LETTERS

Plasma ghrelin is probably not a useful biomarker for risk prediction or early detection of colorectal cancer

A recent study by Murphy *et al* reported an intriguing, time-dependent relationship between ghrelin, an appetite-stimulating hormone, and colorectal cancer (CRC).[1] In the 10 years prior to diagnosis, but not earlier, low serum ghrelin concentrations were associated with a dramatic increase in CRC risk (OR=10.86). This raises the question of whether ghrelin might be a candidate biomarker for clinical use. However, whether ghrelin levels actually go down during the period approaching diagnosis is challenging to assess, requiring repeated prediagnostic blood samples from patients.

We conducted a validation of the study by Murphy *et al*[1] using fasting plasma samples from the Västerbotten Intervention Programme, collected from CRC cases and matched controls within the 5 years preceding diagnosis of the cases. All participants had provided an additional blood sample 10 years earlier. This unique study design, described in detail elsewhere,[2] allowed us to investigate potential changes in ghrelin levels over a long prediagnostic time period, from a generally tumour-free phase of 10–15 years prior to diagnosis to the window of opportunity for early detection.

Repeated plasma samples were available for 60 complete case sets, including 33 colon cancer and 27 rectal cancer cases, from our previous study.[2] Plasma total ghrelin concentrations were analysed using sandwich ELISA (Merck, Germany). Our sample size provides 80% power to detect an OR of 3.3 for a median-split ghrelin variable at P<0.05 (and >99% power to detect an OR of 10). Characteristics of the participants are provided in table 1.

In our dataset, ghrelin was not associated with CRC risk (figure 1A). For samples collected within the 5 years preceding the CRC diagnosis of cases, we observed a multivariable OR and 95% CI of 0.98 (0.41 to 2.35) for levels below versus above the control–participant median of 496 pg/mL (figure 1A). Furthermore, ghrelin levels remained stable over time in both cases and controls (intraclass correlation coefficient=0.74, $P_{interaction}$=0.81, figure 1B,C).

Our study is comparable to the report by Murphy *et al* in many respects.[1] Both

Table 1 Characteristics of colorectal cancer cases and matched control participants, all of whom provided repeated blood samples at 10-year intervals

	Time period prior to diagnosis of cases			
	>10 years		<5 years	
	Cases	Controls	Cases	Controls
N	60	60	60	60
Males (%)	34 (57)	34 (57)	34 (57)	34 (57)
Age (years)	50.0 (40.3 – 50.2)	50.0 (40.2 – 50.2)	59.8 (50.3 – 60.1)	59.9 (50.2 – 60.1)
BMI	25.5 (23.6 – 27.0)	24.6 (22.8 – 28.1)	26.1 (24.4 – 28.9)	25.5 (23.3 – 29.0)
Ever smoker, N (%)	36 (60)	34 (57)	38 (63)	35 (58)
Alcohol intake (g/day)	3.0 (0.9 – 6.3)	3.4 (1.6 – 6.7)	4.6 (1.7 – 8.1)	3.2 (0.9 – 6.3)
No physical activity* (%)	28 (47)	23 (38)	27 (45)	25 (42)
Ghrelin† (pg/mL)	556 (323 – 772)	494 (370 – 695)	527 (338 – 758)	504 (340 – 623)
<LOD, N (%)	10 (17)	6 (10)	12 (20)	6 (10)

Summary statistics are presented as medians and interquartile ranges unless otherwise stated.
*Self-reported exercise frequency during leisure time on a scale from 1 to 5, where 1=none and 5=more than three times/week.
†Total fasting plasma ghrelin concentrations measured by sandwich ELISA (Merck, Germany).
BMI, body mass index in kg/m^2; LOD, limit of detection.

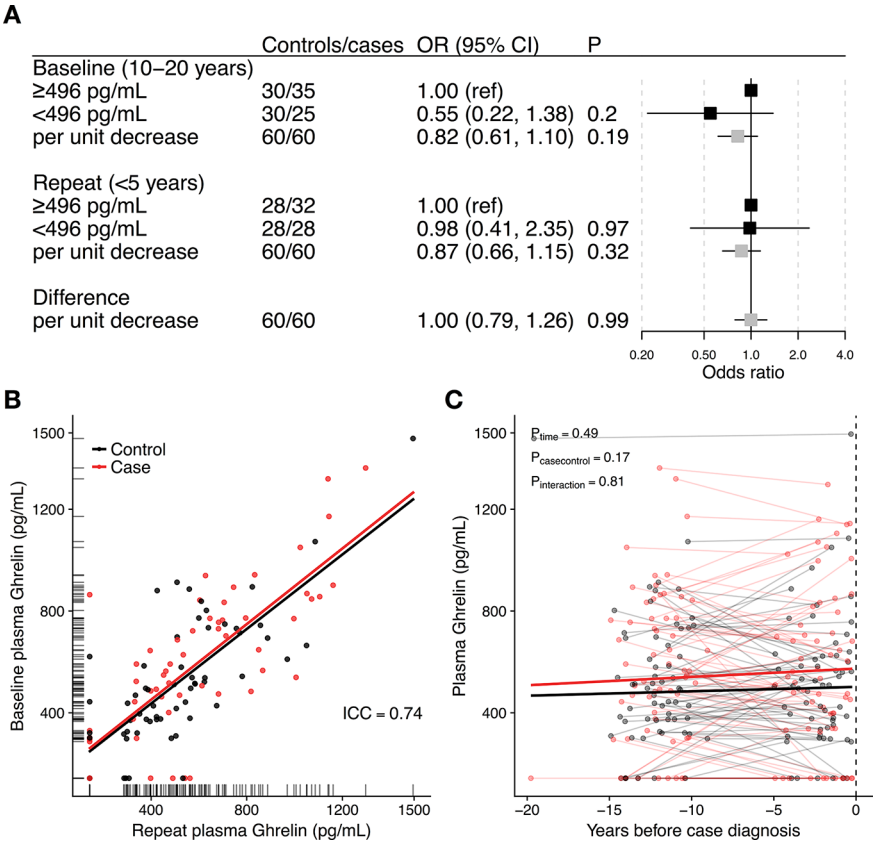

Figure 1 (A) ORs for CRC risk by plasma ghrelin concentrations <5 and >10 years before diagnosis, and by change in ghrelin between measurements. ORs were calculated using conditional logistic regression, conditioned on the case–control sets and adjusted for BMI, smoking, alcohol, physical activity and education. ORs per unit decrease were scaled to half the interquartile range (189 pg/mL for baseline and repeat, 109 pg/mL for difference). (B) Ghrelin intraindividual correlation. (C) Association between ghrelin and time in cases and controls, estimated using mixed models, including time until case diagnosis, case–control status, the interaction term between time and case–control status, BMI, smoking, alcohol, physical activity and education as fixed factors, and participant and case–control set as random factors. BMI, body mass index; CRC, colorectal cancer; ICC, intraclass correlation coefficient.

were based on Scandinavian populations, had a prospective design with long follow-up, extensive data on potential confounders, fasting blood samples and low loss to follow-up, thanks to high-quality cancer registries. Notable differences include the lack of repeated measurements, male smoker inclusion criterion, the alpha-tocopherol, beta-carotene intervention and the radioimmunoassay analysis of serum ghrelin concentrations in Murphy *et al*'s study.[1] Although we had limited power for subgroup analyses, we found nothing to suggest potential major heterogeneity in results based on sex, tumour site or tumour stage (data not shown). Plasma ghrelin concentrations were not associated with smoking in our study, which was in line with Murphy *et al*' s study.[1] Use of ELISA was, if anything, an advantage in our study, as it may have less cross-reactivity, and thereby higher specificity, than radioimmunoassay.[3] Ghrelin concentrations in plasma and serum are reportedly comparable,[4] so the different media are also unlikely to explain the inconsistent findings.

In conclusion, we could not replicate the recently reported novel finding of a very strong association between lower circulating ghrelin concentrations and increased CRC risk in the years approaching diagnosis. Further examination of ghrelin in colorectal carcinogenesis may be warranted. However, our analyses, which were sufficiently powered for the purpose, suggest that ghrelin probably cannot be developed as a biomarker for risk prediction or early detection of CRC.

Anneli Sundkvist,[1] Robin Myte,[1] Richard Palmqvist,[2] Sophia Harlid,[1] Bethany Van Guelpen[1]

[1]Department of Radiation Sciences, Oncology Unit, Umeå University, Umea, Sweden
[2]Department of Medical Biosciences, Pathology Unit, Umeå University, Umeå, Sweden

Correspondence to Dr Bethany Van Guelpen, Department of Radiation Sciences, Oncology, Umeå University, SE-90187 Umeå, Sweden; bethany.vanguelpen@umu.se

Contributors AS, RM, RP, SH and BVG: study design. AS and SH: biochemical analyses. RM: statistical analyses. RM, AS, SH and BVG: data interpretation. AS: wrote the first draft. All authors: reviewed the manuscript and approved the final version.

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