



Glucose-Dependent Insulinotropic Peptide in the High-Normal Range Is Associated With Increased Carotid Intima-Media Thickness

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OBJECTIVE

While existing evidence supports beneficial cardiovascular effects of glucagon-like peptide 1 (GLP-1), emerging studies suggest that glucose-dependent insulinotropic peptide (GIP) and/or signaling via the GIP receptor may have untoward cardiovascular effects. Indeed, recent studies show that fasting physiological GIP levels are associated with total mortality and cardiovascular mortality, and it was suggested that GIP plays a role in pathogenesis of coronary artery disease. We investigated the associations between fasting and postchallenge GIP and GLP-1 concentrations and subclinical atherosclerosis as measured by mean intima-media thickness in the common carotid artery (IMT_{mean}CCA) and maximal intima-media thickness in the carotid bifurcation (IMT_{max}Bulb).

RESEARCH DESIGN AND METHODS

Participants at reexamination within the Malmö Diet and Cancer–Cardiovascular Cohort study ($n = 3,734$, mean age 72.5 years, 59.3% women, 10.8% subjects with diabetes, fasting GIP available for 3,342 subjects, fasting GLP-1 available for 3,299 subjects) underwent oral glucose tolerance testing and carotid ultrasound.

RESULTS

In linear regression analyses, each 1-SD increment of fasting GIP was associated with increased (per mm) IMT_{mean}CCA ($\beta = 0.010$, $P = 0.010$) and IMT_{max}Bulb ($\beta = 0.014$; $P = 0.040$) in models adjusted for known risk factors and glucose metabolism. In contrast, each 1-SD increment of fasting GLP-1 was associated with decreased IMT_{max}Bulb (per mm, $\beta = -0.016$, $P = 0.014$). These associations remained significant when subjects with diabetes were excluded from analyses.

CONCLUSIONS

In a Swedish elderly population, physiologically elevated levels of fasting GIP are associated with increased IMT_{mean}CCA, while GLP-1 is associated with decreased IMT_{max}Bulb, further emphasizing diverging cardiovascular effects of these two incretin hormones.

Incretins are intestinal hormones that potentiate glucose-dependent insulin response following nutrient intake, with subsequent blood glucose-lowering effects. Most of the incretin effect is accounted for by glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), considered regulators of nutrient absorption, appetite, islet function, and energy homeostasis (1). GIP has been demonstrated to play an important role in lipid metabolism (2), and GLP-1 has been demonstrated to have receptor-independent cardioprotective effects in GLP-1 receptor knockout mice (3).

Both experimental and clinical data from studies such as Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results (LEADER), Trial to Evaluate Cardiovascular and Other Long-term Outcomes With Semaglutide in Subjects With Type 2 Diabetes (SUSTAIN-6), HARMONY (A Long Term, Randomized, Double-blind, Placebo-Controlled Study to Determine the Effect of Albiglutide, When Added to Standard Blood Glucose Lowering Therapies, on Major Cardiovascular Events in Patients With Type 2 Diabetes Mellitus), and Researching Cardiovascular Events With a Weekly Incretin in Diabetes (REWIND) support therapeutic benefits of GLP-1 receptor agonists with regard to cardiovascular outcomes in type 2 diabetes (4). Further, a missense variant in the gene encoding GLP-1 receptor has been associated with protection against coronary heart disease (5). The data regarding GIP's involvement in cardiovascular disease (CVD) are conflicting. Emerging evidence indicates that GIP, or direct stimulation of the GIP receptor, can have negative cardiovascular effects. On the other hand, a recent systematic review demonstrated that GIP can exhibit both antiatherogenic and proatherogenic properties *in vitro* (6).

We previously demonstrated that fasting GIP concentrations are significantly higher in individuals with a history of CVD compared with control subjects and that GIP receptor gene (*GIPR*) mRNA expression is higher in the arterial wall of patients with symptoms of CVD (7). Moreover, a common variant in *GIPR* has been associated with increased risk of stroke in patients with type 2 diabetes. Ussher et al. (8) demonstrated that a genetic elimination of *GIPR* improves outcome (improved survival and reduced adverse cardiac remodeling)

following experimental myocardial infarction in mice. Recent data from our group demonstrated that elevated levels of GIP were associated with greater risk of all-cause and cardiovascular mortality within 5–9 years of follow-up, whereas GLP-1 levels were not associated with excess risk in two prospective, community-based studies. Furthermore, in the same study, Mendelian randomization analyses using CARDIoGRAM-plusC4D (Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics) and UK Biobank data suggested a causal involvement of GIP in coronary artery disease and myocardial infarction (9). A suggested mediator of cardiovascular detrimental effects of GIP is osteopontin (OPN), since GIP stimulation increases OPN expression in mouse arteries. Further, individuals with symptomatic CVD have been shown to have higher plaque expression of GIPR and OPN (7). High plasma levels of OPN have been associated with the presence and extent of coronary artery disease in numerous studies (10). However, although emerging data implicate GIP in inducing atherosclerosis, to date, no studies have been performed that examine the association of plasma levels of GIP and GLP-1 with measurements of subclinical atherosclerosis in large human population cohorts.

Therefore, we here explored whether circulating levels of GIP and GLP-1 are associated with subclinical atherosclerosis as measured by intima-media thickness (IMT) in the common carotid artery (CCA) and in the carotid bifurcation. We hypothesized that GIP, but not GLP-1, levels in the high-normal range are associated with increased degree of subclinical atherosclerosis.

RESEARCH DESIGN AND METHODS

Ethics Statement

The study was approved by the Research Ethical Review Board at Lund University. Written informed consent was obtained from all subjects prior to commencement of the study.

Subjects

Between 1991 and 1996, baseline examinations including anthropometrical measurements and blood sample donations were performed within the Malmö Diet

and Cancer (MDC) study, a prospective population-based study ($n = 30,447$) in the city of Malmö, Sweden. In order to study cardiovascular risk factors, a subsample of the study population ($n = 6,103$) was randomized into a substudy, the Malmö Diet and Cancer–Cardiovascular Cohort (MDC-CC). During 2007–2012, a new clinical reexamination including blood sampling and carotid ultrasound was performed within the MDC-CC cohort, with addition of oral glucose tolerance test (OGTT) in 3,734 subjects, and this is the subset used for analyses. For analyses of associations between fasting GIP and mean IMT in the CCA ($IMT_{mean,CCA}$) and maximal IMT in the carotid bifurcation ($IMT_{max,Bulb}$), complete data on all covariates were available in 3,342 and 3,229 subjects, respectively. For analyses of associations between postchallenge GIP and $IMT_{mean,CCA}/IMT_{max,Bulb}$, complete data were available for 2,948 and 2,856 subjects, respectively. As for analyses of associations between fasting GLP-1 and $IMT_{mean,CCA}/IMT_{max,Bulb}$, complete data were available in 3,299 and 3,187 subjects, respectively. A total of 2,893 and 2,828 subjects had complete data for analyses of associations between postchallenge GLP-1 and $IMT_{mean,CCA}/IMT_{max,Bulb}$, respectively. A complete description of the study population has previously been published (11). Statistical analyses in this study have been carried out retrospectively.

Clinical Assessment

Clinical assessment included anthropological measurements and blood samples drawn after overnight fast. BMI was calculated as weight in kilograms divided by the square of height in meters. Diabetes was identified through a self-reported physician's diagnosis of diabetes, use of antidiabetes medications, or OGTT (fasting plasma glucose [FPG] ≥ 7.0 mmol/L or ≥ 12.2 mmol/L following OGTT). Antihypertensive treatment (AHT) was defined as use of β -receptor blockers or ACE inhibitors, calcium antagonists, or diuretics. Lipid-lowering treatment was defined as use of statins, fibrates, or other lipid-lowering medication. Smoking was self-reported and defined as present smoker/no smoker. Blood pressure was obtained after 10 min of rest in the supine position.

OGTT

A standardized 75-g OGTT (12) was performed after an overnight fast (individuals with known diabetes did not undergo the OGTT and subsequently did not have postchallenge blood sampling).

Laboratory Assays

During OGTT, blood samples were drawn for analysis of GIP and GLP-1 at 0 and 120 min between 2007 and 2012. The samples for GIP analyses were collected in serum tubes with a clot activator and serum gel separator and stored at -20°C until analyses (between 9 November 2011 and 7 July 2012). The samples for GLP-1 were collected in tubes with addition of a DPP-4 inhibitor (Diprotin A) and stored at -80°C until analyses (between 8 January 2014 and 15 May 2014). Total plasma GLP-1 concentrations (intact GLP-1 and the metabolite GLP-1 9-36 amide) were determined radioimmunologically as previously described (minimum detection limit 1 pmol/L, intra- and interassay coefficients of variation $<6.0\%$ and $<15\%$, respectively). Identical quality controls and identical batches for all reagents in each analysis set were used in a consecutive sample analysis during 2 months. Serum GIP was analyzed with use of Millipore's Human GIP Total ELISA (cat. no. EZHGIP-54 K) (total, minimum detection level 1.65 pmol/L, intra- and interassay coefficients of variation 1.8–6.1% and 3–8.8%, respectively). FPG was analyzed after an overnight fast with the HemoCue Glucose System (HemoCue AB, Ängelholm, Sweden). Serum insulin was assayed with Dako ELISA kit (minimum detection level 3 pmol/L, intra- and interassay coefficients of variation 5.1–7.5% and 4.2–9.3%, respectively) at the Department of Clinical Chemistry, Skane University Hospital, Malmö. Insulin resistance was estimated by HOMA of insulin resistance (HOMA-IR) (13). HDL cholesterol (HDL) and triglycerides were measured according to standard procedures at the Department of Clinical Chemistry, Skane University Hospital, Malmö, which is attached to a national standardization and quality control system.

Ultrasound

The ultrasound examinations of the right carotid artery were assessed with B-mode ultrasound using an ACUSON Sequoia (Acuson, Mountain View, CA) with a 7-MHz transducer. Three images were saved in a

predefined window, consisting of 3 cm of the distal part of CCA, the bifurcation area, and 1 cm of proximal internal and external carotid artery, respectively. Measurements of $\text{IMT}_{\text{mean}}\text{CCA}$ and $\text{IMT}_{\text{max}}\text{Bulb}$ were performed off-line with the Artery Measurement System (AMS) (14). A mean of three measurements was calculated. Complete description of the ultrasound examination and the measurement procedures is available elsewhere (11).

Statistical Analysis

Where needed, logarithmic transformations were used to normalize the distribution of variables prior to analysis (pre- and postchallenge GIP, pre- and postchallenge GLP-1, pre- and postchallenge insulin, pre-FPG and postchallenge plasma glucose, HOMA-IR, triglycerides, and HDL). Fasting and postchallenge GIP and GLP-1 were further z transformed to facilitate comparisons between variables and their results. Between-group comparisons were carried out with use of one-way ANOVA for continuous variables, and χ^2 tests for binary variables. All linear regression analyses of incretin associations with $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were carried out in three steps: 1) Unadjusted. 2) According to model 1 (age and sex adjusted). 3) Associations between fasting incretins and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were further adjusted for clinically relevant covariates age, sex, BMI, systolic blood pressure (SBP), smoking status, diabetes status, FPG, fasting insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 2a). Analyses of postchallenge incretin associations with $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were further adjusted for age, sex, BMI, SBP, smoking status, diabetes status, postchallenge plasma glucose, postchallenge insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 2b). In the next set of analyses, subjects with diabetes were excluded, and linear regression analyses were carried out for associations between incretins and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ in unadjusted models and adjusted for age and sex (model 1). Linear regression models for associations between fasting incretins and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were thereafter adjusted for age, sex, BMI, SBP, smoking status, HOMA-IR, FPG, fasting insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 3a). Associations between

postchallenge incretins and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were adjusted for age, sex, BMI, SBP, smoking status, HOMA-IR, postchallenge plasma glucose, postchallenge insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 3b).

For exploration of whether relationships between GIP and GLP-1 and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ were linear, GIP and GLP-1 levels were divided into quartiles and were related to $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$ after model 2a and model 2b adjustment using linear regression with quartiles of GIP and GLP-1 as fixed factors.

All analyses were performed in SPSS, Windows, 25.0 (SPSS, Chicago, IL). A two-tailed P value <0.05 was considered statistically significant.

Data and Resource Availability

The data that support the findings of this study are available upon request from Steering Committee of MDC study by contacting its chair, Olle Melander (olle.melander@med.lu.se), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available due to ethical and legal restrictions related to the Swedish Biobanks in Medical Care Act (2002:297) and the Personal Data Act (1998:204).

RESULTS

Baseline characteristics of the study population are listed in Table 1. The population was elderly (mean \pm SD age 72.5 ± 5.6 years), 59.4% were females, and 10.7% either had a previous diabetes diagnosis or had OGTT results consistent with diabetes during the study. In comparisons across quartiles of GIP, differences in all characteristics except for smoking status and sex proportions were observed. No multicollinearity issues were observed, with all variance inflation factors <1.7 .

Associations Between GIP/GLP-1 and $\text{IMT}_{\text{mean}}\text{CCA}/\text{IMT}_{\text{max}}\text{Bulb}$

In linear regression analyses, each 1-SD increment of fasting GIP was significantly associated with increased $\text{IMT}_{\text{mean}}\text{CCA}$ (per mm, $\text{IMT}_{\text{mean}}\text{CCA}$ $\beta = 0.010$, $P = 0.010$) and $\text{IMT}_{\text{max}}\text{Bulb}$ (per mm, $\text{IMT}_{\text{max}}\text{Bulb}$ $\beta = 0.014$, $P = 0.040$) in model 2a (Table 2). Further, each 1-SD increment of fasting GLP-1 was significantly associated with

Table 1—Baseline characteristics of the study population within quartiles of fasting GIP

	Total	Q1	Q2	Q3	Q4	P
<i>n</i>	3,342	832	846	834	830	
Demographics						
Age (years)	72.5 ± 5.6	71.6 ± 5.5	72.5 ± 5.5	72.8 ± 5.6	72.8 ± 5.7	<0.001
Female sex, <i>n</i> (%)	1,985 (59.4)	506 (60.2)	498 (58.5)	489 (57.9)	511 (60.5)	0.620
BMI (kg/m ²)	26.9 ± 4.4	26.4 ± 4.0	26.7 ± 4.0	26.7 ± 4.4	27.8 ± 5.0	<0.001
Laboratory						
GIP (pmol/L) ^f	41.0 (30.4–56.5)	24.3 (20.2–27.4)	35.7 (33.1–38.1)	47.5 (44.2–51.4)	80.0 (63.2–89.4)	<0.001
GLP-1 (pmol/L) ^f	8 (6–10)	7 (6–9)	8 (6–9)	8 (6–10)	8 (6–10)	<0.001
Insulin (pmol/L) ^f	53.5 (37.5–76.4)	47.2 (34.7–66.0)	50.7 (35.4–72.9)	54.2 (38.9–76.4)	65.3 (44.4–93.1)	<0.001
Glucose (mmol/L) ^f	5.9 (5.4–6.5)	5.8 (5.3–6.3)	5.8 (5.4–6.4)	5.9 (5.4–6.4)	6.1 (5.5–6.8)	<0.001
HDL (mmol/L)	1.4 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.001
Triglycerides (mmol/L)	1.0 (0.7–1.3)	0.9 (0.7–1.2)	1.0 (0.7–1.3)	1.0 (0.7–1.3)	1.1 (0.8–1.4)	<0.001
HOMA-IR	2.0 (1.4–3.1)	1.7 (1.2–2.6)	1.9 (1.3–2.8)	2.0 (1.4–3.0)	2.6 (1.7–3.9)	<0.001
Postchallenge values*						
GIP (pmol/L)	222.8(163.5–293.6)	178.5(107.6–270.0)	193.7(129.8–287.5)	208.0(147.3–356.3)	222.9(163.8–293.7)	<0.001
GLP-1 (pmol/L)	16 (12–21)	16 (12–20)	15 (12–20)	16 (12–21)	16 (13–21)	0.463
Insulin (pmol/L)	39.8 (25.8–63.3)	37.6 (24.2–64.8)	40.0 (25.5–63.7)	41.1 (26.4–63.4)	40.3 (26.8–62.8)	0.686
Glucose (mmol/L)	6.8 (5.6–8.2)	6.7 (5.4–8.2)	6.8 (5.6–8.2)	6.8 (5.4–8.1)	6.8 (5.6–8.4)	0.297
Clinical profile						
SBP (mmHg)	139 ± 18	137 ± 17	138 ± 18	139 ± 18	141 ± 19	<0.001
AHT, <i>n</i> (%)	1,684 (50.4)	370 (44.0)	406 (47.7)	438 (51.8)	498 (59.0)	<0.001
Lipid-lowering drugs, <i>n</i> (%)	1,007 (30.1)	226 (26.9)	219 (25.7)	270 (32.0)	309 (36.6)	<0.001
Smoker, <i>n</i> (%)	240 (7.2)	55 (6.5)	53 (6.2)	56 (6.6)	78 (9.2)	0.059
Diabetes, <i>n</i> (%)	356 (10.7)	46 (5.5)	61 (7.2)	88 (10.4)	170 (20.1)	<0.001
IMT _{mean} CCA (mm)	0.889(0.783–1.021)	0.870(0.775–1.001)	0.888(0.780–1.024)	0.893(0.788–1.022)	0.903(0.789–1.049)	0.002
IMT _{max} Bulb (mm)	1.665(1.303–2.274)	1.597(1.256–2.139)	1.662(1.303–2.278)	1.644(1.338–2.210)	1.774(1.316–2.415)	0.001

Data are means ± SD or median (interquartile range) unless otherwise indicated. ^fFasting. *Postchallenge values (2 h postchallenge [at 120 min]) in subjects without diabetes only.

decreased IMT_{max}Bulb (per mm, IMT_{max}Bulb β = -0.016, P = 0.014) but not with IMT_{mean}CCA (per 1-SD change β = -0.003, P = 0.142) in model 2a

(Table 2). Complete data on all variables included in analyses in all models are available in Supplementary Tables 1 and 2.

Table 2—Associations of fasting and postchallenge GIP and IMT_{mean}CCA/IMT_{max}Bulb

		IMT _{mean} CCA		IMT _{max} Bulb					
		Fasting GIP, <i>n</i> = 3,342		Postchallenge GIP, <i>n</i> = 2,948		Fasting GIP, <i>n</i> = 3,229		Postchallenge GIP, <i>n</i> = 2,856	
		β	P	β	P	β	P	β	P
		0.016	3.0 × 10 ⁻⁵ *	0.010	0.012*	0.031	7.0 × 10 ⁻⁵ *	0.011	0.140*
		0.012	0.002†	0.005	0.171†	0.024	2.8 × 10 ⁻⁴ †	0.010	0.162†
		0.010	0.010‡	0.005	0.188§	0.014	0.040‡	0.003	0.668§
		IMT _{mean} CCA		IMT _{max} Bulb					
		Fasting GLP-1, <i>n</i> = 3,299		Postchallenge GLP-1, <i>n</i> = 2,893		Fasting GLP-1, <i>n</i> = 3,187		Postchallenge GLP-1, <i>n</i> = 2,828	
		β	P	β	P	β	P	β	P
		-0.005	0.244*	-0.007	0.097*	-0.005	0.244*	-0.007	0.097*
		-0.005	0.174†	-0.010	0.008†	-0.005	0.174†	-0.010	0.008†
		-0.003	0.426‡	-0.006	0.142§	-0.003	0.426‡	-0.006	0.142§

Data are unstandardized β-coefficients (1-SD increase of incretins per mm IMT_{mean}CCA or IMT_{max}Bulb) unless otherwise indicated. *No adjustment. †Adjustment for age and sex. ‡Adjustment for age, sex, BMI, SBP, smoking status, diabetes status, FPG, fasting insulin, HDL, triglycerides, AHT, and lipid-lowering treatment. §Adjustment for age, sex, BMI, SBP, smoking status, diabetes status, postchallenge plasma glucose, postchallenge insulin, HDL, triglycerides, AHT, and lipid-lowering treatment.

For examination of whether the relationship between fasting GIP and IMT_{mean}CCA was equal across the entire distribution of fasting GIP, analyses of quartiles were performed. The linear trend with increased IMT_{mean}CCA across quartiles of fasting GIP was significant, and most of the increment in effect size was seen for subjects in quartile 4 (Q4) (highest concentrations of fasting GIP) compared with all other subjects (Q1–Q3) in model 2a (Table 3) and compared with subjects in Q1 (Q1 β = -0.091, Q4 β = 0.020) in model 2b, P_{trend} = 0.021.

Further, the highest concentrations of postchallenge GLP-1 (Q4) were inversely associated with IMT_{max}Bulb compared with subjects in all other quartiles (Q1–Q3) and compared with subjects in Q1 (Q1 β = 0.025, Q4 β = -0.020) (Table 3), P_{trend} = 0.042.

Associations Between GIP/GLP-1 and IMT_{mean}CCA/IMT_{max}Bulb in Subjects Free From Diabetes

The association between fasting GIP and IMT_{mean}CCA remained significant when subjects with diabetes (*n* = 365) were excluded from analyses and analyses were adjusted according to model 3a

Table 3—Multivariable analysis of the relation between quartiles of GIP/GLP-1 and $IMT_{mean}^{CCA}/IMT_{max}^{Bulb}$

	Quartiles of fasting GIP		Quartiles of postchallenge GIP*	
	β (SE)	<i>P</i>	β (SE)	<i>P</i>
Associations between quartiles of GIP and IMT_{mean}^{CCA}				
Q1	Referent	—	Referent	—
Q2	0.003 (0.010)	0.758	0.020 (0.011)	0.059
Q3	0.013 (0.011)	0.233	0.012 (0.011)	0.260
Q4	0.023 (0.011)	0.032	0.015 (0.011)	0.180
<i>P</i> _{trend}	0.008 (0.003)	0.021	0.004 (0.004)	0.305
Associations between quartiles of GIP and IMT_{max}^{Bulb}				
Q1	Referent	—	Referent	—
Q2	0.023 (0.018)	0.202	−0.010 (0.019)	0.599
Q3	0.016 (0.018)	0.388	−0.016 (0.020)	0.412
Q4	0.035 (0.019)	0.065	−0.008 (0.020)	0.683
<i>P</i> _{trend}	0.010 (0.006)	0.105	0.002 (0.006)	0.761
Associations between quartiles of GLP-1 and IMT_{mean}^{CCA}				
Q1	Referent	—	Referent	—
Q2	0.002 (0.011)	0.828	−0.002 (0.011)	0.878
Q3	−0.001 (0.011)	0.898	−0.005 (0.011)	0.639
Q4	−0.007 (0.011)	0.541	−0.018 (0.011)	0.100
<i>P</i> _{trend}	−0.003 (0.004)	0.450	−0.006 (0.006)	0.108
Associations between quartiles of GLP-1 and IMT_{max}^{Bulb}				
Q1	Referent	—	Referent	—
Q2	−0.031 (0.019)	0.115	−0.016 (0.020)	0.430
Q3	−0.032 (0.019)	0.103	−0.025 (0.019)	0.200
Q4	−0.038 (0.019)	0.047	−0.041 (0.020)	0.044
<i>P</i> _{trend}	−0.007 (0.006)	0.222	−0.013 (0.006)	0.042

Data are unstandardized β -coefficients (1-SD increase of incretins per mm IMT_{mean}^{CCA} or IMT_{max}^{Bulb}) (β) and SEs. Q1, quartile with lowest values; Q4, quartile with highest values. Analyses of fasting incretins are adjusted for age, sex, BMI, SBP, smoking status, diabetes status, FPG, fasting insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 2a). Analyses between postchallenge GIP and IMT_{mean}^{CCA} are adjusted for age, sex, BMI, SBP, smoking status, diabetes status, postchallenge plasma glucose, postchallenge insulin, HDL, triglycerides, AHT, and lipid-lowering treatment (model 2b). *2 h postchallenge (at 120 min).

($\beta = 0.008, P = 0.033$) (Supplementary Table 3). Likewise, when subjects with diabetes were excluded from analyses and analyses were adjusted according to model 3a, the association between fasting GLP-1 and IMT_{max}^{Bulb} remained significant ($\beta = -0.017, P = 0.016$) (Supplementary Table 4). When analyses were carried out in subjects with diabetes, no significant associations were seen (Supplementary Table 5).

Further, to enable comparison between effect sizes, we z transformed all continuous variables. The effect size of each 1-SD increment of fasting GIP ($\beta = 0.044$) was higher than that of each one 1-SD increment of BMI ($\beta = 0.025$) but lower than that of each 1-SD increment of SBP ($\beta = 0.175$) and each 1-SD increment of age ($\beta = 0.197$) (Supplementary Table 6).

CONCLUSIONS

The key finding of this study is that fasting GIP levels are associated with greater subclinical atherosclerosis as measured by IMT_{mean}^{CCA} and IMT_{max}^{Bulb} , whereas fasting GLP-1 levels are associated with less subclinical atherosclerosis as measured by IMT_{max}^{Bulb} .

The results differ for fasting and postchallenge concentrations of GIP and GLP-1. The associations were seen for fasting GIP and increased IMT at both sites ($IMT_{mean}^{CCA}/IMT_{max}^{Bulb}$); for GLP-1, postchallenge concentrations were associated with decreased IMT in the carotid bifurcation. These findings might be explained by the notion that each of these segments has distinct associations with cardiovascular risk factors, due to their differing geometry resulting in different shear stress rates, which in turn result in diverse cellular constituents of the atherosclerotic process (dominance of cholesterol-rich plaques in the carotid bifurcation versus the dominance of foam cell lesions in the CCA) (15,16). Further, both fasting and postchallenge GIP and GLP-1 are highly familial traits (17), and both basal secretion and GLP-1 response to oral glucose challenge are reduced in prediabetes, type 2 diabetes, and obesity, possibly explaining higher GLP-1 concentrations' associations with lower IMT_{max}^{Bulb} (18). However, our analyses were adjusted for diabetes status.

GIP secretion is near normal in diabetes, but its effect on insulin secretion is impaired. On the other hand, GLP-1 secretion is impaired in subjects with diabetes, but the effect on insulin secretion is preserved (19). Thus, we carried out analyses after exclusion of subjects with diabetes further adjusted for HOMA-IR, with the results essentially unchanged, suggesting an association independent of insulin resistance.

Several randomized controlled trials have demonstrated that GLP-1 analog therapy is beneficial with regard to cardiovascular outcomes (4). On the contrary, the question of whether GIP/GIPR might have untoward effects on cardiovascular biology is raised, given the results from other studies. Nitz et al. (20) demonstrated that a genetic variant in the GIPR gene (rs1800437) is associated with features of CVD and metabolic syndrome, and heritable fasting GIP concentrations have been associated with CVD and increased total and cardiovascular mortality risk (9). However, the role of GIP in CVD is not completely understood (21). A recent systematic review

portrays GIP as both pro- and antiatherogenic (6). Several observational studies reported correlations between GIP levels and severity or presence of atherosclerotic CVDs. In cell culture studies, GIP was reported to exert both anti- and pro-atherogenic effects on vascular endothelial cells (7,22). Antiatherogenic effects of GIP were reported in atherosclerosis animal models (22); however, inactivation of the GIPR improved outcomes in mice following experimental myocardial infarction (8). Further, pharmacological doses of GIP were shown to exert anti-inflammatory effects in adipose tissue, but physiological levels of GIP may promote adipose tissue inflammation (23,24). The observation of GIP's proinflammatory effects in animal models is consistent with findings in humans (7,25). The significant association between GIP and increased subclinical atherosclerosis as demonstrated here corresponds with the findings by Berglund et al. (7) showing that GIP stimulates osteopontin (OPN) expression in the vasculature via endothelin-1 and CREB. OPN has emerged as a biomarker in CVD (26), and it plays an important role in the development of medial thickening and neointimal formation in mice (27). Caesar et al. (28) observed that aortas in OPN knockout mice were protected against Ang II-induced medial hypertrophy and inflammation, despite comparable increases in SBP in both the knockout and wild-type mouse groups. In addition, data from our laboratory demonstrated that GIP increases OPN expression in β -cells in pancreas, with subsequent antiapoptotic and proliferative roles in the pancreatic tissue (29). Further, OPN expression is stimulated by GIP in adipocytes, which was associated with insulin resistance (30). OPN has been associated with the presence and extent of coronary artery disease in numerous studies (31), and OPN is a strong predictor of adverse outcomes in patients with peripheral artery disease (32), myocardial infarction (33), and stroke (34).

Ultrasound measurement of the carotid IMT is a marker for subclinical atherosclerosis and is associated with future cardiovascular events (35). Notably, carotid IMT has shown greater adjusted risk of stroke compared with the coronary artery calcium score (36). The thickening of the IMT in the CCA and the carotid bifurcation might be affected by different mechanisms. Due to the low

shear stress and high shear stress oscillations, the carotid bulb is prone to atherosclerosis development (37). On the other hand, the IMT thickening in the CCA is believed to mainly appear due to intimal thickening and smooth muscle hypertrophy, possibly caused by elevated blood pressure (38), which in turn might be facilitated by the positive association between GIP and systolic pressure as seen here.

Study Limitations

By studying a general, elderly population and adjusting for metabolic risk factors and diabetes, we believe that we illustrated that GIP and GLP-1 may have a role in, or reflect, atherosclerosis, beyond factors included in the metabolic syndrome and diabetes. By additional adjustment for smoking, AHT, and lipid-lowering treatment, all of those being factors that are involved in, or reflect, atherosclerosis, we tried to eliminate other possible confounding factors. However, since atherosclerosis is a multifactorial disease, caution should be taken in drawing conclusions about associations. One limitation of this study is the fact that only the right carotid artery was examined. Examining several vascular sites would obviously improve the accuracy of the measured IMT. Furthermore, all ultrasound examinations and subsequent measurements of IMT are vitiated by subjectivity, resulting in interobserver variability. Another limitation is that this study population consisted of mostly elderly subjects within a narrow age range (mean \pm SD 72.5 \pm 5.6 years). Further, we had no way of ascertaining overnight fasting in each subject in such a large population, although, given the data, we believe that the subjects adhered to the instructions. As blood samples were stored over time, we cannot exclude that incretins in samples stored might show some instability, considering that both GLP-1 and GIP are prone to *in vitro* degradation.

As this is a cross-sectional study, reverse causality cannot be ruled out. Finally, the study was undertaken in individuals of mainly Swedish (European ancestry) descent, and the conclusions may not be generalizable to other populations.

Conclusion

In a Swedish elderly population, increased physiological levels of fasting GIP are

associated with increased carotid IMT, while, on the contrary, GLP-1 is associated with decreasing degree of subclinical atherosclerosis.

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guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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