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Dietary glycemic and insulin scores and colorectal cancer survival by tumor molecular biomarkers

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Abstract

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Accumulating evidence suggests that post-diagnostic insulin levels may influence colorectal cancer (CRC) survival. Yet, no previous study has examined CRC survival in relation to a post-diagnostic diet rich in foods that increase post-prandial insulin levels. We hypothesized that glycemic and insulin scores (index or load; derived from food frequency questionnaire data) may be associated with survival from specific CRC subtypes sensitive to the insulin signaling pathway. We prospectively followed 1,160 CRC patients from the Nurses' Health Study (1980–2012) and Health Professionals Follow-Up Study (1986–2012), resulting in 266 CRC deaths in 10,235 person-years. CRC subtypes were defined by seven tumor biomarkers (*KRAS, BRAF, PIK3CA* mutations, and IRS1, IRS2, FASN, and CTNNB1 expression) implicated in the insulin signaling pathway. For overall CRC and each subtype, hazard ratio (HR) and 95% confidence interval (95% CI) for an increase of one standard deviation in each of glycemic and insulin scores were estimated using time-dependent Cox proportional hazards model. We found that insulin scores, but not glycemic scores, were positively associated with CRC mortality (HR=1.19, 95% CI=1.02 to 1.38 for index; HR=1.23, 95% CI=1.04 to 1.47 for load). The significant positive associations appeared more pronounced among *PIK3CA* wild-type cases and FASN-negative cases, with HR ranging from 1.36 to 1.60 across insulin scores. However, we did not observe statistically significant interactions of insulin scores with *PIK3CA*, FASN, or any other tumor marker (P interaction > .12). While additional studies are needed for definitive evidence, a high-insulinogenic diet after CRC diagnosis may contribute to worse CRC survival.

Introduction

Insulin, which promotes cell proliferation and inhibits cell apoptosis, contributes to the pathogenesis of colorectal cancer (CRC).[1] Growing evidence suggests that among CRC patients, insulin-related signaling pathways may also be implicated in the prognosis of CRC.[2] For instance, major determinants of hyperinsulinemia, such as obesity and physical inactivity, are associated with poor survival.[3, 4] In a meta-analysis, CRC patients with diabetes had a higher all-cause and CRC-specific mortality compared with CRC patients without diabetes.[5] Thus, a post-diagnostic diet resulting in high insulin secretion may have an adverse effect on survival among CRC patients. Consistent with this hypothesis, a prospective study among female CRC patients reported that a reduced consumption of sugar sweetened beverages and juices was associated with a longer overall survival.[6] However, no previous studies have examined survival among CRC patients in relation to dietary glycemic and insulin scores, both of which can capture postprandial insulinogenic effects of foods.

CRC consists of heterogeneous subtypes defined by molecular markers reflecting diverse combinations of genetic and epigenetic alterations in tumor cells.[7] Hence, the effect of a high-insulinogenic diet on survival among CRC patients could differ across tumor molecular markers, particularly those linked to the insulin signaling pathway. Molecular markers relevant to the insulin signaling pathway include two major insulin receptor substrates (IRS1 and IRS2) and downstream signaling molecules such as *KRAS, BRAF*, and *PIK3CA*.[2] Insulin, through the phosphatidylinositol-4,5-bisphosphonate 3-kinase (PI3K) signaling pathway, regulates the expression of fatty acid synthase (FASN),[8, 9] a key enzyme that converts excess carbohydrate into fatty acids.[10, 11] Fatty acids are essential components of cell membranes and thus, are important for proliferating neoplastic cells.[10] Indeed, FASN is

often overexpressed in cancer cells, which is hypothesized to be a selection mechanism for cancer cells to achieve a growth or survival advantage.[10] Additionally, emerging evidence suggests potential cross-talk between insulin and WNT/CTNNB1 (catenin beta 1) signaling pathways.[12, 13] Therefore, we examined the associations of glycemic and insulin scores with survival among CRC patients, accounting for potential heterogeneity in the relationships by aforementioned tumor molecular and immunohistochemical biomarkers.

Method

Study Population

Patients with confirmed colorectal adenocarcinoma were identified from two ongoing prospective cohort studies in the U.S., the Nurses' Health Study (NHS, 121,701 female nurses followed since 1976) and Health Professionals Follow-Up Study (HPFS, 51,529 male health professionals followed since 1986). In both cohorts, participants reported CRC diagnosis on biennial follow-up questionnaires. Upon obtaining participants' permission, study physicians blinded to participants' exposure status reviewed medical records to confirm self-reported diagnosis. Paraffin-embedded tissue blocks were collected from hospitals and were reviewed by a pathologist (S. O.). Through 2008, we documented a total of 3,759 CRC cases (2,437 from NHS and 1,322 from HPFS) from which a total of 1,546 tissue samples (860 from NHS and 686 from HPFS) were obtained. Those who did not provide tissue samples shared similar baseline characteristics as those who provided, except that those without tissue samples had modestly higher proportions of women (because tissue samples were harder to get from earlier periods and NHS cohort was formed much earlier than HPFS cohort), unknown tumor stage or location, high tumor grade, and ever smokers. From the 1,546 CRC patients, we excluded patients who were diabetic at the time of CRC diagnosis (because diabetic patients are recommended to follow a low-insulinogenic diet and have compromised pancreatic beta-cell function), those with missing information on exposure, and those who died within three months after exposure assessment (to minimize reverse causation due to undetected major illness). Thus, this analysis included a total of 1,160 CRC patients (657 from NHS and 503 from HPFS).

Assessment of Exposure

Dietary intake during the preceding year was collected through a validated semiquantitative food frequency questionnaire (FFQ) listing over 130 food items,[14, 15] which was administered in 1980 and 1984 in NHS and every 4 years starting from 1986 in both cohorts.

Glycemic index (GI) for a carbohydrate-containing food is defined as the area under the blood glucose response curve over two hours after eating the food relative to that after consuming the equivalent amount of carbohydrate as glucose or white bread.[16] The GI value for each carbohydrate-containing food on the FFQ was obtained from published estimates[17] supplemented with data from Prof. David J. Jenkins at the University of Toronto. For each participant, overall glycemic load ($GL_{overall}$) during the past year was calculated by multiplying the GI of each carbohydrate-containing food with carbohydrate amount (gram/serving) in the food and food consumption frequency (serving/day) and then by summing the values for reported carbohydrate-containing foods. Overall glycemic index ($GI_{overall}$)

was obtained by dividing the $GL_{overall}$ by the total amount (gram/day) of carbohydrate consumed.

Similarly, insulin index (II) for a calorie-containing food is defined as the area under the blood insulin response curve over two hours after eating 1000 kJ (239 kcal) of the food relative to that after consuming 1000 kJ of the reference food (white bread or glucose).[18] The II value for each calorie-containing food on the FFQ was obtained from published estimates[18] supplemented with data from Prof. Jennie Brand Miller at the University of Sydney. For each participant, overall insulin load ($IL_{overall}$) during the past year was obtained by calculating, for each calorie-containing food reported, the product of its II, calorie content (kcal/serving), and consumption frequency (serving/day) and then by summing the values. Overall insulin index ($II_{overall}$) was obtained by dividing the $IL_{overall}$ by the total calories (kcal/day) consumed.

Of note, postprandial insulin responses differ by a variety of factors including sex, diabetic status, and determinants of diabetes such as obesity and physical inactivity.[19–21] Thus, GI and II estimated among healthy young adults[16, 18] may have compromised applicability to our study populations, which mainly consist of older individuals with varying levels of the aforementioned characteristics. Yet, previous studies in our cohorts showed that glycemic scores ($GL_{overall}$ and $GI_{overall}$) and insulin scores ($IL_{overall}$ and $II_{overall}$) were positively associated with type 2 diabetes[22] or circulating levels of triglycerides (a marker of insulin resistance[23]),[24] which demonstrates the validity of these scores to reflect insulin response to foods in our cohorts.

Assessment of Covariates

Information on prognostic factors such as tumor characteristics (i.e., stage, grade, and location), age at diagnosis, and year of diagnosis (as a marker of advances in cancer treatment) was extracted from medical records by study physicians. Information on potential confounders, which were pre-specified based on known or suspected risk factors for death among CRC patients, was taken from the questionnaire used to assess exposure status. The potential confounders include smoking status, physical activity, aspirin use, alcohol consumption as well as height and weight to calculate body mass index (BMI).

Assessment of Tumor Molecular Markers

Sequencing of KRAS, BRAF and PIK3CA—DNA was extracted from paraffin-embedded tumor tissue. PCR and pyrosequencing targeted for *KRAS* (codons 12, 13, 61, and 146),[25, 26] *BRAF* (codon 600),[27] and *PIK3CA* (exons 9 and 20)[9, 28] were performed as previously described.

***Immunohistochemistry for* IRS1, IRS2, FASN *and* CTNNB1—**Methods of immunohistochemical methods and representative images are detailed in previous studies as follows: IRS1,[29] IRS2,[29] FASN,[30, 31] and CTNNB1.[32] As previously described,[29–32] for each marker, expression levels were graded by a single pathologist, and a selected group of >100 cases was independently reviewed by a second pathologist to assess reproducibility. Both pathologists were blinded to other data. We confirmed reasonable agreements between

the two pathologists, with κ coefficients of .69, .77, .57, and .80, for IRS1, IRS2, FASN and CTNNB1, respectively, at P<.001.[29–32]

Ascertainment of Death

Through 2014, deaths were ascertained based on reports from family or postal authorities or the National Death Index. These methods identified more than 98% of deaths in the cohorts.[33] The cause of death was assigned by study physicians blinded to participants' exposure status after review of medical records or death certificates. Our primary outcome was death from CRC and secondary outcome was death from any cause.

Statistical Analysis

Among all CRC patients and within each strata defined by tumor tissue markers, time-dependent Cox proportional hazards model was used to estimate hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the association of post-diagnostic glycemic and insulin scores with CRC death. Unless stated otherwise (see below), person-time of follow-up was accrued from the date of CRC diagnosis to the date of CRC death, death from others causes, or end of study period (2014), whichever came first. All analyses including univariable analysis were stratified by questionnaire cycle (pre-diagnostic questionnaire, post-diagnostic questionnaire; see below) and by CRC stage at diagnosis, a strong determinant of prognosis.

In this analysis, post-diagnostic values were obtained from the first questionnaire between 1–4 years after diagnosis, in view of evidence that recurrent or metastatic potential of CRC might be largely determined during this timeframe.[34] Additionally, because the vast majority of CRC deaths occur within 5 years after cancer diagnosis, dietary and other lifestyle factors during this time window are most likely relevant to CRC mortality.

Throughout the follow-up, values of exposure and potential confounders were updated once when the aforementioned post-diagnostic values became available (i.e. post-diagnostic questionnaire cycle). From the date of CRC diagnosis to date of this update, we did not use information collected after cancer diagnosis, because diet and other lifestyle may be affected by active cancer treatment in the early survival period. Instead, we obtained information from the questionnaire closest to the time of diagnosis among questionnaires within 4 years before diagnosis (i.e. pre-diagnostic questionnaire cycle). This approach is justifiable, because aggressiveness of CRC that affects recurrent or metastatic potential may be influenced by factors even before the physician-based diagnosis,[35] which depends on many factors including screening, symptoms, and incidental findings.

Of note, availability of the pre- and post-diagnostic data influenced the way person-time of follow-up was accumulated. For patients with only the pre-diagnostic data, they were censored 4 years after diagnosis (i.e. the upper limit of the time frame defining the post-diagnostic questionnaire cycle), because the data are less likely to be a surrogate of the post-diagnostic data beyond this time point. For patients with only the post-diagnostic data, person-time started to be accrued from the date of return of the post-diagnostic questionnaire.

Given a limited number of CRC deaths within strata of molecular markers, small differences in the number of CRC deaths across quantiles could lead to artificial differences in the magnitude of the estimates. Thus, we tested only a linear relationship and presented HRs and 95% CIs for one standard deviation increase in each score. Non-binary prognostic factors or confounders were adjusted for as a continuous variable in view of limited statistical power. The proportional hazard assumption was confirmed to hold through the Wald test on the cross-product term of exposure and survival time since exposure assessment. Potential interactions between glycemic and insulin scores and tumor molecular markers were tested by the Wald test on the cross-product term of the two variables. Additionally, potential heterogeneity in the relationships by anatomic locations of CRC (proximal, distal, and rectum) was explored.

To assess the validity of our findings, several sensitivity analyses were performed. First, to examine the effect of purely post-diagnostic exposure, we repeated the analyses excluding person-time contributions that borrowed information from the pre-diagnostic questionnaire cycle within 4 years before diagnosis. Second, considering that lifestyle factors often track over time, confounding by pre-diagnostic exposure can occur. To address this concern, the multivariable association was examined by additionally adjusting for pre-diagnostic scores, which were estimated by averaging glycemic and insulin scores collected more than four years before CRC diagnosis.

All statistical tests were two-sided, and $P < .05$ was considered statistically significant. However, given multiple hypothesis testing, statistical significance was interpreted conservatively, with P value of .001 – < .01 considered highly significant. Analyses were performed using SAS 9.3 (SAS Institute, Cary, NC).

Results

A total of 1,160 patients with stage I-IV CRC contributed 10,235 person-years and we identified 266 CRC deaths and 495 overall deaths. Baseline characteristics of the patients by $GL_{overall}$ and $IL_{overall}$ are summarized in Table 1. For both measures, those with higher scores tended to be diagnosed at older ages, to be ever smokers, and to drink less alcohol. Those with higher glycemic scores had higher fiber consumption. No apparent association was observed between any of the scores and tumor molecular markers.

For CRC mortality, in general, stronger positive associations were observed in multivariable analysis compared to univariable analysis. An increased CRC mortality was associated with higher insulin scores (HR=1.19, 95% CI=1.02 to 1.38 for $II_{overall}$; HR=1.23, 95% CI=1.04 to 1.47 for $IL_{overall}$), but not with glycemic scores (Table 2). Stratifying by tumor molecular markers, a higher insulin score was associated with an increased CRC mortality among *KRAS*-mutant, *BRAF* wild-type and mutant, and nuclear CTNNB1-negative CRC cases at the significance level of .01 – < .05. Notably, among *PIK3CA* wild-type and FASN-negative cases, the positive association was at the significance level of < .01 and was stronger for $IL_{overall}$ than $II_{overall}$. The HR for one standard deviation increment in $IL_{overall}$ was 1.45 (95% CI=1.17 to 1.79) for *PIK3CA* wild-type cases and 1.60 (95% CI=1.16 to 2.22) for FASN-negative cases. This positive association, particularly with $IL_{overall}$, was not abolished

in sensitivity analyses such as including person-time contributions purely from the post-diagnostic cycle and additionally adjusting for pre-diagnostic insulin scores averaged over the period of more than four years before CRC diagnosis (Supplemental table 1). Yet, the relationship between insulin scores and CRC mortality was not significantly different across *PIK3CA* wild-type and *PIK3CA* mutant and across FASN-negative and FASN-positive (P for interaction > .12). By anatomic site of CRC, an increased CRC mortality associated with insulin scores more was generally more evident with cancers of proximal colon and rectum than distal colon cancer (Supplementary Table 2). Among *PIK3CA* wild-type, the positive association was largely driven by rectal cancer. Glycemic scores were not appreciably associated with CRC mortality in any stratum of the tumor molecular markers tested, except among *PIK3CA* wild-type cases (HR=1.23, 95% CI=1.02 to 1.49 for $GL_{overall}$).

For all-cause mortality, the univariable analysis showed significantly elevated risks associated with glycemic and insulin scores in overall CRC cases and within strata of several tumor markers. However, these associations were no longer statistically significant in the multivariable analysis, except *PIK3CA* wild-type cases (HR=1.14, 95% CI=1.00 to 1.30 for $II_{overall}$; HR=1.17, 95%=1.01–1.35 for $IL_{overall}$) and FASN-negative CRC cases (HR=1.30, 95% CI=1.07 to 1.58 for $II_{overall}$; HR=1.37, 95%=1.08–1.73 for $IL_{overall}$) (Supplementary Table 3). Among FASN-negative CRC cases, a significantly elevated all-cause mortality persisted in all sensitivity analyses, ranging between 31% and 46% per one standard deviation increase in insulin scores (Supplementary Table 4). The positive association between $II_{overall}$ and all-cause mortality among FASN-negative CRC cases was significantly more pronounced (P for interaction=.01) compared to no evidence of an association among FASN-positive CRC cases (HR=0.92, 95% CI=1.07 to 1.58).

Discussion

In this prospective study conducted among CRC patients, higher post-diagnostic insulin scores but not higher glycemic scores were associated with an increased CRC mortality. The positive association was particularly evident among *PIK3CA* wild-type or FASN-negative subtypes. However, statistical interactions of insulin scores with these tumor biomarkers were not significant. Insulin scores were also positively associated with all-cause mortality only among *PIK3CA* wild-type or FASN-negative subtypes, with evidence of a significant interaction between $II_{overall}$ and FASN expression level.

In our study, dietary insulin scores were better predictors of CRC mortality than dietary glycemic scores. Glycemic scores primarily reflect postprandial glucose responses of carbohydrate-containing foods, while insulin scores directly reflect insulin increases induced by any foods containing calories. Thus, our findings may suggest that not glucose but insulin response after food ingestion may be more relevant to the prognosis of CRC patients. Serologic studies conducted among CRC patients showed consistent results, which observed a positive association with pre-diagnostic circulating C-peptide (a marker of insulin secretion),[36] but not with circulating glucose.[37] Of note, postprandial spikes in glucose as indicated by glycemic scores represent only acute hyperglycemia. Thus, we cannot rule out the possibility that chronic hyperglycemia may influence CRC mortality. Indeed, a case-control study observed that, among CRC patients with type II diabetes mellitus for whom

long-term glycemic control was determined by hemoglobin A1c levels, poor glycemic control was associated with a decreased 5-year overall survival compared to good glycemic control.[38]

Alternatively, inconsistent findings between glycemic and insulin scores may be explained by confounding by protein intake. Protein intake, which induces insulin secretion without increasing blood glucose levels,[39–41] is captured by insulin scores but not by glycemic scores. Independent of its insulinogenic effect, protein intake activate mammalian mechanistic target of rapamycin (MTOR), a major downstream effector of the oncogenic insulin pathway.[42, 43] Therefore, insulin score as a surrogate of protein's effect on MTOR, may be associated with an increased CRC mortality. However, the positive associations between insulin scores and CRC mortality in overall patients as well as among *PIK3CA* wild-type and FASN-negative subtypes persisted even after adjusting for protein intake (Supplementary Table 5). Thus, protein *per se* is unlikely to drive the differential findings across glycemic scores and insulin scores.

The positive relationship between insulin scores and CRC mortality did not vary significantly by any of the pre-specified molecular markers, but it was highly significant among patients whose CRC was *PIK3CA* wild-type or FASN-negative. Given statistically non-significant interactions, the heterogeneous findings should not be over-interpreted, but the association of $II_{overall}$ with all-cause mortality varied significantly by FASN expression level. Additionally, in a previous study, an increased risk of developing CRC associated with a high BMI compared to normal BMI was confined to FASN-negative CRC.[44] This study suggests that FASN-negative CRC may develop through a carcinogenic pathway that relies heavily on excess energy and resulting insulin signaling transduction for progression. Furthermore, by virtue of the link between BMI and FASN-negative CRC, patients diagnosed with FASN-negative CRC were more likely to be overweight and obese individuals (65% vs. 55% in FASN-positive), who yield pronounced postprandial insulin spikes after consuming insulinogenic foods.[20] Thus, it is conceivable that prognosis of FASN-negative CRC may as well be sensitive to signals from the insulin pathway. Of note, FASN expression is up-regulated by AKT signaling, which is activated by *PIK3CA* mutation.[9] As such, CRC patients with *PIK3CA* wild-type are likely FASN-negative. This is consistent with our finding that the influence of high-insulinogenic diets on CRC survival was pronounced among patients with *PIK3CA* wild-type or FASN-negative CRC. Thus, in the absence of FASN that confers growth and survival advantage,[10] mitogenic insulin signals activated by high insulinogenic diets may play a particularly important role in promoting CRC recurrence and metastasis.

Additionally, because *PIK3CA* mutation leads to constitutive activation of the insulin pathway regardless of the upstream insulin signal,[45] CRC with *PIK3CA* wild-type but not mutant can respond to changes in insulin concentrations induced by diets. This explanation, of note, may seemingly contradict our finding that a positive association between insulin scores and CRC mortality was pronounced in *BRAF*-mutant CRC compared to its wild type CRC. Because we tested multiple tumor biomarkers, we *a priori* set to give more credence to findings with a high statistical significance. The level of statistical significance for the association among *BRAF*-mutant CRC patients was .01 – < .05. relative to that of .001 – < .

01 for *PIK3CA* wild-type CRC patients. Further, the pronounced association in *PIK3CA* wild-type CRC than in its mutant CRC is mechanistically consistent with that in FASN-negative CRC than in FASN-positive CRC as described above. The conflicting heterogeneity in the association by *BRAF*-mutation status is more likely due to chance, although we cannot rule out the possibility that it may turn out to be real rather than chance in future studies.

There are several limitations in our study. First, because we used pre-diagnostic information to represent information during the early survival period, our finding may not be entirely attributable to post-diagnostic diet. Yet, as justified in the method section, due to somewhat arbitrary components in the timing of the physician-based diagnosis of CRC, the diet-survival relationship around the diagnosis may not differ materially by pre- versus post-diagnosis. Furthermore, compared to an analysis following only CRC patients with post-diagnostic diet, our approach does not premise the survival of CRC patients to fill out a post-diagnostic questionnaire, which reduces potential selection bias and increases power. Second, due to limited availability of tumor marker information and multiple molecular markers tested, our statistical power to detect interactions of glycemic and insulin scores with tumor markers are limited. Given this, we took biological plausibility into account when interpreting our seemingly heterogeneous results that did not reach statistical significance. Third, foods are eaten in combinations and potential interactions among ingested foods influence postprandial insulin response.[41] Thus, glycemic and insulin scores of overall diet, which were calculated based on GI and II values of individual foods when consumed separately, may not fully capture insulin response to mixed foods. Nonetheless, a recent study showed that observed postprandial insulin response to a composite meal was strongly correlated with insulin demand predicted by $GL_{overall}$(γ=.68) and $IL_{overall}$(γ=.78).[46] The stronger correlation with the insulin score may in part explain the better ability of insulin scores to predict CRC survival in our study. Fourth, we lack information on cancer treatment, which could influence dietary choice of cancer patients or modify the diet and survival relationship. However, all of our analyses were stratified by stage at diagnosis, which is the principal determinant of cancer treatment. Lastly, given that acute postprandial insulin spike and chronic high insulin levels are physiologically distinctive concepts, we cannot extrapolate our results into the association between chronic hyperinsulinemia and CRC prognosis.

Our study has several strengths, including prospective design, high follow-up rates and the ability to adjust for a variety of confounders. By using the molecular pathological epidemiology (MPE) approach that integrates tumor biomarker information into the conventional analysis of diet and survival, we could explore whether some patients might be more vulnerable to a high insulinogenic diet or not; this type of information may help identify patients who likely benefit most from dietary interventions. Indeed, our study demonstrated that the possible positive association between insulin scores and CRC mortality might be specific to particular CRC subtypes (i.e. *PIK3CA* wild-type and FASN-negative), which could enhance causal inference.[47] Thus, our study based on the MPE approach has important implications in the era of tumor molecular diagnostics and precision medicine.

In conclusion, we found evidence that a high-insulinogenic diet after CRC diagnosis, as indicated by high insulin scores, may contribute to worse CRC survival, especially when CRC is negative for *PIK3CA* mutation and FASN expression. Because this is the first study to investigate dietary glycemic and insulin scores in relation to CRC survival by tumor biomarkers, our results need to be confirmed in other studies. Additionally, future studies directly examining post-diagnostic post-prandial and fasting insulin levels in relation to CRC survival by tumor biomarkers are warranted to further our mechanistic understanding on the role of insulin in CRC survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Use of standardized official symbols

We use HUGO (Human Genome Organization) - approved official symbols for genes and gene products, including BRAF, CTNNB1, FASN, IRS1, IRS2, KRAS, MTOR, and PIK3CA; all of which are described at www.genenames.org. Gene names are italicized, and gene product names are non-italicized.

Novelty and Impact

Diet is a major determinant of circulating insulin levels, which may influence colorectal cancer (CRC) survival. Additionally, the effect of a diet inducing higher insulin secretion on CRC survival may differ by tumor biomarkers related to the insulin signaling pathway. This prospective cohort study of CRC patients suggests that a high-insulinogenic diet may lead to poor CRC survival mainly among patients with *PIK3CA* wild-type or FASN-negative CRC.

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Table 1

Characteristics of Person-years over Follow-up Period and CRCs according to Overall Glycemic Load and Insulin Load

	Glycemic Load				Insulin Load			
Characteristics[1]	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
Load[2]	91.5(14.6)	114(10.8)	128(12.9)	151(19.2)	627(75.6)	725(68.3)	784(75.2)	876(93.3)
Index[2]	50.5(3.5)	52.1(2.9)	53.8(2.6)	55.3(3.1)	35.5(3.4)	41.0(1.4)	44.3(1.5)	49.3(3.2)
Person-years	2371	2500	2549	2815	2372	2434	2742	2687
Male, %	43.7	42.7	43.2	43.1	43.2	42.9	43.4	43.2
Year of diagnosis	96.3(7.1)	97.9(6.4)	97.6(5.8)	97.6(5.2)	97.6(6.6)	96.9(6.6)	97.3(6.1)	97.7(5.3)
Age at diagnosis	66.9(9.0)	68.5(8.8)	68.8(8.7)	69.6(8.5)	68.2(8.4)	67.7(8.8)	68.4(9.4)	69.5(8.6)
Tumor stage,%								
I	25.1	28.1	26.5	27.7	25.4	25.8	27.1	29.1
II	30.6	34.3	30.5	31.6	32.5	31.5	34.4	28.6
III	23.8	22.1	28.3	25.1	21.9	26.3	24.2	26.9
IV	10.3	7.3	7.5	6.6	9.2	8.5	5.7	8.1
Missing	10.3	8.2	7.2	9.0	11.0	7.9	8.6	7.3
High tumor grade, %	9.6	8.6	10.7	9.2	9.4	10.9	10.1	7.7
Tumor location, %								
Proximal colon	39.7	50.0	46.9	50.8	43.4	46.2	46.5	51.3
Distal colon	33.8	30.1	28.9	30.1	33.3	32.2	29.1	28.4
Rectum	26.4	19.9	24.1	19.1	23.2	21.7	24.4	20.3
Cytoplasmic IRS1 expression[3]								
(−)	31.2	30.5	34.2	31.4	30.5	30.6	34.4	31.9
(+)	12.7	11.1	13.2	15.4	12.7	12.3	12.3	15.0
Cytoplasmic IRS2 expression[3]								
(−)	69.2	71.5	70.3	65.9	68.5	72.6	71.5	64.2
(+)	30.8	28.5	29.7	34.1	31.5	27.4	28.5	35.8
***KRAS* mutation**[3]								
(−)	50.7	47.3	50.2	49.0	50.9	53.0	45.2	48.2
(+)	33.6	35.6	36.2	35.6	34.0	32.2	38.5	36.3

	Glycemic Load				Insulin Load			
Characteristics[1]	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
***BRAF* mutation[3]**								
(−)	77.1	75.7	77.6	75.2	77.2	77.2	76.2	74.9
(+)	10.0	13.1	11.0	12.5	11.0	12.5	11.5	11.7
***PIK3CA* mutation[3]**								
(−)	67.0	71.2	67.5	66.8	66.7	70.7	67.2	68.1
(+)	14.4	12.8	15.1	14.3	15.1	13.3	14.5	13.7
Cytoplasmic FASN expression[3]								
(−)	26.2	24.6	27.9	25.3	24.8	25.4	27.8	26.0
(+)	41.0	40.7	43.0	46.2	38.8	44.4	42.7	44.9
Nuclear CTNNB1 expression[3]								
(−)	36.9	42.3	46.5	45.9	37.9	45.1	41.0	47.6
(+)	39.1	32.1	34.0	34.7	36.8	33.5	36.3	33.3
BMI, kg/m2	26.5(4.3)	25.9(4.0)	26.4(4.2)	25.7(4.2)	26.3(4.1)	26.4(4.3)	26.0(4.3)	25.8(4.0)
Physical activity, MET-hrs/wk	17.5(20.0)	20.9(25.3)	21.5(23.6)	20.0(21.5)	20.7(23.5)	18.9(20.9)	19.8(23.7)	20.5(22.7)
Never smokers, %	66.8	60.0	58.8	51.0	68.2	60.8	56.4	51.1
Regular aspirin use, %	25.5	28.1	28.3	27.5	27.0	25.8	27.8	28.9
Alcohol intake, g/d	18.2(20.7)	9.0(12.1)	6.1(9.6)	3.8(7.4)	20.4(20.7)	8.3(11.2)	5.3(8.6)	3.1(6.3)
Fiber intake,[2] g/d	18.1(5.4)	21.2(6.7)	21.9(5.8)	24.5(7.9)	20.7(8.0)	20.8(6.0)	21.7(6.9)	22.4(6.5)

[1] Values are means (SD) or percentages.

[2] Values were adjusted for total energy intake.

[3] Percentage does not sum up to 100% due to missing information.

Table 2

HR[1] and 95% CI for the Associations of Overall Glycemic and Insulin Scores with CRC Mortality by Tumor Molecular Markers

Marker Status	CRC mortality				
	Death/person-years	Glycemic Index	Glycemic Load	Insulin Index	Insulin Load
All CRC patients					
Univariable	266/10235	1.03 (0.90, 1.17)	1.09 (0.97, 1.23)	1.18 (1.04, 1.34)*	1.14 (1.01, 1.28)*
Multivariable[2]		1.02 (0.89, 1.16)	1.10 (0.94, 1.29)	1.19 (1.02, 1.38)*	1.23 (1.04, 1.47)*
IRS1 expression					
(−)					
Univariable	99/3491	0.96 (0.78, 1.18)	0.98 (0.79, 1.20)	1.14 (0.93, 1.40)	0.98 (0.80, 1.21)
Multivariable[2]		0.97 (0.77, 1.22)	1.06 (0.80, 1.42)	1.15 (0.90, 1.47)	1.21 (0.90, 1.63)
(+)					
Univariable	33/1409	0.98 (0.67, 1.45)	1.50 (1.01, 2.25)*	1.15 (0.77, 1.71)	1.52 (1.01, 2.28)*
Multivariable[2]		1.05 (0.69, 1.59)	1.50 (0.94, 2.41)	1.22 (0.74, 2.02)	1.44 (0.82, 2.53)
IRS2 expression					
(−)					
Univariable	91/3437	1.04 (0.84, 1.29)	1.08 (0.87, 1.36)	1.15 (0.92, 1.43)	1.06 (0.84, 1.33)
Multivariable[2]		1.03 (0.82, 1.29)	1.21 (0.88, 1.68)	1.13 (0.86, 1.49)	1.19 (0.85, 1.67)
(+)					
Univariable	41/1503	0.93 (0.68, 1.27)	1.14 (0.84, 1.56)	1.22 (0.90, 1.66)	1.20 (0.89, 1.61)
Multivariable[2]		0.98 (0.70, 1.38)	1.12 (0.77, 1.62)	1.27 (0.88, 1.83)	1.36 (0.91, 2.05)
***KRAS* mutation**					
(−)					
Univariable	123/5195	1.01 (0.83, 1.23)	0.93 (0.78, 1.11)	1.09 (0.90, 1.32)	1.04 (0.87, 1.24)
Multivariable[2]		1.00 (0.81, 1.23)	1.01 (0.79, 1.29)	1.16 (0.92, 1.46)	1.26 (0.95, 1.66)
(+)					
Univariable	108/3473	1.08 (0.88, 1.33)	1.26 (1.04, 1.53)*	1.36 (1.11, 1.68)**	1.30 (1.08, 1.56)**

Marker Status	CRC mortality				
	Death/person-years	Glycemic Index	Glycemic Load	Insulin Index	Insulin Load
Multivariable[2]		1.06 (0.86, 1.31)	1.21 (0.94, 1.56)	1.28 (1.00, 1.62) *	1.29 (0.98, 1.69)
***BRAF* mutation**					
(−)					
Univariable	205/7826	1.00 (0.86, 1.16)	1.05 (0.91, 1.21)	1.19 (1.02, 1.38) *	1.13 (0.99, 1.30)
Multivariable[2]		0.98 (0.83, 1.14)	1.03 (0.86, 1.24)	1.19 (1.00, 1.41)	1.23 (1.01, 1.50) *
(+)					
Univariable	29/1116	1.09 (0.72, 1.65)	1.23 (0.84, 1.81)	1.37 (0.93, 2.01)	1.40 (0.93, 2.10)
Multivariable[2]		1.12 (0.72, 1.72)	1.49 (0.89, 2.50)	1.47 (0.93, 2.32)	1.81 (1.02, 3.21) *
***PIK3CA* mutation**					
(−)					
Univariable	177/6852	1.08 (0.92, 1.26)	1.16 (1.00, 1.35)	1.31 (1.12, 1.54) ***	1.21 (1.04, 1.41) *
Multivariable[2]		1.07 (0.91, 1.26)	1.23 (1.02, 1.49) *	1.36 (1.13, 1.63) **	1.45 (1.17, 1.79) ***
(+)					
Univariable	40/1435	1.13 (0.78, 1.63)	0.93 (0.68, 1.27)	0.99 (0.71, 1.37)	0.99 (0.73, 1.33)
Multivariable[2]		0.97 (0.66, 1.42)	0.65 (0.37, 1.16)	0.68 (0.42, 1.10)	0.63 (0.35, 1.12)
FASN expression					
(−)					
Univariable	81/2826	1.09 (0.87, 1.38)	0.96 (0.77, 1.21)	1.26 (1.01, 1.58) *	1.07 (0.85, 1.33)
Multivariable[2]		1.08 (0.84, 1.39)	1.32 (0.96, 1.80)	1.47 (1.12, 1.92) **	1.60 (1.16, 2.22) **
(+)					
Univariable	114/4829	0.91 (0.74, 1.12)	1.02 (0.83, 1.25)	1.00 (0.81, 1.23)	1.01 (0.83, 1.24)
Multivariable[2]		0.96 (0.78, 1.18)	0.95 (0.73, 1.22)	1.00 (0.79, 1.27)	1.06 (0.80, 1.40)
Nuclear CTNNB1 expression					
(−)					
Univariable	131/4385	0.98 (0.81, 1.18)	1.04 (0.87, 1.24)	1.19 (0.99, 1.44)	1.14 (0.96, 1.35)

Marker Status	CRC mortality				
	Death/person-years	Glycemic Index	Glycemic Load	Insulin Index	Insulin Load
Multivariable[2] (+)	85/3702	1.00 (0.83, 1.21)	1.03 (0.81, 1.30)	1.19 (0.97, 1.47)	1.31 (1.02, 1.67) *
Univariable		1.13 (0.89, 1.42)	1.11 (0.90, 1.37)	1.14 (0.91, 1.43)	1.12 (0.89, 1.40)
Multivariable[2]		1.06 (0.82, 1.37)	1.07 (0.81, 1.42)	1.02 (0.76, 1.38)	1.02 (0.73, 1.42)

Note: P for trend is marked as

* if .01–<.05;

** if .001–<.01;

*** if <.001

Note: Among tumor markers of which there is evidence of a significant association in the multivariable analysis at least in one stratum, P for interaction was > .12

[1] HR is estimated for one standard deviation increase, which is 3.6 for overall glycemic index, 26.4 for overall glycemic load, 5.7 for overall insulin index, and 118.9 for overall insulin load.

[2] Multivariable analysis was stratified by cancer stage (I, II, III, IV, missing) and questionnaire cycle (pre-diagnostic questionnaire, post-diagnostic questionnaire); adjusted for tumor grade (well-differentiated, poorly-differentiated), tumor location (right-sided, left-sided), age at diagnosis (as a continuous variable), year of diagnosis (as a continuous variable), sex, post-diagnosis values of BMI (as a continuous variable), physical activity (as a continuous variable), smoking status (never, ever), aspirin use (yes, no), alcohol intake (as a continuous variable), and fiber intake (as a continuous variable, for glycemic scores analysis).