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Clinical Research Article

Insulin and Body Mass Index Decrease Serum Soluble Leptin Receptor Levels in Humans

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Abstract

Context: Serum soluble leptin receptor (sOb-R) may protect against future type 2 diabetes or serve as a marker for protective features, but how sOb-R is regulated is largely unknown.

Objective: This work aimed to test how serum sOb-R is influenced by glucose, insulin, body fat, body mass index (BMI), food intake, and physical activity.

Methods: We performed an epidemiological triangulation combining cross-sectional, interventional, and Mendelian randomization study designs. In 5 independent clinical studies (n = 24-823), sOb-R was quantified in serum or plasma by commercial enzyme-linked immunosorbent assay kits using monoclonal antibodies. We performed mixed-model regression and 2-sample Mendelian randomization.

Results: In pooled, cross-sectional data, leveling by study, sOb-R was associated inversely with BMI (β [95% CI] −0.19 [−0.21 to −0.17]), body fat (−0.12 [−0.14 to −0.10), and fasting C-peptide (−2.04 [−2.46 to −1.62]). sOb-R decreased in response to acute hyperinsulinemia during euglycemic glucose clamp in 2 independent clinical studies (−0.5 [−0.7 to −0.4] and −0.5 [−0.6 to −0.3]), and immediately increased in response to intensive exercise (0.18 [0.04 to 0.31]) and food intake (0.20 [0.06 to 0.34]). In 2-sample Mendelian randomization, higher fasting insulin and higher BMI were causally linked to lower sOb-R levels (inverse variance weighted, −1.72 [−2.86 to −0.58], and −0.20 [−0.36 to −0.04], respectively). The relationship between hyperglycemia and sOb-R was inconsistent in cross-sectional studies and nonsignificant in intervention studies, and 2-sample Mendelian randomization suggested no causal effect of fasting glucose on sOb-R.

Conclusion: BMI and insulin both causally decreased serum sOb-R levels. Conversely, intensive exercise and food intake acutely increased sOb-R. Our results suggest that sOb-R is involved in short-term regulation of leptin signaling, either directly or indirectly, and that hyperinsulinemia may reduce leptin signaling.

Key Words: hyperglycemia, hyperinsulinemia, leptin signaling, obesity, soluble leptin receptor

Abbreviations: BMI, body mass index; DXA, dual-energy x-ray absorptiometry; ELISA, enzyme-linked immunosorbent assay; GWAS, genome-wide association studies; HbA_{1c}, glycated hemoglobin A_{1c}; HUVECs, human umbilical vein endothelial cells; Ob-R, leptin receptor; rG, genetic correlation with fasting insulin; sOb-R, soluble leptin receptor; T2D, type 2 diabetes.

Leptin is a 16 kDa hormone mainly produced by adipocytes (1, 2). Leptin inhibits appetite, but also plays a role in reproduction (3), bone metabolism (4, 5) and glycemic control (6). Leptin signaling depends on membrane-bound leptin receptors (Ob-R), which are present in most cell types in the human body. There are 4 human isoforms of membrane-bound Ob-Rs with the same extracellular domain, whereas the intracellular domain varies in length and mode of signaling (7). The soluble Ob-R (sOb-R)

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is formed by shedding of the extracellular domain, which enters the circulation. Increasing evidence suggests that the serum sOb-R reflects the amount of membrane-bound Ob-Rs and that sOb-R acts to prolong leptin half-life (7, 8).

Leptin levels are severely increased in obesity, but appetite inhibition fails, probably due to leptin resistance (9). The exact mechanism of leptin resistance is unknown. Interestingly, sOb-R levels vary inversely with adiposity (10–13), in an opposite manner of leptin, and weight loss has been associated with increased sOb-R levels (12, 14).

In prospective, observational studies, high sOb-R levels are strongly associated with lower risk of future gestational diabetes (15) and type 2 diabetes (T2D) (16), independently of serum leptin levels and other relevant covariates. Furthermore, sOb-R has been associated with cardiometabolic outcomes such as waist circumference, high-density lipoprotein cholesterol, and fasting glucose (17), as well as body mass index (BMI), systolic and diastolic blood pressure, and insulin (18). We have previously reported a strong association between sOb-R and insulin sensitivity measured with the glucose clamp technique in men, independent of leptin levels and body fat depots as measured by magnetic resonance imaging (19). Moreover, sOb-R levels at baseline were independently and positively associated with improved insulin sensitivity following a 12-week intensive exercise intervention (19), although sOb-R levels did not differ before and after the 12-week exercise intervention.

Hence, sOb-R may protect against future T2D or serve as a marker for protective features, and thus provide a potential for T2D prevention. However, how sOb-R is regulated is largely unknown, despite sOb-R's possible preventive potential.

Here, we combine epidemiological study designs to test consistencies in how serum sOb-R is influenced by glucose, insulin, body fat and BMI, food intake, and physical activity.

Materials and Methods

Design

We performed an epidemiological triangulation combining cross-sectional, interventional, and Mendelian randomization study designs (Table 1). The cross-sectional investigation combined data from 5 separate clinical studies: STORK Groruddalen (STORK G) (20), The diabetes intervention trial with vitamin D in subjects of sub-Indian and Nordic ethnicity (DIVINE) (21), Diabetes in Pakistani Immigrants (DIPI) (22), MyoGlu (23), and 4B (24). We studied 4 separate interventions in 3 independent clinical materials: sOb-R response to hyperglycemia (DIVINE), sOb-R response to hyperinsulinemia (DIVINE and DIPI), sOb-R response to food intake (DIPI) and sOb-R response to acute physical activity (MyoGlu). We used publicly available summary data from genome-wide association studies (GWAS) of phenotypes of interest to perform 2-sample Mendelian randomization.

Serum Soluble Leptin Receptor

We quantified serum concentrations of sOb-R in STORK G, DIVINE, DIPI, and 4B, and plasma sOb-R in MyoGlu by enzyme-linked immunosorbent assay (ELISA) (R and D Systems catalog No. DOBR00, RRID:AB_2910202) at the Hormone Laboratory, Oslo University Hospital. Each clinical study was analyzed with the same batch, and all samples from each individual in the same kit. Precision across 3 different concentrations; intra-assay: coefficient of variation percentage, 2.2-6.1; interassay: coefficient of variation percentage, 5.3-8.6.

STORK G

STORK G is a population-based cohort of 823 healthy pregnant women attending 3 public mother and child health clinics for antenatal care in the multiethnic area of Groruddalen, Oslo, Norway (20). Women were eligible if they 1) lived in the study district; 2) planned to give birth at 1 of 2 study hospitals; 3) were less than 20 weeks pregnant; 4) could communicate in Norwegian or any of the 8 translated languages; and 5) were able to give an informed consent. Women with pregestational diabetes, or in need of intensive hospital follow-up during pregnancy, were excluded. The participation rate was 74%, varying from 63.9% to 82.6% across ethnic groups (20).

In pregnancy week 15 ± 3 we measured height with a fixed stadiometer, body weight and percentage total body fat with a calibrated digital scale (Tanita-BC 418 MA, Tanita Corporation), BMI was calculated (weight in kg/height in m^2). From venous blood, fasting glucose was measured with a colorimetric method (Vitros 5.1 FS, Ortho Clinical Diagnostics), and glycated hemoglobin A_{1c} (HbA_{1c}) was analyzed with HPLC (Tosoh G8, Tosoh Corp) at the Akershus University Hospital. Fasting C-peptide was measured by noncompetitive immunofluorometric assays (DELFIA, PerkinElmer Life Sciences, Wallac Oy), and leptin was analyzed with the Luminex xMAP technology (Millipore) at the Hormone Laboratory, Oslo University Hospital. sOb-R was quantified in biobanked fasting venous samples of serum in pregnancy week 15 (15).

DIVINE

The DIVINE study (NCT00992797) was a 6-month randomized, double-blind, placebo-controlled intervention trial investigating the effect of high-dose vitamin D supplementation on insulin sensitivity and insulin secretion in individuals of Nordic and South Asian origin with T2D (World Health Organization 1999) and vitamin D deficiency (21). DIVINE included 61 men and women older than 18 years, on any kind of antidiabetic treatment, of Nordic or South Asian ethnicity, and with vitamin D deficiency (25[OH] vitamin D ≤ 50 nmol/L). Exclusion criteria were HbA_{1c} greater than 11% (97 mmol/mol), BMI greater than 45, hypertension, hypercalcemia, a history of kidney stones, kidney failure, cardiovascular events during the past 6 months, or pregnant/lactating women.

Height was measured with a fixed stadiometer, and body weight and total body fat with a Tanita Body Composition Analyzer BC 418. Plasma glucose was measured by the glucose oxidase method (YSI 2300), HbA_{1c} by HPLC on a Tosoh G7 analyzer (Tosoh Corp), C-peptide by noncompetitive immunofluorometric assays (DELFIA, PerkinElmer Life Sciences, Wallac Oy), plasma leptin with radioimmunoassay from Millipore Corp (formerly from Linco Research Inc). Serum sOb-R was measured at 4 time points during a Botnia clamp (25); 1) fasting state, 2) after a 2-hour tracer equilibration period, 3) hyperglycemic state at end of an intravenous glucose tolerance test (0.3 g/kg glucose bolus), and 4) hyperinsulinemic state after 150 minutes of insulin infusion

Table 1. Overview of combined study designs and methods

Study design	Cross-sectional	Clinical intervention	Two-sample Mendelian randomization
Material	5 independent clinical studies (n = 973) STORK G (n = 823) DIVINE (n = 61) DIPI (n = 39) MyoGlu (n = 26) 4B (n = 24)	3 independent clinical studies, testing 4 interventions: hyperglycemia (DIVINE), hyperinsulinemia (DIVINE and DIPI), food intake (DIPI), acute physical exercise (MyoGlu)	Summary data from published GWAS of • sOb-R (n = 3301) • Fasting glucose (n = 133 010) • Fasting insulin (n = 108 557) • BMI (n = 681 275)
sOb-R quantification	ELISA kit with monoclonal antibodies	ELISA kit with monoclonal antibodies	SomaScan assay
Statistics	Pooled, mixed-model regression, level = study	Mixed-model regression, level = ID + ethnicity/meal type/sex/pre or post 12-wk exercise intervention	Inverse variance weighted. Sensitivity analysis: MR-Egger, weighted median and weighted mode

Abbreviations: BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; GWAS, genome-wide association study; sOb-R, soluble leptin receptor.

(80 mU/m^2/min). Patients were requested to stop oral antidiabetic drugs for 2 days, and insulin for at least 12 hours before examination. Patients were also asked to refrain from strenuous physical activity and alcohol intake during these 2 days, and to arrive fasting for at least 10 hours, from the night before the examination.

DIPI

The DIPI study investigated clinical and pathophysiological characteristics of T2D from 2004 to 2009 (22). DIPI included 39 participants from Norway and Pakistan. Eligible individuals had known T2D (World Health Organization 1999 diagnostic criteria) without any antiglutamic acid decarboxylase or anti-insulinoma antigen 2 antibodies, used any kind of antidiabetic treatment, and lived in the Oslo area. Of the 195 patients invited, 21 Norwegian and 19 Pakistani sex-matched patients were included.

A 2-step euglycemic glucose clamp was performed to test the response to physiological hyperinsulinemia (40 mU/m^2/min insulin infusion) and supraphysiological hyperinsulinemia (400 mU/m^2/min insulin infusion). Participants also underwent 2 meal tests on separate study visits with isoenergetic typical Norwegian and typical Pakistani lunch meals, respectively. Blood samples were collected during 5 hours after food intake.

Height was measured with a fixed stadiometer, weight and total body fat with Tanita Body Composition Analyzer BC 418. Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II, Beckman Instruments), HbA_{1c} by HPLC on a Variant analyzer (Bio-Rad), C-peptide by noncompetitive immunofluorometric assays (DELFIA, PerkinElmer Life Sciences, Wallac Oy), plasma leptin with radioimmunoassay from Millipore Corp (formerly from Linco Research Inc). Serum sOb-R was quantified in samples drawn immediately before and 20, 50, 170, and 320 minutes after food intake, as well as before and during the physiological and supraphysiological hyperinsulinemia steps of the glucose clamp. Patients followed the same fasting regimen as described for DIVINE.

MyoGlu

MyoGlu was an exercise intervention trial (NCT01803568) in 26 sedentary (< 1 exercise session/wk) men aged 40 to 65 years, of Scandinavian origin, performed 2011 to 2012, in Oslo, Norway (23). MyoGlu included 13 dysglycemic men (fasting glucose ≥ 5.6 mmol/L and/or 2-hour glucose ≥ 7.8 mmol/L OR with an insulin sensitivity below 4.5 mg/kg/min, BMI 26-32) and 13 normoglycemic men (fasting glucose < 5.6 mmol/L and < 7.8 mmol/L without family history of diabetes, BMI 21-27). Exclusion criteria were known hypertension, liver or kidney disease, chronic inflammatory diseases or on any medication expected to affect glucose metabolism (lipid lowering, antihypertensive, acetylsalicylic acid, corticosteroids, etc).

Before and after a 12-week period of 4-hour weekly exercise (2 60-minute endurance sessions, and 2 60-minute resistance exercise sessions), participants underwent acute exercise tests (45-minute bicycle test at 70% of maximal oxygen uptake) test (23). The participants refrained from physical exercise and alcohol for 2 days before the tests.

Height was measured with a fixed stadiometer, and weight and fat free mass with a Tanita Body Composition Analyzer BC-418MA, BMI was calculated from weight in kg/height in m^2. Total adipose tissue was measured by magnetic resonance imaging scanning (1.5 T Philips Achieva MR). Fasting glucose was measured by a glucose oxidase method (YSI 2300), and plasma glucose levels were calculated (full blood glucose × 1.119). HbA_{1c} was measured with a Tosoh G7 analyzer (Tosoh Corp), C-peptide with DELFIA (PerkinElmer Life Sciences, Wallac Oy), and plasma leptin with an ELISA (catalog No. KAC2281, Invitrogen). sOb-R was quantified in biobanked plasma from venous samples drawn before, immediately after, and 2 hours after the acute exercise test (19).

4B

4B included patients with and without T2D scheduled for Roux-en-Y gastric bypass at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, from October 8, 2015 to January 27, 2017 (24). Eligibility criteria for Roux-en-Y gastric bypass were BMI greater than or equal to 40 or BMI greater than or equal to 35 with obesity-related comorbidity, age between 18 and 65 years, and failed attempts at sustained weight loss. Participants were excluded if unable to read Norwegian or of non-White ethnicity, had severe psychiatric comorbidity, connective

Table 2. Characteristics of participants in the clinical studies

	STORK G (n = 823)	DIVINE (n = 61)	DIPI (n = 39)	MyoGlu (n = 26)	4B (n = 24)
Age, y	29 ± 5	56 ± 10	40 ± 4	51 ± 6	44 ± 10
Women/men (n)	823/0	37/24	19/20	0/26	18/6
Ethnic origin					
European	379 (47)	43 (70)	21 (54)	26 (100)	24 (100)
South Asian	200 (25)	18 (30)	18 (46)	0	0
Other	232 (29)				
Type 2 diabetes	0 (0)	61 (100)	39 (100)	0 (0)[a]	12 (50)
Body weight, kg	67.9 ± 14.3	93.3 ± 17.0	99.1 ± 20.1	87.1 ± 12.4	120.9 ± 12.6
Height, m	164 ± 7	171 ± 10	168 ± 9	181 ± 7	171 ± 10
BMI	25.3 ± 4.9	32.0 ± 4.8	35.3 ± 7.0	26.5 ± 3.6	41.4 ± 3.4
Fat mass, %	33.5 ± 7.4	33.5 ± 8.4	35.6 ± 8.6	25.0 ± 4.4	48.8 ± 5.6
HbA_{1c}, mmol/mol	33.3 ± 3.3	61.7 ± 15.3	67.2 ± 17.5	5.4 ± 4.4	43.2 ± 9.8
HbA_{1c}, %	5.2 ± 0.3	7.8 ± 1.4	8.3 ± 1.6	5.4 ± 0.4	6.1 ± 0.9
Fasting glucose, mmol/L	4.4 ± 0.4	10.8 ± 3.3	10.6 ± 3.1	5.4 ± 0.5	6.6 ± 1.9
C-peptide, pmol/L	516 (401-666)	1019 (766-1461)	974 (624-1369)	646 (555-944)	1386 (991-1682)
Leptin, ng/mL	1.4 (0.8-2.2)	1.5 (0.9-2.3)	2.1 (1.3-3.7)	2.6 (1.8-3.5)	2.3 (1.8-3.5)
sOb-R, ng/mL	7.0 ± 2.0	6.2 ± 1.2	6.7 ± 1.9	5.0 ± 1.2	4.7 ± 1.2

Mean ± SD, n (%) or median (25th-75th percentile).
Abbreviations: BMI, body mass index; HbA_{1c}, glycated hemoglobin A_{1c}; sOb-R, soluble leptin receptor.
[a]13 dysglycemic, 13 normoglycemic men.

tissue disorders or other hormonal diseases (except T2D), kidney failure (glomerular filtration rate < 30), type 1 diabetes, BMI greater than 47, history of treatment with bone-active substances (bisphosphonates, denosumab, hormone replacement, or parathyroid hormone) or currently receiving anticoagulation or steroid treatment. T2D was defined as HbA_{1c} greater than 48 mmol/mol (6.5%) or use of oral glucose-lowering drug with or without insulin treatment.

HbA_{1c} was analyzed with HPLC (Tosoh G8, Tosoh Corp), fasting glucose with enzymatic photometry (Roche Diagnostics), and C-peptide with Modular E170 (ECLIA) (Roche Diagnostics). Fat mass was measured with dual-energy x-ray absorptiometry (DXA; GE Lunar Prodigy until August 26, 2016, thereafter GE Lunar iDXA [Lunar Corporation]). Body composition performed with GE Lunar Prodigy was re-analyzed with iDXA software to optimize comparability. sOb-R was quantified in biobanked serum from venous blood from 12 participants without, and 12 participants with T2D matched by BMI. Patients followed the same fasting regimen as described for DIVINE.

In Vitro Studies

Because *LEPR* is expressed in arteries and to some extent in muscle cells (www.gtexportal.org), we attempted to measure sOb-R levels in media of human umbilical vein endothelial cells (HUVECs) and myotubes. sOb-R was not detectable in conditioned media from HUVEC, and in myotubes all values were below the detection limit of the kit. Methods and results are presented in Supplementary Fig. S1 (26).

Ethics

All the studies were conducted in accordance with the Declaration of Helsinki and each of them was approved by the Regional Committee for Medical and Health Research Ethics in Norway. Written informed consent was obtained from all participants.

Statistics

Clinical studies

Data are presented as means ± SD if normally distributed and median (25th-75th percentile) if nonnormally distributed. Observations with missing values were omitted. For cross-sectional associations, data from the 5 clinical studies were pooled and analyzed with mixed-model regression using clinical study as random intercept. Intervention data were analyzed with mixed-model regression, using ID as random intercepts and time as a categorical variable. Differential effects in sOb-R response between the ethnic groups, the 2 different lunch meals or sex, were explored by adding interaction terms. Acute exercise data from pre and post 12-week exercise intervention were pooled and analyzed with mixed-model regression using ID as random intercepts. We tested for time × pre/post 12-week intervention and time × group interactions. Effect estimates from regressions are presented as β (95% CI). Analyses were performed using StataSE 16.

Mendelian Randomization

To explore causal relationships, we performed 2-sample Mendelian randomization analyses using the TwoSampleMR package (27) (https://github.com/MRCIEU/TwoSampleMR) in R version 4.1.3 (https://cran.r-project.org/) (28). sOb-R was quantified with an aptamer-based multiplex protein assay (SomaScan Assay, SomaLogic) (29). Since the GWAS of sOb-R was performed in Europeans, we obtained summary statistics from peer-reviewed, published GWAS of European ancestry with the largest sample size available in the TwoSampleMR database for the diabetes-

Table 3. Cross-sectional associations between phenotypes and serum soluble leptin receptor levels (ng/mL) (n = 973)

	Simple			Interaction analysis			
				Phenotype		Phenotype × T2D	
Phenotype	n	β [95% CI]	*P*	β (95% CI)	*P*	β (95% CI)	*P*
Fasting glucose, mmol/L	875	−0.02 (−0.12 to 0.08)	.69	−1.25 (−1.56 to −0.95)	< .001	1.37 (1.04 to 1.69)	< .001
HbA_{1c}, %	867	0.04 (−0.17 to 0.25)	.70	−1.44 (−1.89 to −0.99)	< .001	1.89 (1.37 to 2.41)	< .001
Fasting C-peptide, nmol/L	847	−2.04 (−2.46 to −1.62)	< .001	−2.64 (−3.14 to −2.14)	< .001	1.64 (0.74 to 2.53)	< .001
BMI	877	−0.19 (−0.21 to −0.17)	< .001	−0.21 (−0.24 to −0.19)	< .001	0.14 (0.08 to 0.20)	< .001
Body fat, %	868	−0.12 (−0.14 to −0.10)	< .001	−0.14 (−0.15 to −0.12)	< .001	0.11 (0.07 to 0.15)	< .001

Abbreviations: BMI, body mass index; HbA_{1c}, glycated hemoglobin A_{1c}; T2D, type 2 diabetes.

related phenotypes (Supplementary Table S1 [26]). We used unadjusted summary statistics only, as GWAS summary data adjusted for heritable covariates should be avoided in 2-sample Mendelian randomization because it may introduce bias (30). There were no sample overlaps between individuals in the GWAS of sOb-R (29) and the exposure phenotypes: fasting glucose and fasting insulin (31), percentage body fat (32), or BMI (33). We extracted genome-wide significant instruments ($P < 5e\text{-}08$) for the exposure phenotypes, removed single-nucleotide variations (formerly single-nucleotide polymorphisms) with linkage disequilibrium r^2 greater than 0.001, and excluded single-nucleotide variations that were palindromic with intermediate allele frequencies. Instrument strength in the 2-sample Mendelian randomization was calculated for each genetic instrument as (β/SE^2). Lists of independent genetic variants used as instruments for the exposures with effect size for both exposure and the outcome sOb-R are presented in Supplementary Tables S2 to S5 (26). Due to a lack of independent genetic instruments for sOb-R after pruning and harmonization, we were not able to test the effect of sOb-R on fasting glucose, fasting insulin, BMI, body fat, or T2D. Inverse variance weighted was our primary analysis, and *P* less than .05 was considered statistically significant. For sensitivity analysis, we performed MR-Egger (34), weighted median (35), and weighted mode (36), testing for similar and consistent direction of effects, as these tests have lower statistical power than inverse variance weighted. We tested for heterogeneity across the instrumental variables using the Cochrane *Q*, and for pleiotropy using Egger intercept.

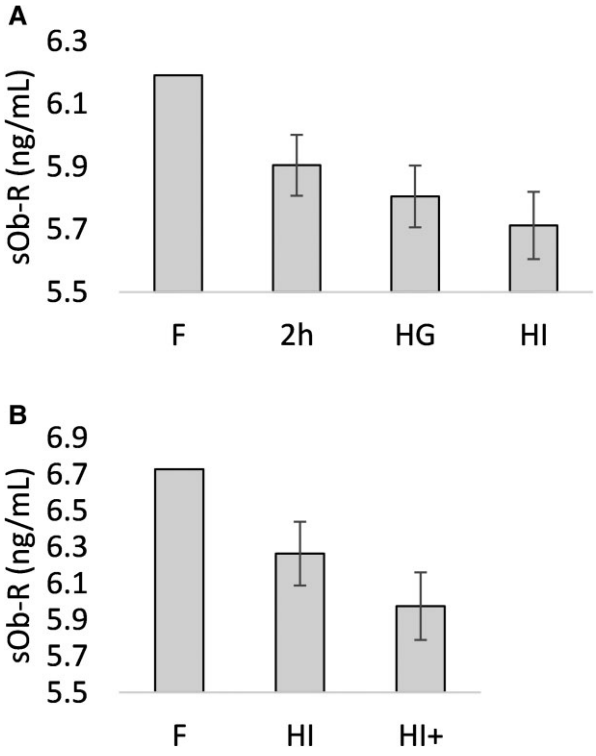

Figure 1. Serum soluble leptin receptor (sOb-R) during glucose clamp in 2 independent clinical studies: A, DIVINE (n = 61), and B, DIPI (n = 39). Data represent β and 95% CIs from mixed-model regression. A: F, fasted state; 2 hours, after 2 hours of tracer; HG, hyperglycemia (during intravenous glucose tolerance test, right before clamp); HI, hyperinsulinemia (end of clamp). B: F, fasted state; HI, physiological hyperinsulinemia (end of first step of clamp, 40 mU/m^2/min insulin infusion); HI+, supraphysiological hyperinsulinemia (end of second step of clamp, 400 mU/m^2/min insulin infusion).

We calculated genetic correlation between sOb-R and its causally related phenotypes with the Complex-Traits Genetics Virtual Lab (37) using full GWAS summary statistics for sOb-R (29), fasting insulin (31), and BMI (33). To test whether causal effects of correlated exposures were independent of each other, we performed multivariable Mendelian randomization using the MendelianRandomization package (38) for R. Genetic variants used as instruments in the multivariable Mendelian randomization are presented in Supplementary Table S6 (26). To test instrument strength and validity in the multivariable Mendelian randomization, we used equation 12 and 13, respectively, from Sanderson et al (39), modified using the intercept from the genetic correlation analysis of BMI and insulin to estimate sampling covariances as suggested by Wu et al 2021 (40) (detailed description in the Supplementary file [26]).

Results

The 5 cohorts differed in age, proportion of men and women, BMI, diabetes status, and ethnic origin (Table 2).

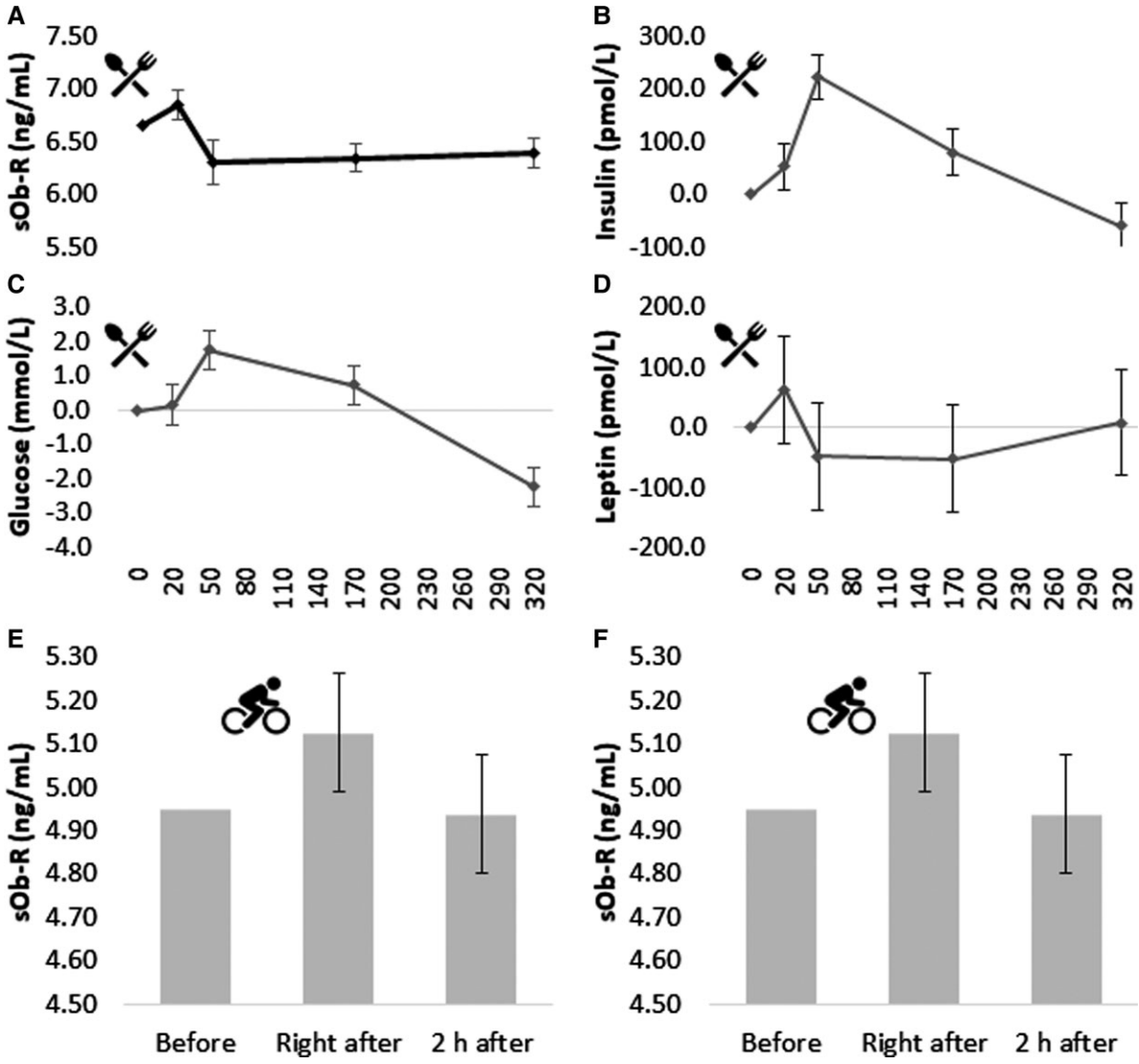

Figure 2. Changes during food intake in A, serum soluble leptin receptor (sOb-R); B, insulin; C, glucose; D, leptin (n = 39), and change in E, plasma sOb-r, and F, plasma leptin during acute intensive exercise (45 min 70% of maximal oxygen uptake) (n = 26). Data are β and 95% CI from mixed-model regression. Food and bicycle images: Flaticon.com.

Cross-Sectional Associations Between Soluble Leptin Receptor and Metabolic Variables

Fasting glucose and HbA_{1c} were not associated with sOb-R in simple regression models, but interaction analysis revealed an opposite association among individuals with and without T2D (Table 3). Fasting C-peptide, BMI, and body fat were all inversely associated with sOb-R, but with a weaker inverse association in participants with T2D (see Table 3).

Effect of Acute Hyperglycemia on Serum Soluble Leptin Receptor

Compared to the fasting state (6.2 ng/mL (reference) [5.9-6.5 ng/mL]), sOb-R decreased by −0.3 ng/mL (−0.4 to −0.2 ng/mL) ($P < .001$) after the 2-hour tracer equilibration period (Fig. 1A). In the hyperglycemic state, before insulin infusion, sOb-R decreased by −0.4 ng/mL (−0.5 to −0.3 ng/mL), but not significantly different from after the 2-hour tracer equilibration. In comparison, leptin levels decreased by −86 (−157 to −16) pg/mL ($P = .016$) in response to hyperglycemia. The response of sOb-R to hyperglycemia did not differ in insulin vs noninsulin users (P for interaction = .31), nor did the leptin response (P for interaction = .64). The response of both sOb-R and leptin levels to hyperglycemia was unchanged after adjustment for age, sex, ethnicity, and percentage of total body fat (n = 61).

Effect of Acute Hyperinsulinemia on Serum Soluble Leptin Receptor

In DIVINE, sOb-R decreased −0.5 ng/mL (−0.7 to −0.4 ng/mL) in the hyperinsulinemic euglycemic state compared to the fasting state (6.2 ng/mL, reference [5.9-6.5 ng/mL]) (see Fig. 1A). In comparison, plasma leptin concentration was unchanged in the hyperinsulinemic euglycemic state (2 [−69 to 72]; $P = .96$). sOb-R response to hyperinsulinemia did not differ in insulin vs noninsulin users (P for interaction = .67), nor did the leptin response (P for interaction = .12). The responses of both sOb-R and leptin levels to hyperinsulinemia were unchanged after adjustment for age, sex, ethnicity, and percentage of total body fat (n = 61).

Correspondingly, in DIPIs, Ob-R decreased in response to physiological hyperinsulinemia (−0.5 [−0.6 to −0.3]; $P <$ .001) and supraphysiological hyperinsulinemia (−0.8 [−0.9 to −0.6]; $P <$ .001), compared to the fasting state (6.7 ng/mL [6.2-7.3 ng/mL]) (Fig. 1B). In comparison, with fasting leptin as reference (2766 [2114-3418]), leptin concentration increased both during physiological hyperinsulinemia (127 [3-251] ($P =$ .045) and supraphysiological hyperinsulinemia (299 [168-431] ($P <$ .001). Response in sOb-R levels to both levels of hyperinsulinemia were unchanged after adjustment for age, sex, ethnicity, and percentage of total body fat. Of note, only 31 of 39 participants were included in the adjusted analysis because of missing covariates.

Due to individual variation in plasma insulin in response to insulin infusion, we explored the relationship between actual changes in plasma insulin and sOb-R from fasting to physiological and supraphysiological hyperinsulinemia (Supplementary Fig. S2 [26]). At physiological infusion of insulin, we observed a negative relationship between Δinsulin and ΔsOb-R levels (rho = −0.38; $P =$ .03), whereas the relationship leveled out at a supraphysiological insulin infusion (rho = 0.05; $P =$ .78) (Supplementary Fig. S2 [26]).

Effect of Food Intake on Serum Soluble Leptin Receptor

Serum sOb-R increased by 0.22 ng/mL (3%) from immediately before the meal to 20 minutes after food intake ($P <$ .0001), adjusted for sex, meal, age, and leptin (Fig. 2A). Thereafter, serum sOb-R decreased by 0.64 mg/mL (9%) from meal start to 50 minutes after food intake, leveling out at 2 hours 50 minutes and 5 hours 20 minutes (see Fig. 2A). In comparison, fasting insulin reached its highest concentration at 50 minutes after food intake (Fig. 2B), fasting glucose at 20 minutes (Fig. 2C), while plasma leptin did not change significantly in response to food intake (Fig. 2D). We observed a weak but statistically significant interaction between ethnic origin and time (Supplementary Fig. S3 [26]). Meal type ($P =$ .44) and sex ($P =$ .77) had no effect on sOb-R response and no interactions were present.

Effect of Acute Physical Activity on Plasma Soluble Leptin Receptor

Acute exercise increased sOb-R by 0.18 ng/mL (0.04-0.31 ng/mL) (4%) immediately after acute exercise ($P =$ .012) (Fig. 2E). In comparison, plasma leptin concentration did not change significantly in response to acute exercise (before to immediately after: −0.26 ng/mL [−0.91 to 0.38 ng/mL]; $P =$ .42; before to 2 hours after: 0.08 ng/mL [−0.55 to 0.73 ng/mL]; $P =$ .79) (Fig. 2F). The increase in sOb-R following acute exercise persisted after adjustment for leptin levels during the acute exercise test (0.17 [0.03-0.30]; $P =$ .017). We did not observe any differential response to acute exercise in dysglycemic vs control individuals (P for interaction = .99), or before and after the 12-week exercise intervention (P for interaction = .92).

Two-Sample Mendelian Randomization

Instrument strength was adequate for all phenotypes tested (median (25th-75th percentile) fasting glucose 68.2 (44.2-96.0), fasting insulin 45.5 (36.3-52.4), body fat percentage 33.8 (32.9-41.4), and BMI 51.0 (38.1-74.2) (see Supplementary Tables S2-S5 for F statistic for each genetic instrument [26]). We found evidence for a causal effect of fasting insulin and BMI decreasing sOb-R levels (Table 4). Sensitivity analyses indicated a consistent pattern with negative βs both for fasting insulin and BMI (see Table 4). Cochrane Q did not indicate heterogeneity for BMI, but possible heterogeneity for insulin (see Table 4). In leave-one-out analysis, all 14 genetic variants for insulin were significantly and inversely associated with sOb-R (β from −2.04 to −1.25; P from 0.0004 to 0.02; Supplementary Table S7 [26]). We could not detect horizontal pleiotropy for BMI (Egger intercept = −0.0008; $P =$ .81) or fasting insulin (Egger intercept = 0.05; $P =$ .72). Fasting glucose was not related to sOb-R, and the direction of effect varied across sensitivity analysis (see Table 4). Body fat percentage was not significantly related to sOb-R, although there was a consistent inverse association across sensitivity analyses.

sOb-R was not genetically correlated with fasting insulin (rG = −0.13; $P =$ .51) or BMI (rG = 0.09; $P =$ .35). Fasting insulin and BMI were genetically correlated (rG = 0.22, $P = 6.96 \times 10^{-7}$). In the multivariable Mendelian randomization, instrument strength (including 209 genetic variants) was adequate both for fasting insulin ($Q = 1170$; $P =$ 9.8e-134) and BMI ($Q = 17\,833$; $P = 0$), and the instrument was valid ($Q = 221$; $P = 0$). The causal effect of BMI on sOb-R levels was unchanged after adjustment for insulin, but no longer statistically significant (−0.21 [−0.43 to 0.01]; $P =$ .058). The causal effect of insulin on sOb-R levels reduced slightly after adjustment for BMI, but remained significant (−1.60 [−2.31 to −0.88]; $P <$ .001).

We tested for consistency of results using summary data for fasting insulin (ebi-a-GCST90002238 (41), n = 151 013), despite being adjusted for BMI. This increased the numbers of instruments from 14 to 38, and presented results similar to our results from the multivariable Mendelian randomization adjusted for BMI (IVW −0.76, SE = 0.35; $P =$ .03, with similar negative effect sizes across sensitivity analyses).

Discussion

This study combined cross-sectional, interventional and Mendelian randomization study designs to explore how sOb-R levels respond to glucose, insulin, body fat, physical activity, and food intake. Across all study designs, higher insulin consistently resulted in lower serum sOb-R. Fat percentage was inversely associated with sOb-R in cross-sectional studies, in line with the inverse causal effect of BMI on sOb-R observed in Mendelian randomization. Fasting glucose was not causally related to sOb-R in Mendelian randomization analysis, in line with inconsistent findings from clinical studies. Serum sOb-R increased immediately after intense physical activity and immediately after ingestion of a meal.

Insulin decreased serum sOb-R consistently across the study designs. Correspondingly, metformin—an oral glucose-lowering drug known to improve insulin sensitivity, thereby requiring lower insulin secretion—increased hepatic *Lepr* expression in mice in a dose-dependent manner, and increased sOb-R levels both in mice and humans with T2D (42). Similarly, incubating hepatic cells (HEK293) with insulin inhibited leptin signaling as measured by reduced JAK-2 phosphorylation (43). Furthermore, serum sOb-R reached its nadir simultaneously with the insulin zenith after food intake, although this occurrence could be related to other metabolic changes during food intake.

Table 4. Results from 2-sample Mendelian randomization of metabolic exposures on outcome soluble leptin receptor (ng/mL) (n = 3301)

			IVW					MR Egger			Weighted median			Weighted mode		
Exposure	n[a]	SNVs[b]	b	SE	*P*	*Q*	P_Q	b	SE	*P*	b	SE	*P*	b	SE	*P*
Fasting glucose, mmol/L	133 010	30	−0.33	0.25	.18	46	.02	0.00	0.53	.998	−0.07	0.29	.81	−0.05	0.31	.88
Fasting insulin, log pmol/L	108 557	14	−1.72	0.58	.003	21	.07	−2.89	3.24	.39	−2.14	0.72	.003	−2.49	1.65	.16
Body fat, %	65 831	10	−0.42	0.24	.08	5	.86	−0.69	1.11	.55	−0.38	0.32	.23	−0.31	0.39	.45
Body mass index (SD)	681 275	490	−0.20	0.08	.01	517	.18	−0.15	0.21	.48	−0.28	0.13	.02	−0.43	0.23	.06

Abbreviations: IVW, instrumental variance weighted; Q, Cochrane *Q* testing for heterogeneity; SNV, single-nucleotide variation; T2D, type 2 diabetes.
[a]Sample size for genome-wide association study of exposure.
[b]Numbers of SNVs available as instrumental variables after harmonization (common for exposure and outcome).

sOb-R increased immediately after the acute exercise, and returned to the starting point 2 hours after the intensive exercise test. Correspondingly, a study from Taiwan showed that sOb-R levels were unchanged 24 and 48 hours after acute exercise (44). Thus, physical activity does not seem to affect sOb-R levels in the long term, whereas acute exercise may increase sOb-R levels. The acute effects of food intake and intense exercise may suggest that sOb-R is involved in short-term regulation of leptin signaling.

Obesity and insulin resistance are tightly linked, resulting in hyperinsulinemia to compensate for the increased insulin need. Only insulin remained significantly associated independent of BMI, while BMI was no longer statistically significant independent of insulin. Several studies have indicated that obesity leads to leptin resistance (9) coexisting with insulin resistance (45), and low concentrations of serum sOb-R may reflect this leptin resistance. Our findings therefore suggest that hyperinsulinemia reduces serum sOb-R levels, which in turn may reduce leptin signaling since sOb-R seem to prolong leptin half-life and reflect the number of Ob-R available at the cell membrane. However, to understand the mechanisms and their clinical implications, future research should explore whether decreased serum sOb-R is due to, for example, reduced shedding of the extracellular domain, or reduction of leptin receptors at the cell membrane due to reduced production, active removal, or other regulatory mechanisms of Ob-R.

A major strength of this study is the integration of results from several epidemiological study designs with different limitations and key sources of bias, to test consistencies in how plasma or serum sOb-R is influenced by glucose, insulin, body fat and BMI, food intake, and physical activity. Altogether, this epidemiological triangulation, and combination of several independent clinical studies, suggested fulfillment of several of Hill's criteria for causation (46), to provide more solid evidence of causality. Our results should have a high level of generalizability, since our findings were consistent across a range of different populations. Serum or plasma sOb-R was measured with the same method across all clinical studies. In the GWAS of sOb-R, however, an aptamer-based multiplex protein assay was used to quantify the serum concentration. Limited sample sizes for some of the GWAS, resulting in limited statistical power and few (insulin and body fat) or no genetic variants for exposures (sOb-R), may hamper definitive conclusions, and should be repeated when larger GWAS of sOb-R become available. Furthermore, the lack of independent genetic instruments for sOb-R made bidirectional Mendelian randomization impossible; thus we cannot currently test whether sOb-R causally increase risk of T2D. The interpretation of our findings is further limited by not knowing the exact role of plasma sOb-R in leptin signaling, although accumulating evidence suggests that they reflect the number of membrane-bound Ob-R.

To conclude, insulin and BMI both seem to causally decrease serum sOb-R, whereas physical activity and food intake acutely seem to increase sOb-R. Our results suggest that sOb-R may be involved in the short-term regulation of leptin signaling, either directly or indirectly. Altogether, existing evidence suggest that the association between sOb-R and future gestational or T2D could be caused by already persisting hyperinsulinemia lowering sOb-R, rather than a protective effect of sOb-R itself. Further research is necessary to conclude whether serum sOb-R is directly involved in the detrimental effects of obesity, insulin resistance, and hyperinsulinemia, or merely a marker of these conditions.

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Disclosures

The authors have nothing to disclose.

Data Availability

The clinical data sets analyzed during the present study are not publicly available but are available from the corresponding author on reasonable request. GWAS summary data used in this study can be accessed via https://gwas.mrcieu.ac.uk/.

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