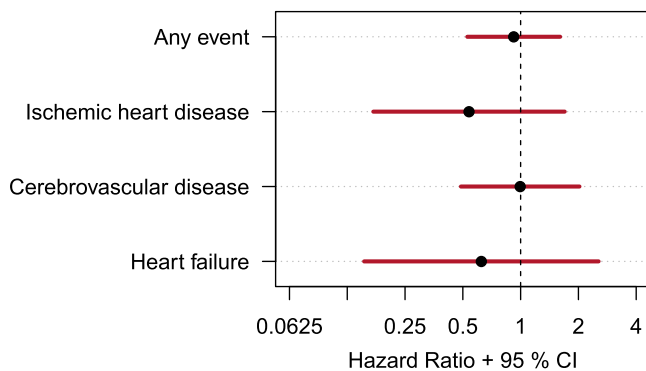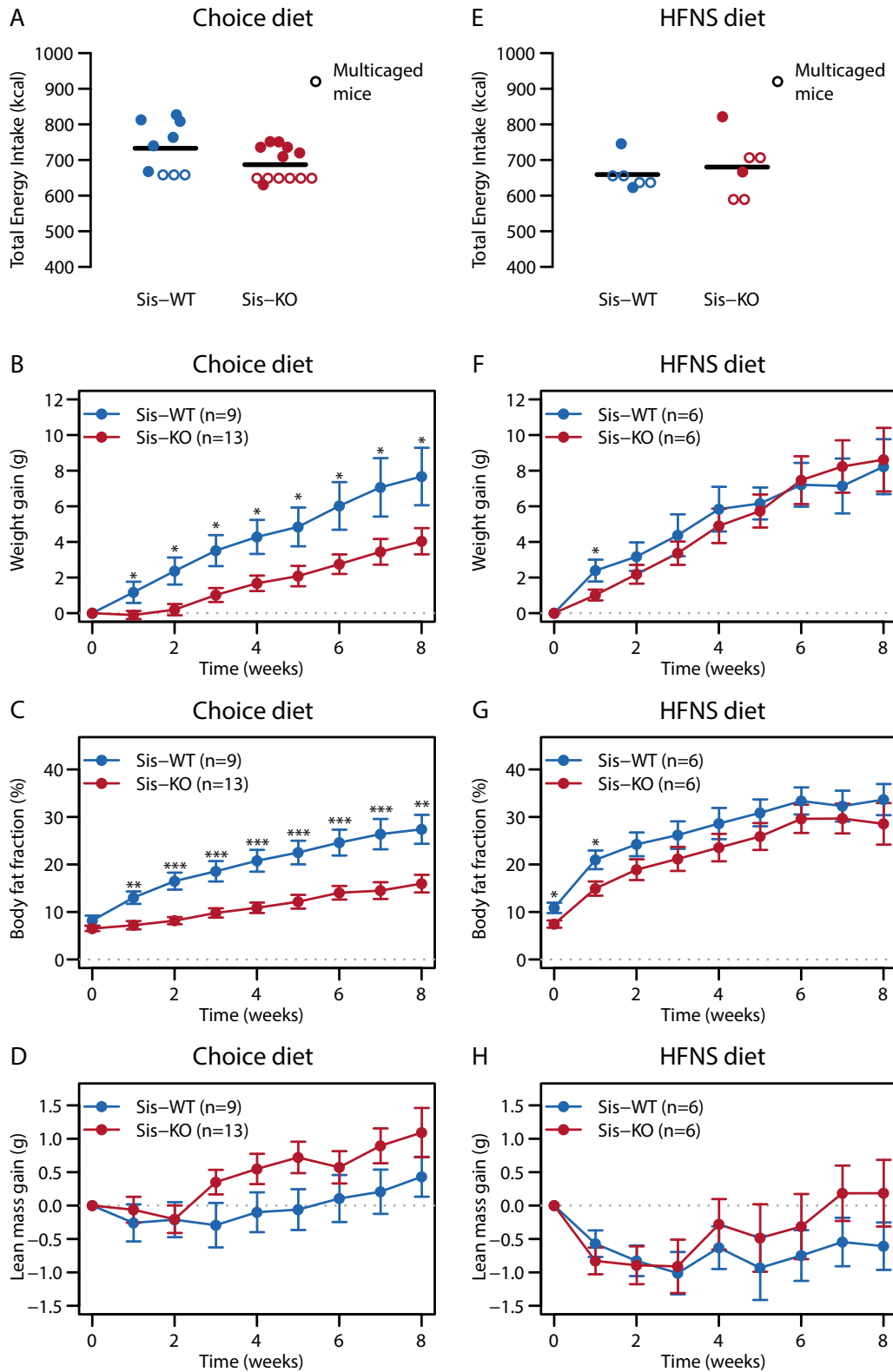

Figure 3. Association between the c.273_274delAG variant and cardiovascular disease events. Effects were estimated with a recessive model as hazard ratios (95% CIs) based on register data from up to 4551 individuals from cohort I (WT/HE/HO, 3355/1000/96). The numbers of individuals with an event according to genotype (WT/HE/HO) was 507/155/13 for any event, 242/64/3 for ischemic heart disease, 261/93/8 for cerebrovascular disease, and 128/30/2 for heart failure. Results for peripheral artery disease and coronary operations are not shown due to nonfinite CIs.

Figure 4. Energy intake and body composition of Sis-KO and Sis-WT mice. Mean total energy intake is indicated by the *horizontal lines* for mice with ad libitum access to (*A*) a choice diet of high-fat 12.6% sucrose, low-fat 17S diet, and low-fat no-sucrose diet (Sis-WT, n = 9 [single/multi-cage, n = 6/n = 3]; Sis-KO, n = 13 [single/multi-cage, n = 7/n = 6]), and (*E*) HFNS diet (Sis-WT, n = 6 [single/multi-cage, n = 2/n = 4]; Sis-KO, n = 6 [single/multi-cage, n = 2/n = 4]). *Closed circles* indicate single-caged mice and *open circles* indicate multi-caged mice. Mean weekly weight gain, body fat fraction, and lean mass gain are indicated by *circles* and SEs are indicated by the *error bars* for (*B–D*) Sis-WT (n = 9) and Sis-KO (n = 13) mice on the choice diet, and (*F–H*) Sis-WT (n = 6) and Sis-KO (n = 6) mice on the HFNS diet. *Asterisks* indicate level of significance: *$P < .05$; **$P < .01$; and ***$P < .001$. HFNS, high-fat no sucrose.
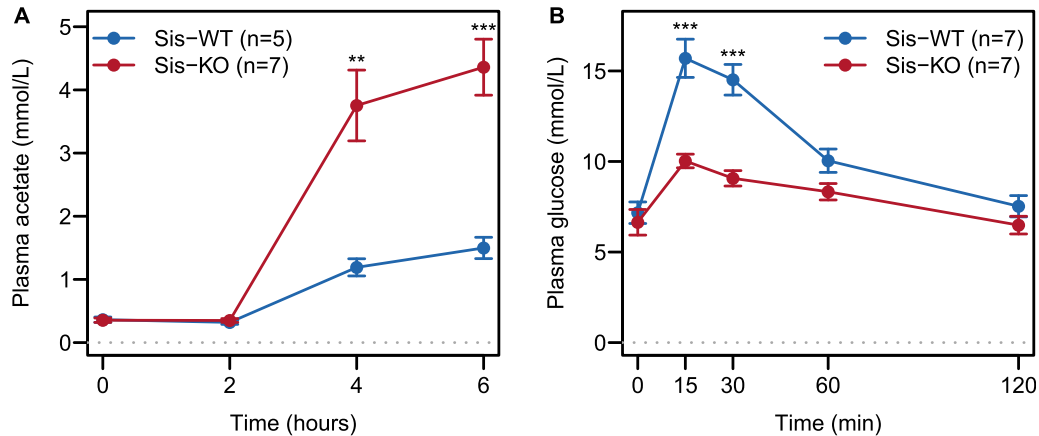
Figure 5. Blood plasma acetate and glucose levels after a sucrose gavage in Sis-KO and Sis-WT mice. Mean values of (*A*) plasma acetate levels (mmol/L) and (*B*) plasma glucose levels (mmol/L) after a sucrose gavage. The points indicate mean values with *error bars* of SEs. *Asterisks* indicate level of significance: **$P < .01$; and ***$P < .001$.

sacrificing the mice, we measured hepatic triglyceride levels and observed approximately 20% lower levels in the Sis-KO mice on the choice diet, but no difference in mice on HFNS diet. This difference was in the same direction and of the same magnitude as those observed in serum in the Greenlanders, but was nonsignificant (Supplementary Figure 5).

To further explore the mechanism underlying the observed phenotype, Sis-KO and Sis-WT mice were gavaged with 3 g/kg sucrose after a 12-hour fast. At 4 and 6 hours post-gavage, plasma acetate levels in the Sis-KO mice had increased to a level several times higher than the level in Sis-WT mice (4 hours, $P = .0037$; 6 hours, $P = 4.0 \times 10^{-4}$; Figure 5 and Supplementary Table 19). A separate gavage experiment showed that the Sis-KO mice took up less sugar in response to a 120-minute sucrose-tolerance test, which resulted in lower levels of plasma glucose (15 minutes, $P = 2.8 \times 10^{-4}$; 30 minutes, $P = 9.1 \times 10^{-5}$; Figure 5 and Supplementary Table 20), compared with Sis-WT mice.

Discussion

We assessed the impact of the *SI* c.273-274delAG loss-of-function variant in Greenlandic adults, and *Sis* knockout in mice. In humans, the c.273-274delAG variant was only observed in Arctic and Siberian populations, and its frequency was estimated to 20% in the Inuit ancestry component of the Greenlanders. Given that we found no signatures of positive selection, this high frequency among Inuit, compared with other populations, has likely been possible due to lack of negative selection pressure combined with strong genetic drift, which is a particularly powerful process affecting small, isolated populations like the Greenlandic. Interestingly, in adults we found that genetic loss of SI function was associated with a substantially healthier metabolic profile, with lower BMI, body weight, and fat percent, as well as a favorable lipid profile. Importantly, we replicated the associations with lower BMI and body weight in an independent cohort of Greenlanders. In this other cohort, we also found that homozygous carriers had markedly higher levels of circulating acetate, which was

likely only detectable due to the lack of fasting in these participants. Notably, the effect of the naturally occurring specific loss of SI function seemed to be greater on weight and levels of triglycerides compared with drug-induced unspecific inhibition of α-glucosidases by acarbose, voglibose, or miglitol,[32–38] and the impact of loss of SI function on triglyceride levels was equal to the reported effect of the lipid-lowering drug statins.[39,40] Moreover, altered high-density lipoprotein metabolism among homozygous carriers suggested increased health-promoting removal of cholesterol from extrahepatic tissues. We also observed lower serum concentrations of alkaline phosphatase among homozygous carriers. This might be a consequence of the lower degree of adiposity,[41] but could potentially be an indication of a healthier liver function. Although SI affects the ability to metabolize sugar, we observed no effect on glucose homeostasis in homozygous carriers of the c.273-274delAG variant. However, a difference in measures from the oral glucose tolerance test is not necessarily expected, as loss of SI function should not affect the uptake of glucose. In response to intake of food containing sucrose or isomaltose, a lower uptake of sugar could be expected, but was not apparent from hemoglobin A1c measures. This could be due to compensatory mechanisms of higher hepatic gluconeogenesis to sustain blood glucose levels, which is in line with observations from previous studies of MGAM-KO mice.[42] When testing for other effects of the variant, we did not find any significant associations with overall self-reported well-being or risk of cardiovascular disease, which could be due to the limited number of events in our analyses. A follow-up study with a larger sample size and longer follow-up is necessary to determine the potential cardioprotective effects of the SI loss of function.

To elucidate the mechanism underlying the healthier metabolic profile associated with loss of SI function, we first investigated intake of selected dietary components in the Greenlanders. These analyses suggested that, compared with other Greenlanders, the homozygous carriers of the c.273-274delAG variant did not have a significantly different intake of total energy or intake of any specific dietary

component, except for added sugar. Hence, the observed differences in lipid levels could not be explained by differences in the composition of dietary fat. Moreover, conditional analyses showed that the lower intake of added sugar did not explain the healthier metabolic profile. Second, we performed analyses conditional on acetate levels. Interestingly, adjusting for acetate attenuated the observed associations with a healthier metabolic profile, suggesting that higher levels of circulating acetate could be part of the functional link between the lack of SI function and improved metabolic health. Third, we performed several mice experiments. In line with the observations in the humans, Sis-KO mice on a diet mimicking a Westernized diet had a slightly lower intake of sucrose, but a similar total energy intake as the Sis-WT mice. Yet, compared with the Sis-WT mice, the Sis-KO mice gained significantly less weight and had lower fat percentage gain, as well as lower liver triglyceride levels. These findings indicated that the healthier metabolic profile linked to loss of SI function, in both humans and mice, is likely caused in part by altered intestinal sucrose uptake, rather than altered amounts of total energy intake. In the mice, a sucrose tolerance test clearly demonstrated that with loss of SI function, sucrose uptake was diminished, indicated by significantly lower levels of plasma glucose. Also, we found that the healthier metabolic profile, associated with loss of SI function, was dependent on presence of dietary sucrose, as Sis-KO mice on an HFNS diet displayed a body composition similar to Sis-WT mice. We, therefore, hypothesized that the metabolic health-promoting effect was mediated by increased colonic bacterial fermentation of undigested carbohydrates, particularly sucrose and isomaltose, escaping small intestinal digestion due to the loss of SI function. Increased bacterial fermentation of these carbohydrates may also explain the markedly higher circulating levels of the short-chain fatty acid acetate, which we observed in humans with loss of SI function. This hypothesis was strongly supported by induction of significantly higher levels of plasma acetate in Sis-KO mice after a sucrose gavage. With the available data, it is not possible to exclude the possibility that other processes, including ketogenesis, contributed to the higher levels of acetate in humans and mice with loss of SI function. However, it seems unlikely that a 12-hour fast and 6 hours of gavage experiment could induce increased acetate production by ketogenesis, as a much longer fast of 48 hours did not induce higher acetate levels in previous mice studies.[43] Moreover, the level of ketone bodies in the Greenlanders did not differ between homozygous carriers and the rest of the study population, which indicated that ketogenesis was not increased among homozygous carriers of the variant. Also, our hypothesis of increased gut bacterial acetate production in response to loss of SI function is supported by previous human studies showing that acarbose treatment is associated with higher fecal concentration of starch and starch-fermenting bacteria, as well as higher levels of short-chain fatty acid in feces and circulation.[44–46] In line with this, a common *SI* missense variant (rs9290264), estimated to reduce the SI enzymatic activity by 35%, has been associated with lower abundance

of the gut bacterial genus *Parabacteroides*,[47] which has been associated with changes in body weight and fat mass.[48]

Whether increased circulating levels of acetate is in fact beneficial has been debated. Some rodent studies have indicated that acetate is linked to increased lipogenesis and possibly induces components of the metabolic syndrome.[49,50] However, a range of studies showed that increased levels of circulating acetate, obtained by direct administration or by increased microbial production induced by diet, are linked to lower body weight and lower levels of plasma cholesterol in most studies of humans and rodents.[51] Whether acetate is beneficial or harmful could depend on the site of acetate catabolism. It has been shown that activated hepatic acetate uptake and catabolism can induce de novo lipogenesis and thereby hepatic lipid accumulation.[50] The fact that we observed markedly higher levels of acetate in circulation could indicate that acetate bypasses hepatic catabolism and thereby reaches systemic circulation, where it might induce beneficial signaling pathways in other tissues, including brain, muscle, and adipose tissue. In humans, colonic infusion of acetate has been shown to increase fat oxidation and inhibit lipolysis, resulting in lower levels of circulating free fatty acids, and lower flux of fatty acids to the liver.[52,53] These effects likely result in reduced hepatic synthesis of triglycerides,[54] in line with our observation of lower levels of fasting serum triglycerides in Greenlanders homozygous for the variant and lower levels of liver triglycerides in Sis-KO mice. Further contributing to improved metabolic health, acetate has been shown to increase resting energy expenditure, affect appetite regulation in humans,[53] and induce adipogenesis in mice.[55] The latter indicating a healthier expansion of the lipid storage capacity in the adipose tissue.

Interestingly, both increasing the level of circulating acetate and targeting the α-glucosidases, including SI, are intensely studied as ways to improve metabolic health and to induce weight loss.[37,51,56–59] And, given the markedly healthier metabolic profile among homozygous carriers of the *SI* loss-of-function variant, it seems relevant to consider specifically targeting SI with a drug to improve metabolic health. SI is a promising drug target, as the enzyme expression is highly specific to the small intestine, which may be favorable compared with targets in the central nervous system affecting appetite regulation, where more undesired effects are expected.[60] Notably, our study constitutes a particularly good first step toward such a consideration because homozygous carriers of the *SI* loss-of-function variant have great predictive value for benefits and adverse effects of targeting SI with a drug to improve metabolic health.[61] Support from naturally occurring human knockouts has even been estimated to double the success rate of drug development.[62] In terms of adverse effects, it seems particularly relevant to consider possible gastrointestinal problems, as CSID is associated with severe gastrointestinal symptoms in children. We were unable to show any differences in self-reported digestive problems in the adult homozygous c.273-274delAG carriers compared with the rest of the Greenlandic study population. This

discrepancy between adults and children may be due to the maturation and growth of the small intestine, increasing the capacity to absorb luminal fluid with increasing age,[16] and to dietary adaptation caused by symptoms in childhood. It has been shown that a common *SI* missense variant (rs9290264) was associated with increased risk of irritable bowel syndrome (IBS),[47] and that patients with IBS show increased prevalence of rare *SI* variants.[63] However, for rs9290264, this conclusion was not supported by analysis of 452,264 individuals from the UK Biobank (http://geneatlas.roslin.ed.ac.uk/) or by analyses of 117,050 individuals from the FinnGen study (http://r3.finngen.fi/), including 6041 and 2727 cases with IBS, respectively. This aspect should be addressed further in large studies with careful IBS phenotyping to verify whether inhibition of SI will result in unwanted adverse effects.

In conclusion, our data indicated that lack of SI function in human adults, and in mice, seems to be specifically linked to altered uptake, and metabolism of dietary components, which result in a healthier metabolic phenotype, likely mediated by decreased intestinal sucrose absorption and possibly also by increased levels of circulating acetate. Our data also indicated that isolated targeting of SI may refine the effects already reported for other α-glucosidase inhibitors, and thus that SI is a potential treatment target to improve metabolic health.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://doi.org/10.1053/j.gastro.2021.12.236.

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Data Availability
The Greenlandic Metabochip-genotype data are deposited in the European Genome-Phenome Archive (https://www.ebi.ac.uk/ega/home) under the accessions EGAS00001002641.

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Conflicts of interest
The authors disclose no conflicts.

Supplementary Methods

Selection Analysis

To assess whether the *SI* variant has been under positive selection, we estimated extended haplotype homozygosity[e1] and integrated haplotype score[e2] at the *SI* c.273_274delAG variant (rs781470490) and across chromosome 3. Estimates were based on analyses of 263 unrelated Greenlanders without European ancestry from cohort I, identified by running an analysis of population structure with ADMIXTURE (version 1.3)[e3] and RELATEADMIX[e4] on Illumina Metabochip single nucleotide polymorphism array data. To construct a data set for this analysis, we first selected all of the 13,195 sites from the Illumina Metabochip on chromosome 3 with <2% missing data. These data were then used for reference-based phasing and imputation with phased reference data from 40 trio-phased Greenlanders of Inuit descent and 190 individuals of European descent from the CEU and GBR populations from the 1000 Genomes (internationalgenome.org).[e5] The genotype data from cohort I was phased with SHAPEIT (version 2.r904)[e6] using this reference panel and the HapMap hg19 recombination map. Genetic variants were imputed onto the phased haplotypes with IMPUTE2 (version 2.3.2).[e7] We used hapbin (version 1.3.0)[e8] to calculate EHH and iHS across chromosome 3.

Estimation of Allele Frequencies in Ancestral Population Components and in Other Data Sets

We estimated the *SI* c.273_274delAG frequency separately for the Inuit ancestry component of the admixed Greenlandic population by estimating ancestry proportions[e3] for the Greenlandic individuals from cohort I, as well as for 50 Danish individuals, assuming 2 ancestral populations—Inuit and Europeans. Moreover, we surveyed the allele frequency of c.273-274delAG in a range of available datasets[18,19,e5,e9–e14] from across the world, by applying SAMtools[e15], BGT[e16], and VCFtools[e17].

Measurement of Plasma Acetate in Mice

The derivatizing reagent was 200 mM N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, 120 mM 3-nitrophenylhydrazine, and pyridine (2% v/v) in 50% acetonitrile. Plasma (10 μL) was mixed with 10 μL of stable isotope labeled internal standards (100 μM of 13C4-acetate in 50% methanol) and derivatizing reagent (20 μL) and incubated for 1 hour at 40°C. Then, the samples were centrifuged at 14,000 rpm for 10 minutes at 4°C, and mixed with 40 μL of 0.1% formic acid. Eight different levels of acetate calibrants were derivatized as the samples. The samples were injected into an ultra-high performance liquid chromatography system (Agilent 1290 Infinity II) connected to a Bruker timsTOF Pro instrument (Bruker, Bremen, Germany). Ions were generated in the negative electrospray ionization mode. Data acquisition was performed with otofControl, version 6.0 and Bruker Compass HyStar, version 5.0 (Bruker Daltonics, Bremen, Germany) and data processing was performed with Bruker TASQ 2021b quantitation software. [M-H]- for acetate and internal standard was used as quantifier (Supplementary Tables 1 to 20)

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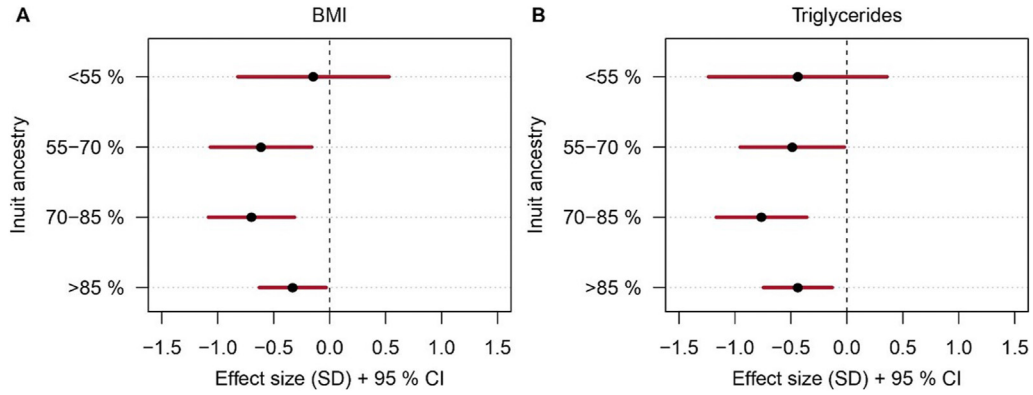
Supplementary Figure 1. Association results for cohort I divided into 4 subgroups according to Inuit ancestry proportion. Results from analyzing association between *SI* c.273_274delAG and (*A*) BMI and (*B*) triglycerides for different subgroups of cohort I using a linear mixed model assuming a recessive effect.
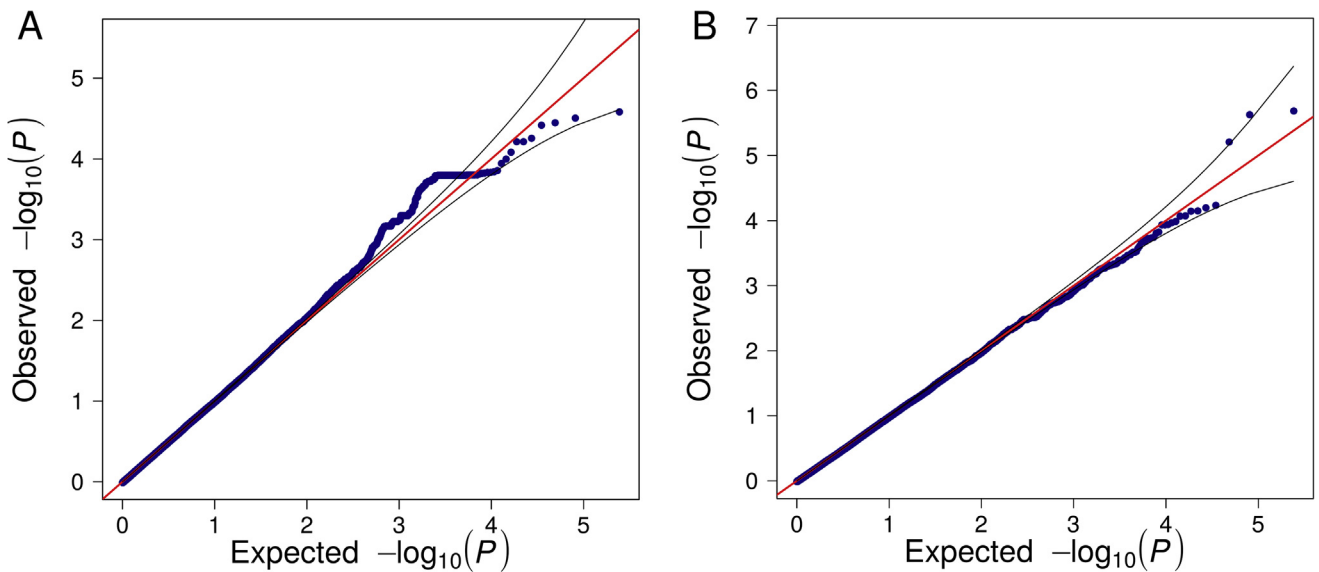
Supplementary Figure 2. Q–Q *plots* of recessive association analyses of (*A*) BMI (lambda, 1.01) and (*B*) triglycerides (lambda, 0.97) performed using a linear mixed model.

A

B
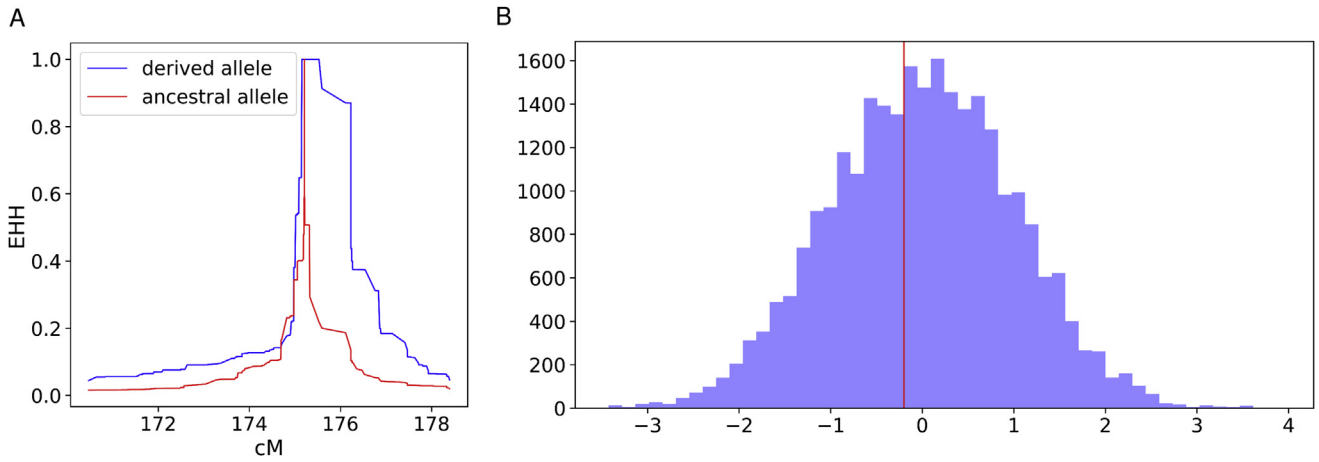
Supplementary Figure 3. Selection scan of the region on chromosome 3 encompassing *SI*. (*A*) Estimated extended haplotype homozygosity (EHH)[1] decay from the *SI* variant. The *blue* and the *red lines* show the decay of EHH for haplotypes carrying the derived and the ancestral allele at the *SI* variant site, respectively. (*B*) Estimated normalized integrated haplotype score (iHS)[2] values on chromosome 3. The *vertical red line* shows the normalized iHS for the *SI* variant (–0.199). As can be seen, the variant is not an outlier in terms of iHS, and thus does not show a signature of recent positive selection.
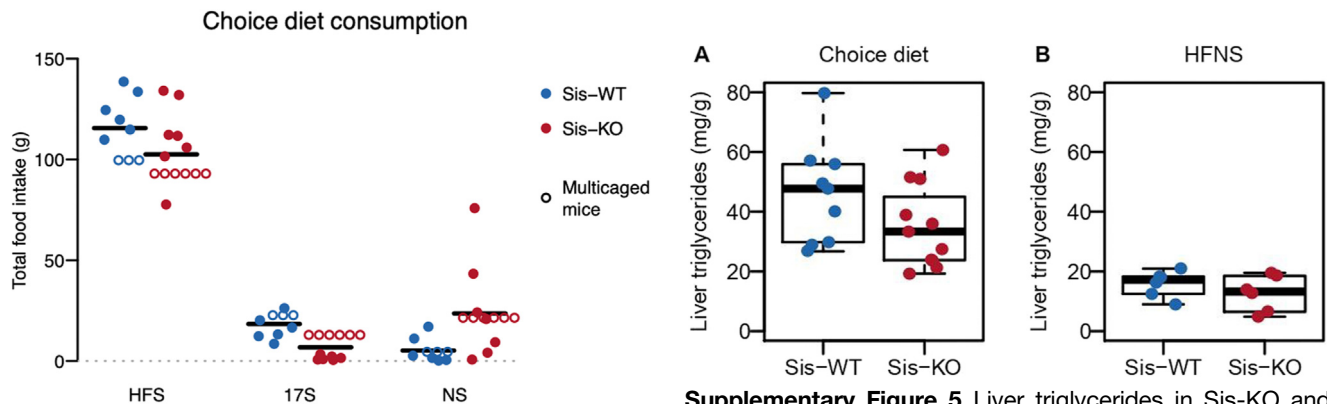
Supplementary Figure 4. Feeding pattern of Sis-KO and Sis-WT mice on choice diet. Average intake per mouse (Sis-KO, n = 13; Sis-WT, n = 9) and per cage of high-fat 12.6% sucrose (HFS) diet, low-fat 17S diet, and low-fat no-sucrose (NS) diet.

Supplementary Figure 5. Liver triglycerides in Sis-KO and Sis-WT mice. Mean liver triglyceride levels for mice with either ad libitum access to (*A*) a choice diet of high-fat 12.6% sucrose (HFS), low-fat 17S, and low-fat no-sucrose (NS) diet (Sis-WT, n = 9; Sis-KO, n = 11), or (*B*) HFNS diet (Sis-WT, n = 6; Sis-KO, n = 6). HFNS, high-fat no sucrose.