Soluble Interleukin-13rα1: A Circulating Regulator of Glucose

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Abstract: Soluble IL-13 receptor alpha 1, or sIL13rα1, is a soluble protein that binds to Interleukin 13 (IL-13) and that has been previously described in mice. The function of sIL13rα1 remains unclear, but it has been hypothesized to act as a decoy receptor for IL-13. Recent studies have identified a role for IL-13 in glucose metabolism, suggesting that a decoy receptor for IL-13 might increase circulating glucose levels. Here we report that delivery of sIL13rα1 to mice by either gene transfer or recombinant protein decreases blood glucose levels. Surprisingly, the glucose-lowering effect of sIL13rα1 was preserved in mice lacking IL-13, demonstrating that IL-13 was not required for the effect. In contrast, deletion of IL-4 in mice eliminated the hypoglycemic effect of sIL13rα1. In humans, endogenous blood levels of IL13rα1 varied substantially, although there were no differences between diabetic and non-diabetic patients. There was no circadian variation of sIL13rα1 in normal human volunteers. Delivery of sIL13rα1 fused to an FC domain provided sustained glucose lowering in mice on a high fat diet, suggesting a potential therapeutic strategy. These data reveal sIL13rα1 as a circulating human protein with an unexpected role in glucose metabolism.
**INTRODUCTION**

IL-13 is a member of the interleukin family, and IL-13 has diverse functions. IL-13 signals through three distinct receptor complexes and binds with highest affinity to a heterodimer receptor comprised of IL-4 receptor α and IL-13 receptor α-1 (3, 6). IL-13 is a central mediator in bronchial asthma due to its role as an inducer of airway hyper-responsiveness and participates in a variety of respiratory diseases including anaphylaxis, emphysema, and chronic obstructive pulmonary disease (20, 22). Recent studies have identified a role of IL-13 in regulating liver and skeletal muscle metabolism (5, 19). Interestingly, genetic deletion of IL-13 in mice leads to hyperglycemia and upregulation of hepatic gluconeogenesis (19). IL-13 induces phosphorylation of STAT-3, and loss of IL13rα1 in hepatocytes abolishes the inhibitory effects of IL-13 on glucose production (19). Collectively, these results suggest glycemic control is mediated through the IL13rα1 receptor.

The presence of a soluble form of IL13rα1 (sIL13rα1) that binds to IL-13 in mice was reported in 1997 (23). sIL13rα1 lacks the transmembrane domain of IL13rα1 and is produced from alternative mRNA splicing that leads to early termination of translation (10). Since the initial report of sIL13rα1, little research has been conducted to elucidate its function. One study identified a role of sIL13rα1 in promoting B cell differentiation and maturation through possible regulation of the IL-6R/gp130 complex (13). The existence of sIL13rα1 has yet to be reported in humans (4). Because sIL13rα1 has been shown to bind to IL-13, it is possible that sIL13rα1 can inhibit IL-13 by acting as a decoy receptor. Given this function, we hypothesized that increasing levels of sIL13rα1 would likely elevate blood glucose levels, similar to the phenotype observed in mice with deletion of IL-13 (19). Unexpectedly, we found using two distinct methods of sIL13rα1 administration, adeno-associated virus (AAV) or purified recombinant protein, that...
sIL13rα1 decreases fasting blood glucose in mice. Injection of sIL13rα1 into mice lacking IL-13 resulted in the same glucose lowering effect as observed in wild type mice, but deletion of IL-4 abolished the effect of sIL13rα1. In addition, administration of sIL13rα1 as a fusion protein with the immunoglobulin fragment crystallizable region (FC) caused a sustained decrease in fasting blood glucose in mice fed a high fat diet. Our results show that sIL13rα1 regulates fasting blood glucose independent of IL-13 and that sIL13rα1 may enhance IL-4 signaling.
MATERIALS AND METHODS

Animals. All experiments were conducted in accordance with the Guide for the Use and Care of Laboratory Animals and approved by the Harvard Medical School Standing Committee on Animals. Mice with deletion of IL-13 were from Andrew N. McKenzie of the MRC Laboratory of Molecular Biology and mice with deletion of IL-4 knockout mice were from Jackson Laboratories. Mice fed a high fat diet were obtained from Jackson Labs and fed with high fat diet for a total of 14 weeks. All mice were C57/BL6 and male.

Human experiments. Human experiments were approved by the Human Research Committee of the Partners HealthCare System and participants gave written informed consent. Circadian Study: Blood samples were collected from 24 healthy non-obese adults (males n=12, females n=12; mean age 22.08 ± 2.32 years, range 18-30) who participated in an inpatient circadian rhythm study designed to assess the phase and amplitude of endogenous physiologic rhythms. Upon awakening after the third baseline night in the laboratory, each participant began a constant routine (CR). The CR protocol is designed to eliminate periodic changes in behavior or the environment that could influence the level of the measure of interest (in this case, sIL13rα1). Throughout the 28 (n=12) or 40 (n=12)-hour CR the participant remained awake, sitting in bed (to control posture and activity level), room lighting remained on at a low level, room temperature was controlled, and food and fluid were distributed across day and night via identical hourly snacks. To verify wakefulness and compliance with the CR protocol, there was a trained staff member in the room with the participant throughout the CR. Blood samples were collected at regular intervals and were placed into heparinized collection tubes on ice for up to an hour before being centrifuged, and the resulting plasma was frozen until analysis. Samples collected at four-hour intervals beginning at the start of the CR were assayed for sIL13rα1.
Following the CR, the participant was scheduled for a recovery sleep episode, and their study continued for an additional 1-3 weeks. Only data from the CR portion of the study are included here. Each sample was assayed twice, and mean concentration at each sampling time was calculated for each participant. All sample mean values for each participant (7-11 samples/participant) were averaged together to get a “participant mean”.

**TIMI Studies**: PROVE IT-TIMI 22- Therapy—Thrombolysis in Myocardial Infarction 22 trial, published in 2004 (1), randomized 4,162 patients stabilized after a recent acute coronary syndrome (ACS) to high-dose atorvastatin 80mg daily or moderate-dose pravastatin 40mg daily.

The SOLID-TIMI-52 – (Stabilization of Plaque Using Darapladib-Thrombolysis in Myocardial Infarction 52; ClinicalTrials.gov NCT 01000727) was published in 2014 (9) and was a multinational, double-blind, placebo-controlled trial that randomized 13,026 participants within 30 days of hospitalization with an acute coronary syndrome at 868 sites in 36 countries. The baseline characteristic of the patients used in this research are summarized in Table 1.

**AAV production.** sIL13rα1 was cloned into the AAV-cDNA6-V5His vector or a null construct and then packaged into the AAV-9 serotype viral vectors by Vector Biolabs (AAV-DJ8/mussIL13rα1, Lot: 2015-0119). Empty viral AAV-9 vectors were also purchased from Vector Biolabs (AAV/DJ8-CMV-Null, Lot 2015-0413).

**Glucose, Insulin, and Pyruvate Tolerance Tests.** Blood glucose concentrations were measured at indicated time points using an Ascensia Elite XL glucometer (Bayer Co). Glucose (2mg/g mouse weight) tolerance tests were performed following overnight fast and plasma glucose levels were measured at 15, 30, 60 and 120 minutes post injection. Insulin (750 U/g) tolerance tests was performed at 12PM following a 4 hour fast. Blood glucose levels were measured at 20 and 40 minutes post injection. For pyruvate tolerance tests, pyruvate (2 mg/g)
and sIL13rα1 (0.5 mg/kg) were injected after overnight fast and blood glucose levels were measured at 15, 30, 45, 60, and 120 minutes post injection. Insulin levels were measured using the Ultra Sensitive Mouse Insulin Elisa Kit (Crystal Chem) according to the manufacturer’s instruction.

Recombinant sIL13rα1 production. The extracellular domain of mouse IL13rα1 was cloned in frame with a GP67 N-terminal secretion signal and a C-terminal 6x His tag for production and secretion in insect cells. The GP67-sIL13rα1-His sequence was then cloned into the pENTR-D vector (Invitrogen). Recombinant sIL13rα1 protein was produced using the Baculodirect Baculovirus Expression System (Invitrogen). To remove surfactants, filtered media was run through the MinimateTM TFF System (Pall) using the manufacturer’s instructions, and sIL13rα1 was exchanged into 1x PBS. Recombinant His-tagged sIL13rα1 protein was isolated from media using the Ni-NTA Purification System (Qiagen). Protein was further purified on a BioLogic DuoFlowTM (BioRad) Fast performance liquid chromatography system in 0.5x PBS through a size exclusion column. Fractions were assessed for the presence of sIL13rα1 and the absence of contaminating proteins by western analysis and coomassie blue staining. Fractions were then combined and concentrated using Amicon® Ultra 15 mL Centrifugal Filters (Millipore) according to manufacturer’s instructions. Samples were determined to be endotoxin-free using the LAL Chromogenic Endotoxin Quantitation Kit (Pierce). Concentrated protein was stored at -80°C until use. The FC-IL13rα1 was purchased from R&D Systems, Cat# 491-IR/CF, Lot # ANG0113011.

sIL13rα1 sequence used for the protein purification

>mus_Gp67_ILECD_His_RNA
ATGCTACTAGTAAATCAGTCACACCAAGGCTTCAATAAGGAACACACAAGCAAGATGGTAAGCGCTATTGTTTTAT
ATGTGCTTTTGGCGGCGGCGGCGCATTCTGCCTTTGCGGCGGATCCCGCCACAGAAG
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RNA extraction and quantitative PCR. Total RNA was isolated using TRIzol reagent (Invitrogen) and PureLink RNA Micro Kit (Invitrogen). cDNA was synthesized from 2μg of total RNA and random hexamers using high capacity reverse transcription kit (Applied Biosystems). Quantitative PCR (qPCR) was performed using Taqman probes (ThermoFisher Scientific) on a CFX384 Thermal cycler (BioRad) with 95 x 10 min, then 40 cycles of 95°C × 15 s, 60°C × 1 min, before ending with 4°C forever. Taqman probe information can be found in Table 2.
ELISA assay. Serum levels of sIL13rα1 were analyzed using the Mouse IL13RA1 ELISA Pair Set Kit (SinoBiological) or the Human IL13RA1 (R&D) according to the manufacturer’s instructions. Repetitive (4 times) freeze-thaw cycles of human serum did not influence the assay. The protein was detectable from 0.062 to 16 ng/ml on serum or serum diluted up to four fold with the manufacturer’s assay buffer (Reagent diluent concentrate 2; Lot:333567; R&D). The assay had a coefficient of variance of 2.1%. All human samples were assayed in duplicate and averaged.

The kit was tested to assure assay linearity between 1-4 ng/ml in human serum and in human serum diluted up to four fold. Serum samples of mice injected with sIL13rα1 were diluted 1:10 or 1:20 for the FC-IL13rα1 injections.

Western Analysis. Western analysis was performed with a SDS-PAGE Electrophoresis System. 20μg protein samples were resuspended in 1x NuPAGE LDS sample buffer (Invitrogen) and 5% 2-mercaptoethanol (sigma) and boiled at 95C for 10 minutes prior to loading on a 4-12% Bis-Tris precast protein SDS-page gel (Invitrogen). The gel was run in MOPS SDS Running Buffer for 2 hours at 120V. Transfer was conducted for 35 minutes at 25V on a semi-dry electrophoretic transfer cell (Bio-Rad). The membrane was blocked in 5% bovine serum albumin (BSA) in PBST (PBS and 1% Tween (Fisher Scientific)) for 1 hour at room temperature. The membrane was incubated in 1:1000 diluted primary antibody (P-STAT-6, Cell Signaling #9361S; STAT-6, Cell Signaling #9362S) in the blocking buffer at 4°C overnight and in 1:10000 secondary antibody for 1 hour at room temperature. The SDS-page was stained with SimplyBlue Coomassie stain (Bio-Rad) for 1 hour. Luminol Enhancer and Stable Peroxide Buffer of Western Lightning ECL Oxidizing Reagent (PerkinElmer) were combined in a 1:1 ratio and added to the
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membrane for 1-2 minutes with agitation. Densitometry measurements of scanned images were conducted using ImageJ software.

**Primary Hepatocyte Culture.** Liver was isolated and hepatocytes were isolated using William’s E media and 90% Percoll Solution as described in (16). Hepatocytes were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

**Statistical Analysis.** All data are presented as mean ± SEM except where noted. Statistical differences between vehicle and sIL13rα1 injection were assessed using 2-tailed Student’s t test. For in vitro assays, the mean and SEM were determined from 3–4 biological replicates. A P value less than 0.05 was considered as significant.
RESULTS

AAV delivery of sIL13rα1 reduces fasting glucose and improves glucose metabolism

Mice lacking IL-13 demonstrate hyperglycemia and insulin resistance that worsens with age, suggesting that IL-13 has a protective role in glucose metabolism (19). We hypothesized that sIL13rα1 would inhibit circulating IL-13 and therefore increasing circulating sIL13rα1 would mimic the hyperglycemia and insulin resistance phenotypes observed in mice with genetic deletion of IL-13 (19). We first increased circulating levels of sIL13rα1 in mice by administration of AAV encoding for sIL13rα1. Circulating levels of sIL13rα1 were measured by ELISA and were elevated approximately 10 fold by AAV delivery compared to the control (6.2±0.4 ng/ml vs. 0.6±0.04 ng/ml) (Fig. 1A). Fed glucose levels and body weights of animals injected with sIL13rα1 AAVs were not significantly different from controls after one week. One week after AAV administration, surprisingly, fasting blood glucose levels were significantly lower in animals injected with sIL13rα1 AAV after a 6-hour fast (Fig. 1B) and an overnight fast (Fig. 1C).

To further characterize the effects of sIL13rα1 on glucose homeostasis, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed. Mice with sIL13rα1 delivered by AAV had improved glucose tolerance (Fig. 1D) as well as increased insulin sensitivity (Fig. 1E). Since we observed significant changes in glucose metabolism in mice treated with sIL13rα1 delivered by AAV, we measured if sIL13rα1 influences insulin secretion. No significant differences were seen in fasting insulin levels between the treatment and control groups (Fig. 1F). We then hypothesized that sIL13rα1 may decrease circulating glucose levels by acting on the liver to inhibit gluconeogenesis. We performed pyruvate tolerance tests (PTT) to assess whether sIL13rα1 might influence gluconeogenesis. The PTT suggested that sIL13rα1-treated
mice exhibited decreased glucose production (Fig. 1G). When livers were harvested and the
expression of gluconeogenesis genes measured by qPCR, we observed significant decreases in
the expression of several key gluconeogenic genes in the sIL13rα1 AAV mice compared to the
null-AAV mice (Fig. 1H). Together, these results indicate that sIL13rα1 may inhibit hepatic
gluconeogenesis.

Recombinant sIL13rα1 reduces fasting glucose and improves glucose metabolism

To explore the role of sIL13rα1 on the regulation of fasting glucose levels, we expressed
and purified recombinant sIL13rα1 (sIL13rα1) protein with a baculovirus system (Fig. 2A). A
single injection of sIL13rα1 decreased glucose levels in fasting mice compared to vehicle-
injected mice for nearly 24 hours, until the animals were fed again (Fig. 2B). Similar to the mice
with sIL13rα1 delivered by AAV, we did not observe any differences in circulating insulin levels
between vehicle- and sIL13rα1-injected mice (1.4±0.14 ng/ml vs. 1.7±0.21 ng/ml; n=7).

Since we observed a decrease in expression of gluconeogenic genes in mice treated with
AAV expressing sIL13rα1, we repeated the PTT experiment in mice injected with recombinant
sIL13rα1 protein. Purified sIL13rα1 (or vehicle control) and pyruvate were injected after an
overnight fast. Glucose levels were significantly lower in the sIL13rα1-injected group compared
to controls (Fig. 2C). In addition, livers were harvested and gluconeogenic gene expression was
measured by qPCR. Similar to the mice with sIL13rα1 delivered by AAV, mice injected with
recombinant sIL13rα1 had decreased expression of gluconeogenic genes (Fig. 2D). Using these
two different delivery models, we conclude that administration of sIL13rα1 by either AAV or
recombinant protein can reduce fasting blood glucose levels in wildtype mice.

sIL13rα1 reduces fasting glucose levels independently of IL-13
Because sIL13rα1 can interact with IL-13 (6, 23), we hypothesized that sIL13rα1 affects glucose metabolism through IL-13. To test whether the metabolic effects of sIL13rα1 require IL-13 signaling, we injected recombinant sIL13rα1 into mice with deletion of IL-13 and measured blood glucose levels following injection. Surprisingly, administration of sIL13rα1 significantly decreased blood glucose levels at two hours post injection in IL13−/− mice, similar to the effect in wildtype mice (Fig. 3A). Serum sIL13rα1 levels were the same between the two groups (Fig. 3B), revealing that sIL13rα1 reduces fasting glucose levels in a manner that is independent of IL-13.

**sIL13rα1 enhances IL-4 signaling**

IL-13 is closely related to IL-4, and these two cytokines share common receptors, leading to overlapping functions and activities in vitro and in vivo (6, 8). We tested the possibility that the glucose lowering effect of sIL13rα1 requires the IL-4 ligand. Administration of sIL13rα1 significantly decreased blood glucose in wildtype control mice, but deletion of IL-4 in mice abolished this effect (Fig. 3C). Serum levels of sIL13rα1 were similar (Fig. 3D), indicating that reduction of fasting glucose by sIL13rα1 requires IL-4 signaling. IL-4 can improve glucose tolerance and insulin sensitivity in a manner that is STAT-6 dependent and restricted to hepatocytes (14). To determine if sIL13rα1 can affect IL-4 signaling, we measured STAT-6 phosphorylation in the liver (Fig. 4A) and in the skeletal muscle (Fig. 4B). Wildtype or IL-4−/− mice were injected with either recombinant sIL13rα1 or vehicle as control, and STAT-6 phosphorylation was measured. STAT-6 phosphorylation was significantly enhanced in the liver of sIL13rα1 injected wildtype mice compared to controls (Fig. 4A), a phenotype that was not present in IL-4−/− mice (Fig. 4A). Interestingly, STAT-6 phosphorylation did not change in the skeletal muscle of sIL13rα1 injected wildtype mice and IL-4−/− mice compared to controls (Fig.
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In addition, mRNA levels of IL-4ra, a downstream target of STAT-6 and a receptor for IL-4 (12), increased significantly in the liver of the sIL13rα1-injected wildtype mice compared to control, while no difference was measured in sIL13rα1-injected IL-4-/mice compared to control (Fig. 4C). Furthermore, we measured the expression of gluconeogenic genes in the livers of wildtype and IL-4-/mice injected with either vehicle or sIL13rα1 (Fig. 4D). In sIL13rα1-injected mice, we observed a significant decrease in glucose-6-phosphatase catalytic subunit (G6pc) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (Ppargc1α) mRNA expression in wildtype mice, while no change was observed in IL4-/ mice (Fig. 4D).

To test if sIL13rα1 can directly enhance IL-4 activity, primary hepatocytes were isolated from rats. Hepatocytes were isolated, cultured, and treated with different concentrations of IL-4 (0.5ng/ml, 1ng/ml, 5ng/ml, 10ng/ml, 25ng/ml and 50ng/ml). Treatment with 1ng/ml of IL-4 significantly enhanced STAT-6 phosphorylation but did not reach plateau (maximal) levels. Hepatocytes were further treated with recombinant sIL13rα1 (300 ng/ml), IL-4 (1ng/ml), or both and STAT-6 phosphorylation was measured. No significant changes of STAT-6 phosphorylation were observed between hepatocytes from IL-4 treated or both IL-4 and sIL13rα1 treated mice, revealing that sIL13rα1 may have an effect on IL-4 signaling in the liver that is not apparent in cultured hepatocytes (Fig. 4E).

**Human levels of sIL13rα1 are similar in diabetic and non-diabetic patients.**

The existence of a soluble form of IL13rα1 has been described previously only in mice (23). In order to assess sIL13rα1 in humans, we used an ELISA system to measure sIL13rα1. We first measured serum levels of sIL13rα1 in diabetic patients (n=148) and non-diabetic patients (n=150) in a previously performed randomized trial in patients with coronary disease (PROVE IT-TIMI 22) (1). We observed a strong trend toward lower sIL13rα1 levels in the diabetic
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patients ($p = 0.051$), with 1.52 +/- 9.96 ng/ml (mean +/- standard deviation) in controls versus 0.77 +/- 1.44 ng/ml (mean +/- standard deviation) in the diabetic population. To determine if this strong trend was present in another population, we measured sIL13rα1 levels in a second larger cohort of patients with coronary disease (SOLID-TIMI 52)(9), with 292 patients in each group (Table 1). In this 584 patient study, there were no differences between diabetic patients, with 2.86 +/- 26.38 ng/ml (mean +/- standard deviation) and non-diabetic patients with 2.92 +/- 28.39 in sIL13rα1 levels ng/ml (mean +/- standard deviation). Furthermore, additional analyses of the SOLID cohort stratifying by obesity and there was no evidence for an interaction ($p=0.54$), n=207 obese subjects (BMI $\geq 30$), n=373 non-obese subjects (BMI $< 30$). Accounting for obesity using conditional regression the relationship with sIL13rα1 remained non-significant ($p=0.84$).

Because IL-13 levels have been reported to vary in a circadian manner (17), we also measured if there was a circadian variation to sIL13rα1 levels in normal human volunteers. Blood samples were collected from 24 healthy adults (mean age 22.1 ± 2.3 years, range 18-30) who participated in an inpatient circadian rhythm study. The plasma sIL13rα1 levels for males were not significantly different from females (1.31 ± 1.52 ng/ml vs. 1.24 ± 1.08 ng/ml, mean +/- standard deviation, $p = 0.89$), and there was no apparent variation in sIL13rα1 levels across a 40-hour sample collection period where the participants were in constant conditions.

We further determined the levels of the sIL13rα1 serum levels in the high fat diet (HFD) mouse model compared to the regular diet, and there was no significance difference between the groups with 1.19 +/- 0.09 ng/ml (n=8; mean +/- standard error) with the regular diet and with 1.30 +/- 0.06 ng/ml in the HFD group (n=10; mean +/- standard error). Also, the serum levels of sIL13rα1 were similar after an overnight fast and after feeding (1.08 +/-0.06 ng/ml vs. 1.11 +/-
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0.06 ng/ml, respectively, n=8, N.S). Thus, in humans and mice, sIL13rα1 levels are similar between the diabetic and non-diabetic state.

Administration of sIL13rα1 fused to FC reduces fasting glucose for 1 week in high fat diet mice

Because sIL13rα1 can decrease blood glucose levels in mice normal breeding diet, we asked if exogenous sIL13rα1 can reduce glucose levels in mice fed a high fat diet for 14 weeks, a model of human type 2 diabetes (21). Since FC domains can prolong protein half-life in vivo (15, 18), we administered an FC-IL13rα1 chimeric protein to mice fed a high fat diet mice. Elevations in sIL13rα1 levels after injection were approximately 10-fold greater than changes in dose due to differences in body weight. Four hours after administration, blood glucose levels were significantly lower in FC-IL13rα1-injected mice compared to vehicle (Fig. 5A). The glucose lowering effect was significant for five days post injection (Fig. 5B), until circulating sIL13rα1 levels in FC-IL13rα1-injected mice fell (Fig. 5C). These results suggest that FC-IL13rα1 can lead to a sustained glucose lowering effect.
DISCUSSION

sIL13rα1 was first identified in 1997 and described as a binding protein to IL-13 with the potential to act as a decoy receptor (23). sIL-13rα1 lacks the transmembrane domain of IL13rα1 and is produced from alternative mRNA splicing that leads to early termination of translation (10). Since that time, little research has been conducted on sIL13rα1, except for its participation in B cell differentiation (13, 23). In this study, we describe an unexpected role of sIL13rα1 in glucose metabolism. Using two different methods of administration, sIL13rα1 decreased fasting blood glucose in mice. In addition, sustained delivery of sIL13rα1 improved glucose tolerance and insulin sensitivity and decreased the expression of key gluconeogenic genes in the liver.

Because sIL13rα1 lack the transmembrane domain we hypothesized that increasing levels of sIL13rα1 would likely elevate blood glucose levels, similar to the phenotype observed in mice with deletion of IL-13 (19). However, our results show that the ability of sIL13rα1 to reduce fasting blood glucose is independent of IL-13. IL-4 and IL-13 are structurally and functionally related. In non-hematopoietic cells, IL-4 and IL-13 signals through the type II receptor that composed of IL-4Rα and IL-13Rα1. Biochemical studies using human IL-13rα1 that lacks the transmembrane domain, have revealed that the extracellular domain of IL13rα1 has only moderate affinity to IL-13 compared to the affinity of IL-4 for the IL-4ra receptor (6). In contrast, IL-4 binds IL-4Rα with high affinity and the presence of IL-13Rα1 provides little additional affinity (6). Interestingly, although IL-4 and IL-13 share receptor subunits, they have overlapping but also unique functions in vitro and in vivo (3, 8).

Our experiments showed that administration of sIL13rα1 is dependent upon IL-4 and that sIL13rα1 may enhance IL-4 signaling. In addition, sIL13rα1 enhanced phosphorylation of STAT-6, a key event downstream of the IL-4 receptor (7, 11). We also showed that sIL13rα1
increased the expression of IL-4ra, a subunit of the IL-4 type I receptor and a downstream response of STAT-6 activation (12). Binding of IL13/IL-4 to the type II receptor triggers the activation and gene transcription of the STAT6 pathway. Interestingly, when IL13rα1 expression is high, IL-4 is more potent at stimulating the tyrosine phosphorylation of STAT6; however, in cells where IL13rα1 is limiting, IL-13 can become more potent than IL-4 (6). It has been shown that IL-4 treatment significantly improves glucose tolerance and insulin sensitivity and this depends on STAT-6 (2, 14) suggesting that sIL13rα1 could mediate its decrease in fasting glucose through the IL-4/STAT-6 axis.

STAT-6 is expressed in metabolic tissues including liver and adipose tissue; however, the ability of IL-4 to stimulate STAT-6 signaling is primarily restricted to hepatocytes, and adipocytes lack the IL-4ra receptor (14). Although we observed enhancement of STAT-6 phosphorylation in vivo, we did not observe this effect in cultured hepatocytes treated with sIL13rα1 and IL-4, suggesting that sIL13rα1 might regulate IL-4 signaling in an indirect manner. For example, it has been shown that in lymphocytes, IL-4 activation leads to IL-4ra expression in a manner that is dependent on IL-6 (12). In addition, induction of IgG2a and IgG2b in murine germinal center B cells by sIL13rα1 is dependent on IL-6 expression (13).

In our initial study of 298 patients, we found a strong trend toward lower sIL13rα1 levels in the diabetic patients, consistent with the concept that increasing sIL13rα1 levels might decrease glucose levels in diabetic patients. However, in a larger experiment using the blood samples of 584 patients, sIL13rα1 were not different from diabetic patients and controls. Even if sIL13rα1 levels are not different in diabetic patients, it is plausible that raising sIL13rα1 could lower glucose levels, and it is thus important to understand what controls sIL13rα1 blood levels. Although further work is necessary to precisely define how sIL13rα1 regulates glucose
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metabolism, our experiments indicate potential inhibition of hepatic gluconeogenesis as a mechanism. Delivery of sIL13rα1 fused to an FC domain appears to sustain glucose lowering in mice fed a high fat diet, suggesting possible benefits in type 2 diabetes. Defining how sIL13rα1 can improve glucose metabolism could yield new approaches to metabolic diseases.
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**GRANTS:** This article was supported by the Blavatnik Biomedical Accelerator of Harvard University, the National Institutes of Health/National Heart, Lung, Blood Institutes grant R01 HL117986, and the National Institutes of Health/National Institutes of Aging grant R01 AG047131. Inbal Rachmin was supported by an EMBO and ISEF Fellowship. The human circadian experiments were supported by NIH grant R01 HL080978 and by grant HPF 00402 from the National Space Biomedical Research Institute; they were carried out in the Brigham and Women’s Hospital Center for Clinical Investigation (formerly a General Clinical Research Center supported by NIH grant M01 RR02635), supported by Harvard Catalyst | The Harvard Clinical and Translational Science Center (National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health Award UL1 TR001102) and financial contributions from the Brigham and Women’s Hospital and from Harvard University and its affiliated academic healthcare centers. The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic healthcare centers, or the National Institutes of Health.

**Conflict of Interest:** None.

**AUTHOR CONTRIBUTIONS:** I.R., C.C.O., and R.T.L., designed the research and analyzed the data. J.R.P., performed the Elisa calibration. I.R., C.C.O., Y.F., J.R.P., E.M.R., and E.M.C., performed the experiments. J.F.D., K.M.Z., C.A.C., C.P.C., M.L.O., and D.A.M., led the human studies. I.R., Y.F., and R.T.L., wrote the paper, and all authors edited and approved the final manuscript.
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References:


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**Fig. 1. AAV delivery of sIL13rα1 reduces fasting glucose and glucose metabolism**

sIL13rα1 or Null AAV-9 viral constructs were administered to 10-12 week old adult male C57/Bl6 mice (males) fed on normal breeding diet by tail vein injection. Serum levels of sIL13rα1 seven days post AAV injection (A). Blood glucose levels of mice after 6 hour fast, seven days following AAV administration (B). Blood glucose levels of mice after overnight fast (C). Glucose Tolerance Test: Mice were fasted overnight and fasting glucose levels were measured with 59.7 +/- 3.8 in the AAV- sIL13rα1 compared to 73.9 +/- 5.3 mg/dL in the AAV null group (n=8, p<0.05). Mice were injected with glucose, blood glucose levels were measured at 15, 30, 60 and 120 minutes post injection (D). Insulin Tolerance Test: At 12pm mice were injected with insulin. Blood glucose levels were measured at 20 and 40 minutes post injection (E). Blood insulin levels of mice at baseline and after 2, 4 or 6 hour fast seven days following AAV administration (F). Pyruvate Tolerance Test: Mice were fasted overnight and injected with pyruvate. Blood glucose levels were measured at 15, 30, 45, 60 and 120 minutes post injection (G). Liver gluconeogenic gene expression was measured using qPCR. Tata box binding protein was used for normalization (H).

*p<0.05, **p<0.01, ***p<0.001. Data is presented as mean ± SEM.

**Fig. 2. Recombinant sIL13rα1 decreases circulating glucose levels**

Recombinant sIL13rα1 protein production and purification using Baculovirus Expression Vector System and isolation by FPLC (A). 0.5mg/kg sIL13rα1 recombinant protein was injected to 8-10 weeks old C57BL/6 mice (males), i.p, 3 hours post fasting. C57BL/6 mice were injected with sIL13rα1 demonstrate significantly decreased circulating blood glucose levels compared to vehicle over the course of 22 hours (B). Pyruvate Tolerance Test: Mice were fasted overnight
and injected with pyruvate (2g/kg) and sIL13rα1 (0.5mg/kg). Blood glucose levels was measured at 15, 30, 45, 60 and 120 minutes post injection (C). Liver gluconeogenesis gene RNA expression four hours post sIL13rα1 injection (D).

*p<0.05, **p<0.01, ***p<0.001. Data presented as mean ± SEM.

**Fig. 3. sIL13rα1 reduction of fasting glucose is IL-4 dependent**

Three hours post fasting, 0.5mg/kg sIL13rα1 recombinant protein was i.p injected into IL13-/-(A-B) or IL4-/-(C-D) and their wildtype littermates (8-10 weeks old C57BL/6 mice; males) and 1 hour post injections serum levels of sIL13rα1 were measured (B, D). Fasting glucose blood levels were measured at 15, 30, 60, and 120 min post injections (A, B). *p<0.05, **p<0.01, ***p<0.001. N.S , no significance. Data presented as mean ± SEM.

**Fig. 4. sIL13rα1 enhance STAT-6 signaling in an IL-4 dependent manner**

Wildtype and IL-4−/− mice (8-10 weeks old C57BL/6 mice; males) were fasted overnight and saline or sIL13rα1 was injected. Livers (A) and Skeletal muscles (B) were collected immediately after 30 minutes, homogenized, STAT-6 phosphorylation and total STAT-6 were measured with western analysis. Densitometry analysis was done using Image J software, and each lane represents a different mouse. IL-4ra gene RNA expression was measured four hours post sIL13rα1 injection in wildtype and IL-4−/− mice (C). Liver gluconeogenesis gene expression four hours post sIL13rα1 injection in IL-4−/− mice and their wildtype littermates (D). Livers were isolated and perfused. Hepatocytes were isolated, cultured, and treated with sIL13rα1 (300 ng/ml), IL-4 (1ng/ml), or both. STAT-6 phosphorylation was measured with western analysis. (E).
sIL13rα1 regulates fasting glucose levels

*p<0.05, **p<0.01, ***p<0.001. Data is presented as mean ± SEM.

Fig. 5. Single injection of FC-sIL13rα1 on high-fat diet mice reduces fasting blood glucose for nearly a week

Mice were fed a high-fat diet (58% of energy derived from fat) for 14-weeks (17 weeks old mice, males). Saline or sIL13rα1 (0.5 mg/kg) was administered to the mice and glucose levels were measured post injection (A). Blood glucose levels after a 6 hour fast on different days after injection (B). Circulating sIL13rα1 levels days after injection (C).
**Table 1. Baseline characteristic of case (diabetic) and control (non diabetic) subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (N=292)</th>
<th>Controls (N=292)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs (median, IQR)</td>
<td>62.50 (55.50, 69.00)</td>
<td>63.00 (56.00, 69.00)</td>
<td>0.024</td>
</tr>
<tr>
<td>BMI (kg/m2) (median, IQR)</td>
<td>29.2 (25.7, 32.7)</td>
<td>26.8 (24.5, 30.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI&gt;=30 (kg/m2) (no(%))</td>
<td>125 (43.1%)</td>
<td>82 (28.1%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidemia (no(%))</td>
<td>197 (67.5%)</td>
<td>179 (61.3%)</td>
<td>0.13</td>
</tr>
<tr>
<td>Peripheral Arterial Disease (no(%))</td>
<td>21 (7.2%)</td>
<td>40 (13.7%)</td>
<td>0.009</td>
</tr>
<tr>
<td>Prior PCI (no(%))</td>
<td>71 (24.3%)</td>
<td>81 (27.7%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Hypertension (no(%))</td>
<td>239 (81.9%)</td>
<td>199 (68.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL) (median, IQR)</td>
<td>148 (124, 176)</td>
<td>151 (131, 185)</td>
<td>0.11</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL) (median, IQR)</td>
<td>71 (56, 91)</td>
<td>76 (61, 102)</td>
<td>0.008</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL) (median, IQR)</td>
<td>41 (35, 46)</td>
<td>44 (37, 53)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL) (median, IQR)</td>
<td>150 (110, 219)</td>
<td>127 (95, 168)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eGFR&lt;60 (mL/min) (no(%))</td>
<td>33 (11.5%)</td>
<td>19 (6.6%)</td>
<td>0.058</td>
</tr>
<tr>
<td>Current Smoker (no(%))</td>
<td>42 (14.4%)</td>
<td>55 (18.8%)</td>
<td>0.14</td>
</tr>
<tr>
<td>Prior MI (no(%))</td>
<td>92 (31.5%)</td>
<td>109 (37.3%)</td>
<td>0.13</td>
</tr>
</tbody>
</table>

**Index Event**

<table>
<thead>
<tr>
<th></th>
<th>Cases (N=292)</th>
<th>Controls (N=292)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable Angina (no(%))</td>
<td>39 (13.4%)</td>
<td>43 (14.7%)</td>
<td>0.64</td>
</tr>
<tr>
<td>Non-STEMI (no(%))</td>
<td>133 (45.6%)</td>
<td>137 (46.9%)</td>
<td>0.74</td>
</tr>
<tr>
<td>STEMI (no(%))</td>
<td>120 (41.1%)</td>
<td>112 (38.4%)</td>
<td>0.49</td>
</tr>
<tr>
<td>PCI Performed at QE (no(%))</td>
<td>223 (76.4%)</td>
<td>220 (75.3%)</td>
<td>0.78</td>
</tr>
<tr>
<td>Aspirin (no(%))</td>
<td>279 (95.6%)</td>
<td>284 (97.3%)</td>
<td>0.28</td>
</tr>
<tr>
<td>Lipid-lowering agents (no(%))</td>
<td>277 (94.9%)</td>
<td>275 (94.2%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Beta Blocker (no(%))</td>
<td>258 (88.4%)</td>
<td>249 (85.3%)</td>
<td>0.26</td>
</tr>
<tr>
<td>ACE or ARB (no(%))</td>
<td>247 (84.6%)</td>
<td>223 (76.4%)</td>
<td>0.012</td>
</tr>
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</table>

Summary of patient information used in this study from the SOLID TIMI-52 trial
Table 2.

<table>
<thead>
<tr>
<th>Gene</th>
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<tbody>
<tr>
<td>TBP Mm01277042_m1</td>
<td>1542567</td>
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<tr>
<td>Fbp1 Mm00490181_m1</td>
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<tr>
<td>G6Pc Mm00839363_m1</td>
<td>1442679</td>
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<tr>
<td>Pck1 Mm01247058_m1</td>
<td>1548867</td>
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<tr>
<td>Ppargc1 Mm01208835_m1</td>
<td>1415978</td>
</tr>
<tr>
<td>GCK Mm00439129_m1</td>
<td>1517173</td>
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<tr>
<td>Gys2 Mm00523953_m1</td>
<td>1208131</td>
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Quantitative PCR (qPCR) was performed using Taqman probes (ThermoFisher Scientific)