Insulin-Like Growth Factor-I and Insulin Are Associated With the Presence and Advancement of Adenomatous Polyps

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Background & Aims: Insulin and insulin-like factor-I (IGF-I) affect proliferation, differentiation, and apoptosis and are potential risk factors for colorectal cancer (CRC). Visceral obesity, possibly via hyperinsulinemia, has also been linked to CRC risk. We evaluated the relationship of insulin, IGF-I, insulin-like growth factor binding protein (IGFBP) 3, and visceral adipose tissue (VAT) in subjects with adenomatous polyps, the precursor lesion of colorectal cancer. *Methods:* Participants were asymptomatic subjects who underwent screening flexible sigmoidoscopy (FSG) within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Subjects underwent single-slice, computerized tomography scanning to measure VAT and serum fasting insulin, IGF-I, and IGFBP-3 measurements. *Results:* Four hundred fifty-eight subjects were enrolled, of which 202 subjects had an adenoma, 70 of which were an advanced adenoma. IGF-I ($P = .02$), IGF-I/IGFBP-3 ratio ($P = .003$), and insulin ($P = .02$) were significantly increased in subjects with adenomas compared with controls. In an unadjusted logistic regression analysis using sex-specific quartile cut points, subjects in quartile 4 in comparison with quartile 1 of IGF-I (odds ratio [OR] = 1.7; [95% CI: 1.0–2.9], Ptrend = .03), IGF-I/IGFBP-3 ratio (OR = 1.9 [95% CI: 1.1–3.3], Ptrend = .01), and insulin (OR = 2.1 [95% CI: 1.2–3.6], Ptrend = .04) were at increased risk of adenoma. When limiting the case group to advanced adenomas, the effect was more pronounced: IGF-I (OR = 2.8 [95% CI: 1.3–6.2], Ptrend = .006), IGF-I/IGFBP-3 ratio (OR = 2.3, [95% CI: 1.0–5.2], Ptrend = .04), and insulin (OR = 2.3 [95% CI: 1.1–4.9], Ptrend = .14). Visceral adipose tissue was not associated with adenoma risk. *Conclusions:* Levels of IGF-I, ratio of IGF-I/IGFBP-3, and insulin are associated with adenomas and even more so with advanced adenomas. These data support the hypothesis that insulin and IGF-I may contribute to the development and advancement of adenomatous polyps.

E nvironmental risk factors for colorectal cancer (CRC) including fat, fiber, or micronutrient intake do not explain the link between obesity,[1–3] diabetes,[4–6] or physical activity[1,7,8] and CRC risk. An alternative hypothesis that incorporates these factors into a pathophysiologic model for CRC risk uses insulin resistance and hyperinsulinemia to link obesity and CRC risk.[9,10] Support of the "insulin hypothesis" of CRC is provided by in vitro[11–13] and epidemiologic data showing a relationship between diabetes and obesity and especially visceral obesity and CRC.[3,5,6,14] Studies have demonstrated an association between waist circumference or waist-to-hip ratio, surrogate measures of intraabdominal fat or visceral adipose tissue (VAT), and subsequent development of CRC[8,15] and large adenomatous polyps ($\geq$1cm in size).[14] Visceral obesity is strongly associated with increased insulin levels.[16–19] Physical inactivity is associated with an increased amount of visceral adipose tissue[20,21] and has also been linked to increased risk for CRC.[1,7,8]

An association between serum insulin[15] or serum C-peptide and incident CRC[22,23] has also been observed. In the Cardiovascular Health Study cohort, the risk of subsequent colon cancer was 2.0- to 2.6-fold increased in subjects in the highest quartile compared with the lowest in waist circumference, waist to hip ratio, fasting and 2-hour postprandial glucose, and 2-hour postprandial insulin.[15] A biologic basis for a central role for insulin in CRC pathogenesis has been established because insulin and insulin-like growth factors (IGFs) stimulate cell proliferation in the colonic mucosa and in carcinoma cell lines and affect apoptosis.[9,11–13] IGFs and insulin-like growth factor binding proteins (IGFBPs) also have important roles in cell cycle regulation and possess mitogenic and antiapoptotic properties.[24] IGF-I, IGFBP-3, and the ratio of the 2 have been implicated as risk factors for CRC, although not consistently.[25] Because insulin and IGF-I can be measured in blood, these factors could

have public health impact if they were determined to be a risk factor for adenoma or cancer.

The association of insulin and insulin-like growth factor to adenomatous polyps has been examined previously in a limited fashion, in small numbers of patients.[26–28] Because of the growth-promoting effects of insulin and IGFs and their possible association with invasive CRC, it is important to examine the relationship of these factors to adenomatous polyps, the precursor lesion of CRC. We concurrently evaluated the relationship of insulin, IGF-I, IGFBP-3, and VAT in asymptomatic subjects who presented for screening flexible sigmoidoscopy.

Materials and Methods

Population

Subjects in this study are drawn from enrollees, originally recruited through mass mailings, in the intervention arm of the Pittsburgh site of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), a multicenter, randomized clinical trial evaluating the effect of cancer screening tests on site-specific cancer mortality.[29] Over 154,000 subjects have been enrolled in the PLCO trial nationally, and nearly 17,000 were enrolled in the broader Pittsburgh region between 1993 and 2000. Subjects in the intervention arm of the trial undergo periodic cancer screening tests including chest x-ray, flexible sigmoidoscopy, and, for men, digital rectal exam and PSA and, for women, CA-125 and vaginal ultrasound. Subjects are referred to personal physicians for evaluation of screen-detected abnormalities and tracked to determine the results from subsequent diagnostic workups. Pathologic findings are based on the local, community pathologist's interpretation and not subject to central review. Participants in the PLCO trial met the following eligibility criteria: (1) age 55–74 years; (2) not currently undergoing treatment for cancer except basal cell or squamous cell skin cancer; (3) no known prior cancer of the colon, rectum, prostate, lung, or ovaries; (4) no surgical removal of colon, lung, ovary, or prostate; (5) not participating in another cancer screening or cancer prevention trial; (6) males not taking Proscar in the past 6 months, females not taking Novaldex in the past 6 months; (7) able to provide informed consent; (8) no more than 1 PSA test in the past 3 years (for subjects randomized after April 1995); and (9) no colonoscopy, sigmoidoscopy, or barium enema in the past 3 years (for subjects randomized after April 1995).

Subjects for this substudy underwent a screening flexible sigmoidoscopy as part of the trial and were invited by mail to participate. Subjects with a "polyp" on sigmoidoscopy were preferentially approached (69.2%) in comparison with subjects who had a negative flexible sigmoidoscopy exam (30.8%), to increase the population with an adenoma, but without knowledge of the specific pathologic findings at diagnostic colonoscopy at the time of recruitment. To encourage balance between cases and controls, we randomly selected controls according to

sex, age (5-year age blocks), and PLCO recruitment period (3 month blocks) of the cases. Subjects were recruited over a 2-year period between January 1998 and November 1999.

Participants were asked to undergo a single-slice CT scan through the L4-L5 interspace for quantification of visceral adipose tissue and, at a separate visit, a fasting blood draw and subcutaneous adipose tissue aspiration. Case subjects who underwent diagnostic colonoscopy for a polyp found on sigmoidoscopy participated in this study after their diagnostic workup was complete and their polyps had been removed. The median time from polypectomy to blood draw in subjects with adenomas was approximately 1 year. The intended sample size calculated for detecting a difference in visceral adipose tissue between subjects with adenomatous polyps and controls was 450. Overall, 480 of 981 (48.9%) subjects invited by mail agreed to participate. Control subjects included those with nonadenomatous findings on colonoscopy and subjects with a negative screening sigmoidoscopy. Subjects who underwent colonoscopy were characterized on the basis of the most advanced finding and grouped into advanced adenoma, nonadvanced adenoma, and nonadenomatous polyp (eg, hyperplastic polyp) categories. An adenoma was defined as advanced if it contained villous features (villous or tubulovillous), was large ($\geq$1 cm as estimated by the endoscopist), or had severe dysplasia.

To investigate the potential for selection bias created as a consequence of nonparticipation, we compared, among men and women, the age, race, marital status, education, cigarette history, history of diabetes, and body mass index (BMI) of enrolled and nonenrolled individuals. Older men and younger women approached to serve as potential control subjects were more likely to enroll ($P < .05$), but no other factor was associated with differential enrollment.

Measurement of IGF-I, IGFBP-3, and Insulin

The serum was initially frozen at $-70°C$ and shipped in bulk for analysis. Analyses were performed on serum from the first thaw cycle. IGF-I was extracted using acid ethanol cryoprecipitation to remove residual IGFBPs from the serum[30] (http://members.mint.net/ea6bii/rogue/Methods.html). Subsequently, the supernatant was assayed for IGF-I using a polyclonal Ab to IGF-I (Nichols Institute, San Juan Capistrano, CA). IGFBP-3 was analyzed by an immunoradiometric assay (IRMA) methodology (DSL, Webster, TX). All samples were extracted and assayed in duplicate by personnel blinded to their case control status. Each batch of 38 samples was analyzed simultaneously with 2 in-house controls. The interassay coefficient of variation for IGF-I was 7.1% and for IGFBP-3 4.5%. The molar ratio was calculated by multiplying 3.7*IGF-I/IGFBP-3. Fasting insulin was measured via a ^{125}I radioimmunoassay (Linco Research, Inc.), with a coefficient of variation of 2.6%.

Measurement of Visceral Adipose Tissue

Subjects were scanned with a 9800-CT scanner (General Electric, Milwaukee, WI). The L4-L5 interspace was de-