Plasma Concentrations of Gut Hormones Acyl Ghrelin and Peptide YY and Subsequent Risk of Colorectal Cancer and Molecular Tumor Subtypes

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ABSTRACT

Obesity and metabolic dysfunction are implicated in colorectal cancer development. Appetite-regulating gut hormones might have a role in colorectal cancer risk. We investigated whether circulating levels of the gut hormones ghrelin (analyzed as acyl ghrelin) and Peptide YY (PYY) were associated with subsequent colorectal cancer risk, including clinical and molecular tumor subtypes. We also provide descriptive data on these hormones in relation to background participant characteristics and metabolic biomarkers. This population-based study included 1,010 matched case–control pairs with a median of 12.3 years of follow-up. Acyl ghrelin and PYY were measured by multiplex immunoassay. Data on *KRAS* and *BRAF* mutations and microsatellite instability (MSI) status were available for 704 and 708 cases, respectively. Conditional logistic regression models estimated association to colorectal cancer risk. Partial correlation and linear regression were used to investigate relationships between background and metabolic variables and variation in plasma gut hormone concentrations. Acyl ghrelin was not clearly associated with colorectal cancer risk (multivariable OR per 1 SD increase: 1.11; 95% CI, 1.00–1.23). Positive associations were observed for specific subtypes, in particular *BRAF*-mutated colorectal cancer and right-sided colon cancer, although with nonsignificant heterogeneity. PYY was not related to colorectal cancer risk (multivariable OR per 1 SD: 1.04; 95% CI, 0.95–1.14) or any tumor subtype. In the control participants, ghrelin was inversely correlated with BMI, and PYY was positively correlated with C-peptide and insulin levels. These findings provide limited support for a possible role for ghrelin in colorectal cancer development, primarily in specific anatomical and molecular tumor subtypes.

Prevention Relevance: The findings of this study do not support a major role for the metabolic gut hormones ghrelin and PYY in colorectal cancer development but suggest the possibility of an involvement for ghrelin in specific tumor subtypes. Elucidating subtype-specific risk factors and mechanisms of carcinogenesis may have implications for precision prevention.

Introduction

Obesity and excess body fat rank among the most well-established lifestyle-related risk factors for colorectal cancer (1, 2). Plausible biological mechanisms for an independent role of adiposity include insulin resistance, inflammation, and alterations in adipokines and gut microbiome (2). However, metabolic markers related to these potential mechanisms, including growth factors, adipocyte-derived cytokines, and markers of insulin resistance, have shown inconsistent associations with colorectal cancer risk in studies from prospective cohorts, using pre-diagnostic samples (3–6). To help elucidate any underlying etiologic mechanisms, novel biomarkers of energy metabolism and metabolic functions related to body size are of interest. Biomarkers reflecting biological mechanisms might also have a potential application for risk stratification for precision screening or as targets for precision pharmacoprevention.

Probably the best-studied gut hormone is the appetite-regulating hormone leptin, highly associated with obesity and also secreted by adipose tissue (7). High circulating levels of leptin have been suggested to play an etiologic role in carcinogenesis, including colorectal cancer development (8). However, leptin is not established as a single mediator between obesity and colorectal cancer risk (5, 9). Ghrelin, an appetite-regulating gut hormone with metabolic functions, is secreted pre-prandially with decreasing levels shortly after a meal (10), and peptide YY (PYY), a satiety protein secreted from enteroendocrine cells in response to nutrient intake (11) are other

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less investigated gut hormones, both in general and in relation to colorectal cancer risk specifically. Ghrelin may have a proliferative effect on colorectal cancer cells (12–14) but findings for circulating ghrelin concentrations in relation to subsequent colorectal cancer risk have been mixed (15–17). PYY has, to our knowledge, not been investigated in a prospective setting.

The main objective of this study was to determine whether plasma concentrations of the gut hormones acyl ghrelin (the biologically active form of ghrelin) and PYY were associated with subsequent risk of developing colorectal cancer, including molecular and clinical tumor subtypes. Secondary objectives included assessment of the relationship between acyl ghrelin, PYY and background variables among the control participants, as well as between plasma acyl ghrelin and total ghrelin concentrations in a subset of participants.

Materials and Methods

Study population

The study participants were selected from two prospective population-based cohorts, the Västerbotten Intervention Programme (VIP, 91.8% of participants) and the Monitoring Trends and Determinants in Cardiovascular Disease Study (MONICA, 8.2% of participants). Both cohorts were initiated in the mid-1980s and are part of the Northern Sweden Health and Disease Study (NSHDS), previously described in more detail (5, 18). The NSHDS currently has approximately 135,000 participants and 240,000 sampling occasions, including >50,000 participants with repeated measurements, and up to 35 years of follow-up (19–21). In VIP, all residents in Västerbotten are invited to a health examination at their primary health care center when they turn 40, 50, and 60 years old (until 1996, also 30-year-olds were invited). In the MONICA study, participants of Västerbotten and Norrbotten ages 25 to 74 years are randomly invited every 4 to 5 years. VIP and MONICA follow very similar protocols, and in both cohorts, the vast majority of blood samples are collected after >8 hours of fasting, then aliquoted and frozen within an hour. Long-term storage is at a central location in −80° freezers. Data collected at the health examination also include extensive lifestyle and health questionnaires, anthropometry measurements, measurements of blood fats and blood pressure, and an oral glucose tolerance test.

Incident colorectal cancer cases diagnosed after cohort participation and prior to May 31, 2016, were identified by linkage to Swedish national registries as described previously (22). This study included 1010 cases with a verified primary colorectal cancer diagnosis and a prediagnostic plasma sample available, as well as 1:1 control participant matched by sex, age, year of sampling, fasting status and, for the large majority (89.5%) of samples, number of freeze–thaw cycles (**Fig 1**). Participants with a previous cancer diagnosis, other than nonmelanoma skin cancer, were excluded.

For all 1,010 case–control pairs, baseline measurements of plasma acyl ghrelin and PYY were available (**Fig. 1**). Of those, 259 case–control pairs also had a second measurement collected at least 5 years, and generally 10 years, after the baseline measure. In addition, a subset of the 518 participants with repeated measurements also had data on plasma total ghrelin concentrations available from an earlier study ($n = 119$) (16). The data subsets are illustrated in **Fig. 1**.

Ethics statement

The study protocol for the present investigation was approved by the Regional Ethical Review Board at Umeå University. All participants provided a written informed consent to use their blood samples for research purposes. All data handling complies with the European Union General Data Protection Regulation. The study conforms with the Code of Ethics of the World Medical Association (Declaration of Helsinki), printed in the *British Medical Journal* (July 18, 1964).

Plasma and tumor analyses

Levels of acyl ghrelin and total PYY were measured in EDTA plasma using a custom-designed multiplex immunoassay from mesoscale discovery (MSD). The assay was run according to the manufacturer's instructions, as described previously (5). Two aliquots of a pooled plasma control sample were included on each 96-well plate for calculation of coefficients of variation (CV). Inter- and intraassay CVs were, for acyl ghrelin 5.0% and 1.7% respectively, and for PYY 2.9% and 0.9% respectively. In the subset of participants with data on plasma total ghrelin, concentrations were analyzed using sandwich ELISA (Merck). Inter- and intraassay CVs for total ghrelin were 7.9% and 3.1%, respectively. For all plasma analyses, samples were sorted by matched case–control sets, with random placement of the samples within each set.

Colorectal cancer cases with available archival formalin-fixed, paraffin-embedded tumor tissue ($n = 841$, 83%) were analyzed for $BRAF^{V600E}$ and *KRAS* (codon 12 and 13) mutations and microsatellite instability (MSI) status. As described previously, *KRAS* mutations in codons 12 and 13 were detected by Sanger sequencing, *BRAF* V600E mutations were detected by TaqMan allelic discrimination or digital droplet PCR, and MSI status was determined by IHC or by a PCR-based method (22). As *KRAS* and *BRAF* are generally mutually exclusive, cases were classified as either *KRAS* mutated, *BRAF* mutated, or *KRAS/BRAF* wild type. Cases lacking molecular tumor data were generally due to unavailable or insufficient amount of tumor tissue ($n = 169$), mutations in both *KRAS* and *BRAF* ($n = 5$, considered to be different clones in the same tumor), or inconclusive tumor data ($n = 132$ for *KRAS* or *BRAF* mutation status and $n = 133$ cases for MSI status, generally due to insufficient amounts of DNA). Of the 836 cases left, 704 had *KRAS/BRAF* data of whom 24% were *KRAS* mutated and 22% BRAF mutated, and 708 had MSI data of whom 13% were MSI.

Additional variables

The following potential confounding variables, measured by a health-care professional at cohort participation, were

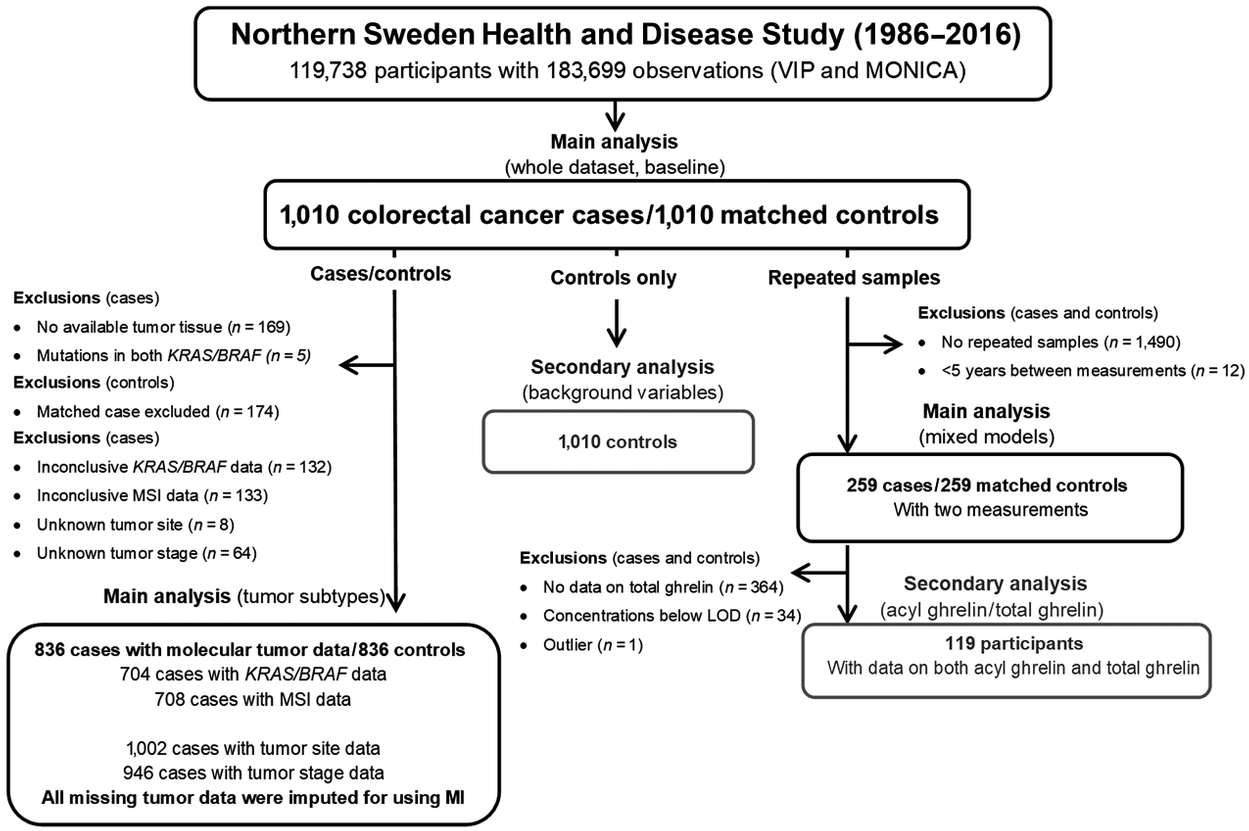

Figure 1.

Selection of study participants. The flowchart provides an overview of the study design and presents the inclusions and exclusions for each set of participants and samples in the main and subanalyses. LOD, limit of detection; MI, multiple imputation. MONICA, Monitoring Trends and Determinants in Cardiovascular Disease Study; MSI, microsatellite instability; VIP, Västerbotten Intervention Programme.

considered; systolic and diastolic blood pressure, fasting plasma glucose, oral glucose tolerance test (2 hours after a 75-g oral glucose load), total cholesterol, triglycerides, and BMI (kg/m^2, height and weight measured in light clothes and without shoes). Questionnaire data collected at the time of blood sampling were used for smoking status (current, former, and never smoker), alcohol consumption (abstainers, below-, or above sex-specific median), recreational physical activity on a scale from 1 to 4 based on frequency (1: never; 2: now and then; 3: up to 2 times/week; 4: 3 times/week or more and more vigorous training) and dietary consumption of fiber, whole grain, and red and processed meats in g/day.

Statistical analysis

Missing data for variables at baseline and the repeated sampling occasion were assumed to be missing at random. Therefore, imputation was performed using the fully conditional specification approach (also known as multiple imputation by chained equations, MICE) in SPSS (23). In total, 30 imputed sets were generated in which continuous variables were predicted using predictive mean matching and categorical variables using logistic regression. The imputation models included the exposures ghrelin and PYY, and the covariates age, sex, cohort, sample year, fasting status, smoking status, physical activity, alcohol intake, and BMI. Numbers of missing values for these variables are shown in **Table 1**.

Associations between acyl ghrelin, PYY, and colorectal cancer risk were evaluated using conditional logistic regression to estimate ORs per sex-specific 1 SD increase in biomarker plasma concentration as well as across groups of sex-specific quartiles, based on the distribution of the exposure in the control group. In quartile-based analyses, the median of each quartile was used as a continuous variable in logistic regression models to test for linear trends. None of the potential confounders had a material impact on the ORs for ghrelin or PYY in relation to colorectal cancer risk. Three multivariable logistic regression models, conditioned on the matched case sets, were therefore constructed, comprising a limited set of covariates that we considered to have strong theoretical potential relevance. Model 1 was a crude model only conditioned on matched case–control pairs; Model 2 was adjusted for smoking status, physical activity level, and alcohol consumption; and Model 3 additionally included BMI. BMI was included in the multivariable model in a separate step because although it is, perhaps, the most important potential confounder, the relationship between BMI and gut hormones is not fully

Table 1. Plasma concentrations of acyl ghrelin and PYY according to baseline characteristics of cancer-free control participants ($n = 1{,}010$).

	Acyl ghrelin (pg/mL)			PYY (pg/mL)		
	***n* (%)**	**Median (25–75th percentage)**	***P***	**Median (25–75th percentage)**	***P***	**Missing, *n* (%)[c,d]**
All controls	1,010	45.4 (21.8–78.4)		25.9 (16.8–37.7)		
Cohort			0.629[a]		0.473[a]	0 (0)
VIP	927 (91.8)	45.2 (21.5–78.4)		25.9 (17.0–37.7)		
MONICA	83 (8.2)	47.5 (23.5–77.1)		26.3 (16.2–34.0)		
Fasting status			0.046[b]		0.008[b]	0 (0)
0–4 hours	35 (3.5)	38.8 (17.6–51.9)		33.3 (23.2–44.2)		
4–8 hours	177 (17.5)	54.3 (24.7–84.5)		28.2 (17.7–42.1)		
>8 hours	798 (79.0)	44.8 (21.1–77.7)		24.9 (16.4–36.6)		
Sex			<0.001[a]		0.002[a]	0 (0)
Men	525 (52.0)	37.9 (16.3–65.0)		27.2 (18.2–41.6)		
Women	485 (48.0)	56.6 (28.9–93.7)		24.6 (15.6–34.7)		
Age (years)			<0.001[a]		0.452[a]	−0 (0)
≤55	504 (49.9)	53.8 (27.4–85.6)		26.5 (16.7–39.2)		
>55	506 (50.1)	39.2 (17.1–70.6)		25.7 (17.0–36.1)		
BMI (kg/m^2)			<0.001[b]		0.073[b]	10 (1.0)
<25	447 (44.2)	55.8 (31.1–98.1)		25.7 (16.4–34.9)		
25–30	414 (41.0)	39.3 (18.3–72.3)		25.8 (16.6–38.6)		
>30	139 (13.8)	33.8 (11.5–58.6)		29.4 (19.4–44.1)		
Smoking			0.189[b]		0.028[b]	23 (2.3)
Nonsmoker	443 (43.9)	46.6 (20.0–74.4)		24.6 (15.8–35.8)		
Ex-smoker	321 (31.8)	42.9 (21.4–79.8)		27.5 (18.9–37.8)		
Current smoker	223 (22.0)	53.0 (24.3–84.9)		27.4 (17.2–42.8)		
Physical activity[e]			0.226[b]		0.266[b]	87 (8.6)
None	375 (37.1)	41.8 (17.8–81.7)		26.2 (16.6–38.8)		
Low	248 (24.5)	48.2 (22.4–76.4)		28.4 (18.6–39.0)		
Moderate	252 (25.0)	51.9 (25.8–79.5)		25.7 (16.4–37.6)		
High	48 (4.8)	46.6 (27.3–77.3)		23.3 (14.7–32.7)		
Alcohol intake[f]			<0.001[b]		0.988[b]	150 (14.9)
None	73 (7.2)	45.5 (20.7–84.7)		27.4 (17.8–34.5)		
Below median	357 (35.3)	38.1 (17.7–66.9)		25.8 (16.8–37.9)		
Above median	430 (42.6)	53.6 (24.9–82.2)		25.5 (16.6–37.9)		

[a]Independent two-sample Mann–Whitney *U* test.
[b]Independent two-sample Kruskal–Wallis test.
[c]Missing category not included in significance tests.
[d]The corresponding number and proportion missing of the 1,010 cases were: cohort: 0 (0%), fasting status 0 (0%), sex 0 (0%), age 0 (0%), BMI 10 (1.0%), smoking 20 (2.0%), physical activity 74 (7.3%), and alcohol intake 148 (14.7%).
[e]On the basis of self-reported recreational physical activity on a scale from 1 to 4 based on frequency (1, none; 2, now and then; 3, up to 2 times/week; 4, 3 times/week or more and more vigorous training).
[f]On the basis of self-reported food frequency questionnaire, median (among users) in women: 1.81 g/day, and in men: 4.80 g/day. BMI, body mass index; PYY, Peptide YY.

understood and we cannot exclude the possibility of a mediating role (i.e., gut hormones impacting on body constitution and thereby affecting colorectal cancer risk). We also tested correcting the continuous ORs from Model 3 for regression dilution bias by multiplying the log OR with the reciprocal of the intraclass correlation coefficient (ICC) (24). ICCs, defined as the proportion of between person-variance to total-variance, were estimated by fitting mixed effects models on the subset of individuals with repeated measures of acyl ghrelin and PYY (25). Mixed effects models included case status and age as fixed factors and participant identification code as a random factor. Nonlinear associations were tested using restricted cubic splines with five knots placed at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles.

To investigate whether associations between levels of gut hormones and risk of colorectal cancer risk differed by *BRAF/KRAS*-status, MSI-status, anatomical subsite, clinical stage of the disease, and time from blood sampling to diagnosis, we estimated subgroup-specific ORs with conditional logistic regression with each control participant matched to its case. For subgroups of BMI (<25, 25–30, >30), we broke up case–control pairs and used logistic regression models, further adjusted for matching factors (age, sex, cohort, sample year, and fasting status) in Model 1 and additionally for smoking status, physical activity, and alcohol consumption in Model 2. Subgroup heterogeneity was tested using the likelihood ratio test of models with and without interaction terms.

Changes in acyl ghrelin and PYY concentrations over time were analyzed using linear mixed models, in addition to calculating ICCs. Log-transformed gut hormone concentrations were modeled, including participant identification codes and case sets as random factors, and case–control status, time from sampling to case diagnosis, smoking, recreational physical activity, alcohol intake, and BMI as fixed factors. An interaction term between case–control status and time was included to test for intraindividual average differences in biomarker concentrations over time between cases and controls. Associations were tested using regression coefficient *t* tests with degrees of freedom from Satterthwaite approximation.

Our study included two secondary objectives, one concerning the relationship between acyl ghrelin, PYY, and background variables, and the other concerning the relationship between acyl ghrelin and total ghrelin concentrations in plasma. When assessing how acyl ghrelin and PYY related to the background variables, we used linear regression to estimate the proportion of the variation in plasma acyl ghrelin and PYY concentrations that can be predicted from background variables and other metabolic biomarkers. We also calculated partial correlations (using nonimputed data) between metabolic factors and biomarkers adjusted for age, sex, and BMI with Spearman correlations coefficient. These analyses were all conducted using data solely from the control participants. When comparing plasma acyl ghrelin and total ghrelin concentrations, we used the subset of participants from our previous study with data on total ghrelin (16). Linear regression was used to model the relationship between acyl- and total ghrelin at baseline and at the repeated sampling occasion. To preserve statistical power, these analyses included both cases and controls. However, case–control status did not significantly modify the association between acyl- and total ghrelin and was therefore excluded from the model.

Analyses of baseline characteristics and baseline linear regression models were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp.). For conditional logistic regression, the "survival" R-package was used and for partial correlation network the "ppcor" R-package was used. Mixed models were fitted using the lme4R-package in R v.3.5.0 (R Foundation for Statistical Computing). All statistical tests for significance were two-sided and a *P* value of below 0.05 was considered statistically significant.

Data availability statement

The data generated in this study are not publicly available due to Swedish Authority for Privacy Protection regulations (the national supervisory authority under the European General Data Protection Regulation, GDPR). Data may be available upon reasonable request to the corresponding author.

Results

Participant characteristics

Median and 25th and 75th percentile plasma concentrations of acyl ghrelin and PYY of the 1,010 control participants at baseline are presented according to background variables in **Table 1**. Levels of acyl ghrelin were higher in women than in men and in participants ≤55 compared with >55 years of age, with a BMI <25 kg/m^2 compared with participants with higher BMIs, and with alcohol intakes above the median compared with below (all $P < 0.001$). PYY levels were higher in men than women ($P = 0.002$) and lower in never compared with ever smokers ($P = 0.028$). Characteristics of cases and controls at baseline have been described in detail elsewhere (5). In brief, the median age for cases and matched controls at baseline was approximately 56.3 years (50.6 and 60.5 in VIP and MONICA participants, respectively), and the median age at diagnosis of cases with colorectal cancer was 66.4 years (66.1 and 72.6 in VIP and MONICA participants, respectively). The median time between sampling and case diagnosis was 12.3 years at the baseline measurement, and 5.8 years at the repeat measurement. Of the participants in this study, 48.0% were women (48.4% and 43.4% in VIP and MONICA, respectively).

Biomarkers and colorectal cancer risk, including molecular and clinical subtypes

Plasma acyl ghrelin and PYY concentrations were not clearly associated with subsequent colorectal cancer risk. Adjusted ORs per 1 sex-specific SD increase in concentration were, for acyl ghrelin 1.11 (95% CI, 1.00–1.23) and for PYY 1.04 (95% CI, 0.95–1.14; **Table 2**). ORs per quartile of acyl ghrelin and PYY were all nonsignificant as were *P* for trend ($P_{trend} > 0.05$; **Table 2**).

In subgroup analyses for acyl ghrelin, shown in **Table 3**, we found positive associations with the risk of colorectal cancer with *BRAF*-mutation (OR, 1.40; 95% CI, 1.05–1.87), and MSS (OR, 1.17; 95% CI, 1.01–1.34), as well as right-sided colon cancer (OR, 1.29; 95% CI, 1.05–1.60), stage I and II tumors (OR, 1.17; 95% CI, 1.01–1.36), and in the subgroup of participants with BMI <25 (OR, 1.14; 95% CI, 1.00–1.30). However, none of the associations were significantly different from the other corresponding subgroups (all $P_{heterogeneity} > 0.05$, Supplementary Tables S1 and S2). For men and women, and lag-time stratification, associations for acyl ghrelin were similar across subgroups (**Table 3**; Supplementary Table S1), and for PYY, no subgroup-specific associations were observed (**Table 4**; Supplementary Table S2).

Adjusting for potential confounders in models 2 and 3 had marginal impact on the risk estimates compared with the "crude" model (Supplementary Tables S1 and S2). Correcting for regression dilution generally increased the magnitude of ORs and widened confidence intervals (**Table 2**; Supplementary Tables S1 and S2). Testing for nonlinear relationships between acyl ghrelin, PYY, and colorectal cancer risk, none of the restricted cubic spline models were significantly different from models consisting of linear terms only ($P_{nonlinear} > 0.05$).

The mixed models analyses for acyl ghrelin and PYY involving the 259 case–control pairs with repeated measurements 10 years apart, and adjusted for the same set of potential confounders as the fully adjusted conditional logistic regression

Table 2. Conditional logistic regression presenting the OR and 95% CI for risk of colorectal cancer in all, men, and women per 1 sex-specific SD increase and by sex- specific quartiles (based on the control participants) of acyl ghrelin and peptide YY (PYY) measured in prediagnostic plasma samples.

	Acyl ghrelin										
	Per SD increase		Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	n[a]	OR (95% CI)	*n*[a]	Ref	*n*[a]	OR (95% CI)	*n*[a]	OR (95% CI)	*n*[a]	OR (95% CI)	P_{trend}[f]	P_{het}[g]
All	1010/1010		255/254		246/252		237/252		272/252			0.97
Model 1[b]		1.07 (0.97–1.18)		–		0.97 (0.75–1.26)		0.94 (0.72–1.22)		1.09 (0.83–1.41)	0.51	
Model 2[c]		1.07 (0.97–1.18)		–		0.94 (0.73–1.22)		0.93 (0.71–1.22)		1.06 (0.81–1.38)	0.60	
Model 3[d]		1.11 (1.00–1.23)		–		0.96 (0.74–1.25)		0.98 (0.75–1.29)		1.14 (0.86–1.51)	0.27	
Model 3 corr[e]		1.15 (1.00–1.33)										
Men	525/525		130/132		135/131		113/131		147/131			
Model 1[b]		1.08 (0.94–1.23)		–		1.06 (0.73–1.53)		0.88 (0.60–1.29)		1.16 (0.79–1.69)	0.51	
Model 2[c]		1.07 (0.93–1.23)		–		1.07 (0.73–1.56)		0.88 (0.60–1.31)		1.12 (0.76–1.66)	0.65	
Model 3[d]		1.08 (0.93–1.25)		–		1.07 (0.73–1.56)		0.89 (0.60–1.33)		1.14 (0.77–1.69)	0.59	
Model 3 corr[e]		1.11 (0.91–1.36)										
Women	485/485		125/122		111/121		124/120		125/121			
Model 1[b]		1.06 (0.92–1.22)		–		0.89 (0.62–1.28)		1.00 (0.70–1.44)		1.02 (0.70–1.47)	0.78	
Model 2[c]		1.07 (0.93–1.23)		–		0.87 (0.60–1.25)		1.03 (0.71–1.49)		1.02 (0.70–1.49)	0.71	
Model 3[d]		1.14 (0.98–1.33)		–		0.90 (0.62–1.32)		1.13 (0.77–1.64)		1.19 (0.80–1.78)	0.25	
Model 3 corr[e]		1.21 (0.97–1.49)										

	PYY										
	Per SD increase		Quartile 1		Quartile 2		Quartile 3		Quartile 4		
	n[a]	OR (95% CI)	*n*[a]		*n*[a]	OR (95% CI)	*n*[a]	OR (95% CI)	*n*[a]	OR (95% CI)	P_{trend}[f]	P_{het}[g]
All	1,010/1,010		228/255		243/251		274/252		265/252			0.63
Model 1[b]		1.06 (0.97–1.16)		–		1.10 (0.85–1.42)		1.22 (0.95–1.57)		1.19 (0.92–1.54)	0.18	
Model 2[c]		1.05 (0.96–1.15)		–		1.08 (0.84–1.41)		1.20 (0.93–1.54)		1.17 (0.90–1.52)	0.23	
Model 3[d]		1.04 (0.95–1-14)		–		1.08 (0.83–1.40)		1.18 (0.91–1.52)		1.15 (0.88–1.49)	0.30	
Model 3 corr[e]		1.08 (0.91–1.28)										
Men	525/525		120/132		121/131		154/131		130/131			
Model 1[b]		1.04 (0.92–1.18)		–		1.03 (0.71–1.49)		1.30 (0.91–1.84)		1.10 (0.76–1.59)	0.50	
Model 2[c]		1.02 (0.90–1.16)		–		1.02 (0.70–1.48)		1.25 (0.88–1.79)		1.05 (0.73–1.52)	0.69	
Model 3[d]		1.02 (0.90–1.16)		–		1.02 (0.70–1.48)		1.25 (0.87–1.78)		1.05 (0.72–1.52)	0.71	
Model 3 corr[e]		1.04 (0.81–1.33)										
Women	485/485		108/123		122/120		120/121		135/121			
Model 1[b]		1.08 (0.95–1.22)		–		1.17 (0.81–1.68)		1.14 (0.79–1.63)		1.28 (0.89–1.83)	0.23	
Model 2[c]		1.07 (0.94–1.22)		–		1.11 (0.77–1.62)		1.08 (0.75–1.56)		1.26 (0.87–1.81)	0.24	
Model 3[d]		1.06 (0.94–1.21)		–		1.10 (0.75–1.60)		1.06 (0.73–1.54)		1.22 (0.84–1.76)	0.31	
Model 3 corr[e]		1.12 (0.88–1.43)										

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio; Ref, reference; SD, standard deviation.

[a]Number of cases/controls.

[b]Conditioned on matched case-control pairs (matched on age, sex, cohort, sample year, and fasting status.

[c]Additionally adjusted for smoking status, recreational physical activity, and alcohol intake.

[d]Additionally adjusted for BMI.

[e]ORs corrected for regression dilution.

[f]The median of each quartile was used as a continuous variable in logistic regression models to test for linear trends.

[g]$P_{heterogeneity}$ between estimates for subgroups calculated from likelihood ratio tests.

Table 3. Conditional logistic regression presenting the OR and 95% CI for clinical and molecular subgroups of colorectal cancer per 1 sex-specific SD increase and by sex-specific quartiles of acyl ghrelin concentrations measured in prediagnostic plasma samples.

	Acyl Ghrelin											
	All (OR per 1 SD increase)			Quartile 1		Quartile 2		Quartile 3		Quartile 4 (highest)			
	n[a]	OR (95% CI)[b]	P_{het}[c]	n[a]	Ref	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	P_{trend}[d]	P_{het}[c]
BRAF/KRAS status			0.22										0.84
BRAF-mutated	156/156	1.40 (1.05–1.87)		39/45	–	36/37	1.10 (0.57–2.13)	37/42	1.07 (0.55–2.11)	44/32	1.85 (0.89–3.83)	0.10	
KRAS-mutated	167/167	1.24 (0.94–1.65)		40/46	–	40/38	1.37 (0.70–2.71)	42/42	1.42 (0.71–2.84)	45/41	1.44 (0.73–2.87)	0.36	
Wildtype	381/381	1.06 (0.89–1.27)		95/96	–	103/101	0.93 (0.60–1.45)	86/93	0.89 (0.56–1.4)	97/91	1.11 (0.70–1.76)	0.61	
MSI status			0.81										0.80
MSI	96/96	1.10 (0.76–1.59)		29/30	–	21/24	1.01 (0.43–2.37)	24/21	1.68 (0.68–4.17)	22/21	1.06 (0.38–2.96)	0.62	
MSS	612/612	1.17 (1.01–1.34)		140/160	–	162/145	1.31 (0.92–1.86)	137/152	1.10 (0.77–1.57)	173/155	1.38 (0.96–1.98)	0.17	
Tumor location			0.30										0.45
Right colon	318/318	1.29 (1.05–1.60)		84/96	–	82/84	1.16 (0.74–1.82)	72/72	1.21 (0.75–1.96)	80/66	1.59 (0.95–2.66)	0.08	
Left colon	296/296	0.99 (0.81–1.22)		76/63	–	67/74	0.65 (0.40–1.05)	68/86	0.62 (0.37–1.04)	85/73	0.89 (0.53–1.49)	0.95	
Rectum	388/388	1.08 (0.92–1.25)		91/94	–	96/93	1.16 (0.72–1.85)	94/91	1.17 (0.74–1.87)	107/110	1.13 (0.71–1.81)	0.73	
Tumor stage			0.29										0.34
Stage I-II	478/478	1.17 (1.01–1.36)		119/128	–	111/124	0.99 (0.68–1.44)	110/106	1.23 (0.82–1.86)	138/120	1.42 (0.94–2.14)	0.06	
Stage III-IV	468/468	1.06 (0.91–1.25)		118/113	–	122/114	0.99 (0.66–1.47)	106/125	0.84 (0.57–1.24)	122/116	1.04 (0.69–1.57)	0.90	
Lag time			0.35										0.42
<9 years	320/320	1.09 (0.92–1.30)		88/87	–	79/81	0.91 (0.58–1.44)	73/79	0.91 (0.56–1.48)	80/73	1.07 (0.66–1.74)	0.69	
9–15 years	338/338	1.16 (0.95–1.41)		81/81	–	79/95	0.82 (0.51–1.32)	89/87	1.17 (0.73–1.88)	89/75	1.33 (0.79–2.25)	0.13	
>15 years	352/352	1.07 (0.89–1.28)		86/86	–	88/76	1.23 (0.78–1.94)	75/86	0.87 (0.54–1.4)	103/104	1.05 (0.66–1.66)	0.89	
BMI[e]			0.97										0.79
<25 kg/m^2	392/448	1.14 (1.00–1.30)		67/84	–	70/99	0.85 (0.54–1.33)	110/120	1.12 (0.74–1.71)	145/145	1.28 (0.85–1.93)	0.08	
25–30 kg/m^2	462/423	1.07 (0.92–1.26)		130/119	–	124/112	1.10 (0.69–1.44)	94/100	0.87 (0.59–1.28)	114/92	1.13 (0.77–1.67)	0.61	
>30 kg/m^2	156/139	0.98 (0.67–1.42)		58/51	–	52/41	1.10 (0.61–2.00)	33/32	0.90 (0.47–1.74)	13/15	0.82 (0.33–2.00)	0.59	

Abbreviations: BMI, body mass index; MSI, microsatellite instability; MSS, microsatellite stability; OR, odds ratios; PYY, Peptide YY; ref, reference; SD, standard deviation.

[a]Number of cases/controls.

[b]ORs are from multivariable Model 3 conditioned on matched case-control pairs (matched on age, sex, cohort, sample year, and fasting status) and adjusted for smoking status, recreational physical activity, alcohol intake, and BMI. Results for minimally and fully adjusted models (with and without BMI) as well as corrected for regression dilution are shown in Supplementary Table S1.

[c]$P_{heterogeneity}$ between estimates for subgroups calculated from likelihood ratio tests.

[d]The median of each quartile was used as a continuous variable in logistic regression models to test for linear trends.

[e]Logistic regression adjusted for matching variables age, sex, cohort, sampling year, and fasting status (since matched case–control pairs were split in the subgroup-analysis for BMI) and additionally adjusted for smoking status, recreational physical activity, and alcohol intake.

Table 4. Conditional logistic regression presenting the OR and 95% CI for clinical and molecular subgroups of colorectal cancer per 1 sex-specific SD increase and by sex-specific quartiles of Peptide YY (PYY) measured in prediagnostic plasma samples.

	PYY											
	All (OR per 1 SD increase)			Quartile 1		Quartile 2		Quartile 3		Quartile 4 (highest)			
	n[a]	OR (95% CI)[b]	P_{het}[c]	n[a]	Ref	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	P_{trend}[d]	P_{het}[c]
***BRAF/KRAS* status**			0.43										0.10
BRAF-mutated	156/156	1.11 (0.88–1.41)		41/35	–	33/49	0.49 (0.24–1.00)	39/37	0.79 (0.40–1.57)	43/35	0.97 (0.51–1.88)	0.50	
KRAS-mutated	167/167	1.07 (0.82–1.41)		27/42	–	44/40	1.92 (0.91–4.06)	56/42	2.27 (1.09–4.74)	40/43	1.71 (0.76–3.83)	0.45	
Wildtype	381/381	0.98 (0.85–1.11)		89/89	–	96/93	1.02 (0.66–1.56)	101/102	0.95 (0.63–1.44)	95/97	0.91 (0.60–1.39)	0.60	
MSI-status			1.00										0.22
MSI	96/96	1.01 (0.76–1.35)		22/22	–	20/30	0.64 (0.23–1.80)	30/20	1.48 (0.57–3.84)	24/24	0.98 (0.37–2.57)	0.62	
MSS	612/612	1.04 (0.93–1.17)		136/146	–	152/147	1.12 (0.79–1.58)	162/160	1.06 (0.76–1.48)	162/159	1.07 (0.76–1.50)	0.88	
Tumor location			0.55										0.91
Right colon	318/318	1.08 (0.91–1.28)		76/83	–	80/88	0.99 (0.60–1.63)	77/74	1.11 (0.69–1.77)	85/73	1.23 (0.76–1.96)	0.32	
Left colon	296/296	1.08 (0.93–1.26)		63/76	–	64/68	1.19 (0.74–1.90)	88/72	1.48 (0.93–2.38)	81/80	1.27 (0.79–2.05)	0.37	
Rectum	388/388	0.98 (0.84–1.14)		85/93	–	98/93	1.15 (0.75–1.77)	108/104	1.11 (0.74–1.67)	97/98	1.05 (0.69–1.60)	0.98	
Stage			0.65										0.92
I–II	478/478	1.03 (0.91–1.17)		110/116	–	122/128	1.03 (0.70–1.52)	124/120	1.08 (0.74–1.57)	122/114	1.09 (0.74–1.60)	0.65	
III–IV	468/468	1.08 (0.94–1.23)		104/125	–	104/109	1.12 (0.76–1.65)	132/112	1.32 (0.91–1.93)	128/122	1.23 (0.85–1.79)	0.27	
Lag time			0.58										0.58
<9 years	320/320	1.06 (0.90–1.25)		65/74	–	67/76	0.98 (0.62–1.57)	101/79	1.43 (0.91–2.25)	87/91	1.03 (0.65–1.64)	0.75	
9–15 years	338/338	1.06 (0.92–1.22)		78/84	–	88/89	1.08 (0.69–1.70)	93/94	1.03 (0.66–1.60)	79/71	1.16 (0.73–1.85)	0.56	
>15 years	352/352	0.98 (0.83–1.16)		85/97	–	88/86	1.15 (0.72–1.83)	80/79	1.11 (0.71–1.74)	99/90	1.21 (0.78–1.88)	0.47	
BMI[d,e]			0.73										
<25 kg/m^2	392/448	0.98 (0.86–1.11)		103/116	–	99/113	0.97 (0.66–1.43)	95/118	0.91 (0.62–1.33)	95/101	1.02 (0.69–1.51)	0.93	
25–30 kg/m^2	462/423	1.08 (0.93–1.25)		104/112	–	109/104	1.11 (0.76–1.63)	133/100	1.41 (0.97–2.07)	116/107	1.17 (0.80–1.71)	0.41	
>30 kg/m^2	156/139	1.18 (0.96–1.47)		21/27	–	35/34	1.63 (0.75–3.57)	46/34	1.92 (0.91–4.11)	54/44	1.77 (0.86–3.68)	0.24	

Abbreviations: BMI, body mass index; MSI, microsatellite instability; MSS, microsatellite stability; OR, odds ratios; PYY, Peptide YY; ref, reference; SD, standard deviation.

[a]Number of cases/controls.

[b]ORs are from multivariable Model 3 conditioned on matched case-control pairs (matched on age, sex, cohort, sample year, and fasting status) and adjusted for smoking status, recreational physical activity, alcohol intake, and BMI. Results from minimally and fully adjusted models (with and without BMI) as well as for corrected for regression dilution are shown in Supplementary Table S2.

[c]$P_{heterogeneity}$ between estimates for subgroups calculated from likelihood ratio tests.

[d]The median of each quartile was used as a continuous variable in logistic regression models to test for linear trends.

[e]Logistic regression adjusted for matching variables age, sex, cohort, sampling year, and fasting status (since matched case control pairs were split in the subgroup-analysis for BMI) and additionally adjusted for smoking status, recreational physical activity, and alcohol intake.

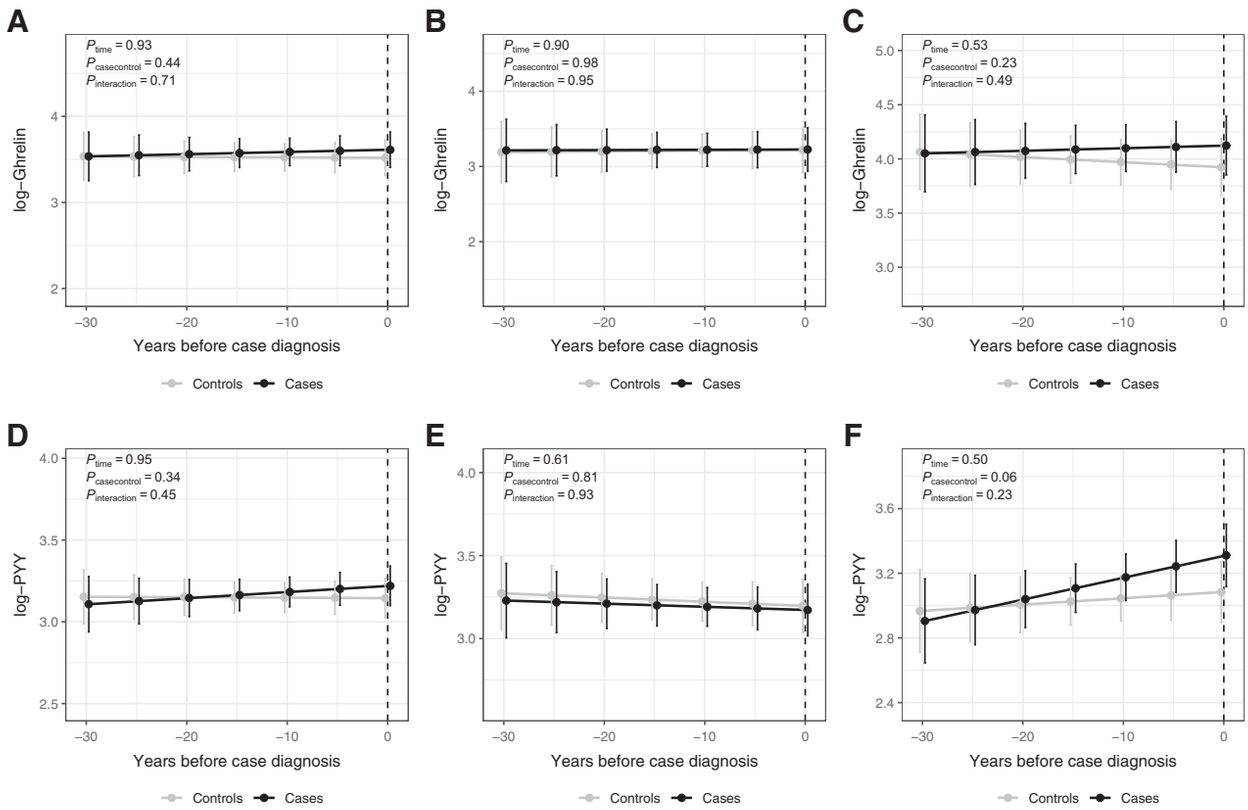

Figure 2.

Estimated average time trajectories for log-ghrelin and log-PYY concentrations in 259 colorectal cancer cases and 259 time-matched controls (including 146 male and 113 female case–control pairs). **A–C**, The results for ghrelin overall (**A**), in men (**B**), and in women (**C**). **D–F**, The results for PYY overall (**D**), in men (**E**), and in women (**F**). Linear mixed models were used to estimate marginal effects of time and 95% CIs in 259 matched case–control pairs with matched repeated measurements prior to case diagnosis. The x-axis displays years before colorectal cancer diagnosis, where 0 = year of diagnosis. Imputed data were used in a mixed model including participant and matched case–control pairs as random factors, and time between sample collection and case diagnosis, case–control status, an interaction term between time and case–control status, smoking status, recreational physical activity, alcohol intake, and BMI, which were included as fixed factors.

model, are presented in **Fig. 2**. Intraindividual concentrations of both biomarkers were generally stable over time in both cases and controls (**Fig. 2A** and **D**, respectively). The reproducibility in terms of ICC was 0.71 (95% CI, 0.66–0.75) for acyl ghrelin and 0.52 (95% CI, 0.45–0.58) for PYY.

Determinants of gut hormone concentrations

To assess the contribution of background variables and other metabolic factors and biomarkers to the variation in plasma acyl ghrelin and PYY concentrations, coefficients of determination (R^2) were calculated in the 1,010 control participants (Supplementary Table S3). Corresponding partial correlations focusing on energy metabolism variables are illustrated in a correlation network in Supplementary Fig. S1. BMI explained 6.1% of the variation in acyl ghrelin ($R^2 = 0.061$, multivariable $P < 0.001$), with a correlation coefficient of $r = -0.2$. Furthermore, serum triglycerides explained 3.2% ($P = 0.024$) and insulin explained 3.1% ($P = 0.004$) of the variation in acyl ghrelin. Age and female sex explained 2.6% and 5.5% of the variation in acyl ghrelin, respectively (both $P < 0.001$). With respect to PYY, C-peptide explained 4.4% of the variation in plasma PYY concentrations ($P = 0.042$) with a correlation coefficient of $r = 0.3$. Although insulin did not contribute to variation in PYY in the multivariable analysis ($P = 0.275$), a positive correlation was observed between these variables ($r = 0.3$). Female sex, diabetes, and ex-smoker status were all statistically significant determinants of PYY in the multivariable model ($P < 0.05$), but each explained less than 1% of the variation in PYY concentrations. Acyl ghrelin and PYY concentrations were weak but statistically significant determinants of each other in the multivariable linear models.

Comparison of plasma acyl ghrelin and total ghrelin concentrations

In the subset of individuals with repeated measurements ($n = 518$), $n = 119$ had data for both acyl and total ghrelin after exclusions ($n = 364$ no total ghrelin, $n = 34$ below the level of detection for total ghrelin, and $n = 1$ outlier; **Fig. 1**). In these plasma samples, acyl ghrelin accounted for on average 10% (SD 6%) of total ghrelin concentrations. In linear regression models, acyl ghrelin explained 63% of the variation in total ghrelin concentrations at the first sampling time point and 72% at the

second time point (Supplementary Fig. S2). Slope coefficients ranged from 4.74 to 4.99.

Discussion

In this nested case–control study of 1,010 matched colorectal cancer case–control pairs from a population-based cohort in northern Sweden, we found no clear associations between prediagnostic plasma concentrations of acyl ghrelin or PYY and subsequent risk of colorectal cancer. There were indications of subtype-specific positive associations for acyl ghrelin, particularly for *BRAF*-mutated colorectal cancer and right-sided colon cancer. However, there was no evidence for a heterogenous association across subtypes in any significance test. In the secondary descriptive analyses, acyl ghrelin was inversely associated with BMI, and PYY was positively associated with C-peptide. In a subset of the participants, plasma levels of acyl and total ghrelin were strongly associated, with acyl ghrelin explaining a moderate to substantial percentage of the variation in total ghrelin levels.

Interest in a potential role for ghrelin status in determining colorectal cancer risk was raised by the Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, using prospectively collected blood samples from male smokers. In that study, a time-dependent association was observed between low levels of circulating total ghrelin concentrations and increased colorectal cancer risk for samples collected within 10 years prior to diagnosis, but a positive association for samples collected >20 years before diagnosis (15). We were not able to replicate these findings in a previous analysis of 60 case–control pairs with matched, repeated prediagnostic samples collected 10 years apart (16). In this study, which included the majority of these case–control pairs and with additional data on acyl ghrelin concentrations in a much larger study population, we found no evidence suggesting heterogeneity of association estimates based on time point of sample collection prior to diagnosis.

The analysis of acyl rather than total ghrelin, selected to allow multiplexing with other circulating biomarkers being analyzed simultaneously, may have contributed to the differences in findings in our study compared with the study from the ATBC cohort (17). Acyl ghrelin is rapidly enzymatically degraded to des-acyl ghrelin at room temperature prior to freezing (26). However, in the subset of plasma samples in this study with data on both acyl and total ghrelin, high coefficients of determination (R^2) were observed, and the proportion acyl ghrelin/total ghrelin was on average 10% (SD 6%), as would be expected (17), supporting the utility of acyl ghrelin analyzed in our biobank samples. We used a custom multiplex immunoassay to analyze plasma acyl ghrelin concentrations, and a sandwich ELISA for total ghrelin. Although data directly comparing these methods to the traditional radioimmunoassay are scarce, CVs were low in our analyses, and sandwich ELISA has been reported to perform better than radioimmunoassay in terms of cross-reactivity and specificity (27). Furthermore, although des-acyl ghrelin has previously been considered to be inactive, reports increasingly suggest possible roles in biological processes relevant for carcinogenesis (28). Thus, consideration of the relative proportions, and potentially different effects, of acyl and des-acyl ghrelin *in vivo* may be necessary to fully elucidate the role of ghrelin in colorectal cancer development.

Preclinical and observational clinical data on the role of ghrelin in cancer have been contradictory, suggested to act as both promotor and inhibitor in various cancers (14, 29, 30), and a limited role as a risk biomarker was raised in a recent review with respect to mixed results in earlier prospective studies (17). In well- and moderately differentiated colorectal cancer tumor samples, enhanced expression of ghrelin has been described (12). In human intestinal epithelial cell lines, the promotion of proliferation was shown to be independent of the ghrelin receptor GHS-R1a (13). Furthermore, ghrelin may act as a mediator in malignant tissue by increasing proliferation and anti-apoptotic activity, and the ghrelin–GHS-R axis may have an autocrine–paracrine role in cancer growth and function (31).

In contrast to ghrelin, PYY has not, to our knowledge, been studied in relation to colorectal cancer risk in a prospective setting. In a small case–control study, circulating PYY concentrations were higher in patients with colorectal and gastric cancer compared with control participants, but statistically significant only for gastric cancer (32). In this study, we observed no association between plasma PYY concentrations and subsequent colorectal cancer risk. In the subset of participants with repeated samples collected approximately 10 years apart, PYY levels appeared to rise in female cases compared with controls during the years approaching diagnosis, which could be consistent with the previous case–control study (32) but similar to that study, our finding was not statistically significant. Lower levels of PYY have been hypothesized to predispose to the development and/or maintenance of obesity, and have been shown to partly mediate the reduced appetite and subsequent weight loss after bariatric surgery (33). However, results have not been entirely consistent (34, 35). Intracolonic PYY concentrations appear to be unaffected in human colorectal carcinoma compared with benign colorectal tissue (36). Furthermore, plasma PYY levels have been reported to be similar in patients with colorectal cancer and healthy controls (32), which does not support a role for PYY in colorectal cancer progression.

As appetite-regulating hormones, relationships between both ghrelin and PYY and colorectal cancer risk could be confounded and/or mediated by body size. We considered body size in two different ways, as a separate addition to the multivariable model (with no material effects on risk estimations) and through subgroup analyses stratified by BMI categories. A positive association was observed between acyl ghrelin and colorectal cancer risk solely in participants with BMI <25 kg/m^2, although the test for heterogeneity was not significant. Circulating levels of acyl ghrelin were higher in

participants with BMI <25 kg/m^2 compared with the overweight (BMI 25–30 kg/m^2) and obese (BMI >30 kg/m^2) groups in our study, in contrast to the Finnish study of male smokers in which BMI did not vary across quartiles of total serum ghrelin concentrations (15). Given the relative paucity of participants with BMI under 20 or above 35, our results are generalizable to normal and overweight individuals and may not capture associations occurring at more extreme body weights.

A secondary aim of this study was to present population-based descriptive data for ghrelin and PYY in relation to participant characteristics, in the 1,010 cancer-free controls. Ghrelin has been reported to correlate with several metabolic factors including BMI (positive), insulin (negative), high-density lipoprotein (positive) (37), and also to have a role in alcohol addiction (positive) (38). The correlation with BMI was replicated in our data and for ghrelin in relation to insulin, we observed an inverse association in the multivariable linear regression analysis. Interestingly, there was also a clear distinction between lower versus higher alcohol consumers (</> median) for the association to plasma ghrelin levels in univariable regression. For PYY, positive correlations with waist-circumference and body fat percentage have been found in women but not in men (35). We observed a positive association between PYY and BMI in the linear regression analyses, but only in the univariable model. In addition, we report novel positive correlations between PYY and both insulin and C-peptide, and the association with insulin remained in the multivariable linear regression model.

A weakness in this study was the lack of more detailed data on body fatness and fat distribution, such as body fat percentage or waist circumference. Information on regular use of aspirin which has been described to reduce the risk for *BRAF* and *KRAS* wild-type colorectal cancer by around 30% (39), was also lacking, as was information on presence of atrophic gastritis in participants, which can decrease ghrelin levels (40). Although molecular tumor variables were available for only 83% of the cases, potentially limiting generalizability of results and increasing the risk of chance findings due to multiple testing, this proportion of missing data is similar to other molecular pathologic epidemiologic studies (4).

Major strengths of this study were the use of prospectively collected exposure data and high-quality blood samples in a population-based setting. The cohorts are highly representative of the background population, reducing the risk of selection bias (41). Blood samples were collected and handled according to strict protocols, generally after at least 8 hours of fasting and aliquoted and frozen within an hour of collection, and the fast processing, efficient multiplex assay (MSD) with high sensitivity yielded low CVs. These aspects ensured high quality not only for the investigation of the gut hormones in relation to colorectal cancer risk, but also for the secondary aim of providing descriptive data on their relation to background characteristics and metabolic biomarkers. The large sample size was another important strength, with 1,010 participants who developed colorectal cancer after blood sampling, and 1,010 matched control participants. In particular, archival tumor tissue was acquired and successfully analyzed for molecular markers in approximately 700 cases. Although subtypes of colorectal cancer can be investigated by other means, such as Consensus Molecular Subtypes (42), the features used in the present study are clinically relevant and are frequently used in molecular pathologic epidemiology studies of colorectal cancer (43, 44). In addition, the long range of follow-up times between blood sampling and diagnosis (median 12.3 years) allowed for time-stratified analyses, to account for the possibility of reverse causation or differential effects on tumor initiation and progression. Finally, we leveraged repeated samples for a subgroup of the study participants for the evaluation of intra-individual stability and reliability of the gut hormones over time and to investigate prediagnostic time trajectories.

In conclusion, in this large, population-based study, plasma concentrations of the gut hormones acyl ghrelin and PYY showed no clear associations with subsequent risk of colorectal cancer. For acyl ghrelin, subtype-specific positive associations were observed, particularly for *BRAF*-mutated colorectal cancer and right-sided colon cancer, although with nonsignificant heterogeneity. In a subset of participants with repeated measures, approximately 10 years apart, time trajectories were generally similar in cases and controls for both acyl ghrelin and PYY during the years prior to colorectal cancer diagnosis of the cases. Finally, we replicated previous reports of an inverse association between circulating ghrelin concentrations and BMI and made a novel observation of a positive relationship between circulating PYY concentrations and C-peptide and insulin.

Authors' Disclosures

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Authors' Contributions

S. Bodén: Conceptualization, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **J. Harbs:** Formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **A. Sundkvist:** Conceptualization, investigation, writing–original draft, writing–review and editing. **K. Fuchs:** Investigation, writing–review and editing. **R. Myte:** Formal analysis, visualization, methodology, writing–review and editing. **B. Gylling:** Data curation, investigation, writing–review and editing. **C. Zingmark:** Investigation, writing–review and editing. **A. Löfgren Burström:** Investigation, writing–review and editing. **R. Palmqvist:** Data curation, funding acquisition, investigation, writing–review and editing. **S. Harlid:** Conceptualization, data curation, supervision, investigation, writing–review and editing. **B. Van Guelpen:** Conceptualization, data curation, supervision, funding acquisition, methodology, writing–original draft, writing–review and editing.

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Note

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