

Nonsteroidal Anti-inflammatory Drug Use, Body Mass Index, and Anthropometry in Relation to Genetic and Flow Cytometric Abnormalities in Barrett's Esophagus¹

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Abstract

A dramatic increase in the incidence of esophageal adenocarcinoma has occurred among men in the United States over the last two decades. The underlying reasons remain largely unknown, although the increasing prevalence of obesity likely plays a role. Most adenocarcinomas arise in a metaplastic epithelium termed Barrett's esophagus (BE) that develops in approximately 10% of persons who have chronic gastroesophageal reflux. Persons with BE are at high risk (0.5–1.0%/year) of progressing to cancer. In a cross-sectional study of 429 persons with BE, we evaluated the associations between increased body mass index, anthropometric measures, cigarette smoking, use of nonsteroidal anti-inflammatory drugs (NSAIDs) and markers of increased risk, including aneuploidy, increased 4N fraction, loss of heterozygosity (LOH) of 17p and 9p alleles, and high-grade dysplasia (HGD). In logistic regression models adjusting for age, gender, NSAID use, and cigarette smoking, increasing waist:hip ratio was related to increasing risk of aneuploidy (trend $P = 0.01$), 17p LOH (trend $P = 0.005$), and 9p LOH (trend $P = 0.007$). The odds ratios comparing highest to lowest quartiles were 4.3 [95% confidence interval (CI), 1.2–15.6] for aneuploidy, 3.9 (95% CI, 1.3–11.4) for 17p LOH, and 2.7 (95% CI, 1.2–6.3) for 9p LOH. A nonsignificant trend was also observed for increased 4N fraction, whereas little association was found for HGD. Similar patterns of risk were noted for other anthropometric measures such as

waist:thigh and abdomen:thigh ratios. There was no evidence that elevated body mass index increased risk of any of the biomarkers. Suggestive evidence also was found for a protective effect of NSAID use. The odds ratios for current users, compared with those who never used NSAIDs regularly, were 0.6 (95% CI, 0.3–1.4) for increased 4N, 0.6 (95% CI, 0.3–1.3) for aneuploidy, 0.3 (95% CI, 0.1–0.7) for 17p LOH, and 0.7 (95% CI, 0.4–1.2) for HGD. There was no association between NSAID use and risk of 9p LOH. We conclude that an abdominal distribution of body fat, which is more common in men and is termed male-pattern obesity, may be a strong predictor of risk of neoplastic progression among persons with BE and may account in part for the male predominance of BE and esophageal adenocarcinoma. We also conclude that NSAID use may reduce the risk of progression to cancer in this population. Prospective studies are needed to confirm these results.

Introduction

A dramatic increase in the incidence of EA⁴ has occurred in the United States and Western Europe over the last two decades (1). The highest rate occurs among white males, whose incidence increased more than 350% in the United States between 1974 and 1994. Similar increases have been observed for white females and black males. However, their incidence rates remain substantially lower, with a male:female incidence rate ratio of 8 and a white:black ratio of approximately 5 (1). Once considered a relatively rare diagnosis, EAs now comprise more than three of every five new esophageal cancers (1). The underlying reasons for the rapid increase in incidence and the unusual gender and race patterns remain largely unknown.

Most EAs arise in a metaplastic epithelium termed BE that develops in approximately 10% of persons who have chronic gastroesophageal reflux disease (2, 3). Each year, approximately 0.5–1% of persons with BE progress to EA, a rate estimated to be 30–40 times higher than the general population (4–13). However, there has been little systematic research into understanding why the esophageal epithelium in some persons progresses to cancer, whereas in others it remains in a relatively benign state indefinitely (14).

One of the strongest and most consistent risk factors identified for EA is increased BMI. A 3- to 7-fold higher risk has been associated with the highest quartile of BMI in recent case-control studies (15–17). It is not yet clear how being

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⁴ The abbreviations used are: EA, esophageal adenocarcinoma; BE, Barrett's esophagus; BMI, body mass index; OR, odds ratio; CI, confidence interval; NSAID, nonsteroidal anti-inflammatory drug; LOH, loss of heterozygosity; HGD, high-grade dysplasia; GI, gastrointestinal; IGF, insulin-like growth factor; COX, cyclooxygenase.

overweight increases risk. One hypothesis is that it exacerbates gastroesophageal reflux through increased intra-abdominal pressure (18) and the development of hiatal hernia (19). Because fat distribution favoring the abdomen (which is more common in men and is termed male-pattern obesity) is thought to produce more severe reflux than fat distributed to the thighs and hips, some of the gender differences in incidence of EA may be explained by gender-related differences in the distribution of excess adipose tissue. This issue cannot be easily explored in case-control studies of cancer because anthropometric measurements would be of doubtful relevance in persons with cancer.

A large body of evidence from both human and experimental animal studies indicates that aspirin and other NSAIDs can reduce the incidence of colorectal cancer, as well as the number and size of polyps in persons with familial adenomatous polyposis (20–33). The possible role of NSAIDs in reducing cancer risk at other sites, including the esophagus, has only recently received attention. Nevertheless, several studies suggest a possible protective effect for esophageal cancer as well (25, 34, 35).

In this cross-sectional analysis of persons with BE, we examined the association between BMI, body fat distribution, NSAID use, and other potential risk factors and several biomarkers demonstrated or suspected to be predictive of subsequent cancer development. These intermediate markers included abnormalities of histology (HGD), flow cytometry (aneuploidy and increased 4N), and genetics [LOH of 9p, targeting *p16*, and 17p, targeting *p53* (36–38)]. These abnormalities are among the most common in EA, and all develop in premalignant Barrett's epithelium before cancer (39–44). Studies of the genetic and flow cytometric biomarkers suggest that they typically arise in the order 9p LOH, 17p LOH, increased 4N (tetraploidy), and aneuploidy, although there are exceptions to this order (45–47). A recent study showed that lesions affecting *p16*, including 9p LOH, are the earliest known clonal genetic abnormalities in BE (48). Furthermore, 17p LOH has recently been shown to identify a subset of patients with BE who are at increased risk for progressing to tetraploidy, aneuploidy, and EA during prospective surveillance (38). Similarly, in prospective studies, tetraploid cell populations predict subsequent development of aneuploidy and EA, and aneuploid cell populations predict development of EA (36, 46, 49).

Materials and Methods

Study Participants. This study was based on persons participating in the Seattle BE Project, a dynamic cohort study of persons with BE that began in 1983. Each participant in this ongoing program of cancer surveillance periodically undergoes an endoscopy with multiple biopsies according to a standard protocol (36). Beginning in February 1995, the study was expanded to include an extensive personal interview with dietary assessment, anthropometric measurements, and collection of a blood sample. All new and ongoing participants underwent a baseline evaluation at their initial visit on or after February 1, 1995. At subsequent visits, they underwent a shorter follow-up evaluation that updated information obtained at baseline. This report is based on baseline evaluations carried out between February 1, 1995 and March 17, 1999 on 429 participants with BE. Of these, 124 (29.1%) were ongoing participants who had been under surveillance for a median of 5.6 years (range, 0.1–12.4 years). The remaining 305 (70.9%) were participants who received their initial evaluation after February 1, 1995. This study was approved by the Institutional Review Board of

the University of Washington, with reciprocity from the Fred Hutchinson Cancer Research Center.

Assessment of Intermediate Markers. The methods for endoscopy, biopsy, and flow cytometric analyses have been described previously (36, 50, 51). For most participants, four quadrant biopsies were obtained from every other centimeter of the Barrett's segment. For those with a history of HGD, these were obtained every centimeter. One tissue sample from every 2 cm of the Barrett's segment was frozen in 10% DMSO, stored at -80°C , and used for subsequent flow cytometric and LOH analysis. The remaining four quadrant tissue samples were placed in Hollande's solution for subsequent histological examination. Biopsies were interpreted by a single pathologist who was blinded to the flow cytometric and genetic results (51–53). Participants were classified according to the maximum histological abnormality detected in any biopsy at a given endoscopy. Because there was little evidence of differential prognosis associated with a diagnosis less than HGD [including negative for dysplasia, indefinite, and low-grade dysplasia (36)], in this report histology was analyzed as HGD or less than HGD. Histological information was available for all 429 participants.

Flow cytometric histograms were interpreted by a single observer, without knowledge of histological or genetic results (51, 53). A diagnosis of aneuploidy was made if discrete peaks were observed on the histogram, representing aneuploid and diploid populations, and the aneuploid peak represented at least 2.5% of the cells in the biopsy specimen (36, 53). An abnormal 4N fraction was defined as $>6\%$ of cells with a DNA content from 3.85–4.1N (49). A patient was classified as having aneuploidy or increased 4N if the particular abnormality was observed in one or more biopsies from a given endoscopy. Flow cytometric data were available for 385 persons.

After purification of tissue samples by Ki67/DNA content multiparameter flow sorting, DNA was extracted and replicated by whole genome amplification (54). As described previously, LOH analyses were performed using fluorescence-tagged PCR primers and automated DNA sequencers (47). A patient was considered to have 9p (*p16*) or 17p (*p53*) LOH if at least one sample in the Barrett's epithelium had clear LOH spanning the *p16/INK4a* or *p53* locus, respectively, based on objective measures of allele intensity ratios (47). 9p and 17p LOH data were available for 283 and 281 persons, respectively.

Interview and Anthropometric Data. All 429 subjects underwent structured interviews carried out in person by trained staff. The interviews usually occurred in the clinic before the endoscopy or occasionally in the participant's home. The baseline interview took approximately 45 min to complete and obtained information on medical history, family history of cancer and several GI disorders, medication history and current medication usage, past and current tobacco and alcohol use, current occupation, and demographics. Questions regarding medication history, including use of prescription and over the counter drugs containing aspirin or other NSAIDs, were modeled after the questionnaire used in the United States collaborative case-control study of EA and made use of show cards to facilitate recall, as described previously (34, 55).

Height, weight, and anthropometric measurements were taken at baseline and follow-up visits using a standardized protocol. Participants were instructed to stand erect, with their arms at their sides, feet together, and abdomen relaxed. The measures were taken at the end of a normal expiration, with the tape pulled tight but not compressing the skin. The abdominal measurement represented the largest circumference below the

Table 1 Intermediate marker status by selected characteristics of study subjects

	Histology		Flow cytometry				Allelic loss			
	HGD		Increased 4N		Aneuploidy		9p LOH		17p LOH	
	<i>n</i>	% ^a	<i>n</i>	% ^a	<i>n</i>	% ^a	<i>n</i>	% ^a	<i>n</i>	% ^a
Total	429	14.9	385	9.6	385	10.4	283	55.1	281	19.6
Age (yrs)										
30–44	34	8.8	31	6.5	31	6.5	22	63.6	21	23.8
45–54	103	9.7	90	6.7	90	5.6	63	55.6	63	12.7
55–64	103	12.6	90	7.8	90	8.9	61	60.7	60	18.3
65–74	119	19.3	110	13.6	110	13.6	86	55.8	86	22.1
≥75	70	21.4	64	10.9	64	15.6	51	43.1	51	23.5
Gender										
Male	334	15.3	302	10.3	302	10.9	220	56.8	219	18.3
Female	95	13.7	83	7.2	83	8.4	63	49.2	62	24.2
Education										
<High school	32	25.0	27	11.1	27	22.2	24	50.0	24	37.5
High school graduate	118	19.5	107	9.3	107	15.9	76	55.3	74	20.3
Some college	110	10.9	98	7.1	98	8.2	72	61.1	72	20.8
College graduate	168	12.5	152	11.2	152	5.9	110	52.7	110	14.5
BMI (kg/m ²)										
<25.0	65	21.5	54	11.1	54	11.1	45	48.9	45	20.0
25.0–27.4	85	12.9	82	7.3	82	12.2	61	49.2	60	26.7
27.5–29.9	104	15.4	97	13.4	97	12.4	79	62.0	78	16.7
≥30.0	166	13.3	143	8.4	143	7.7	93	57.0	93	17.2
Waist:hip ratio										
Quartile 1	107	12.1	95	5.3	95	5.3	71	49.3	72	18.1
Quartile 2	107	12.1	95	8.4	95	7.4	77	48.1	76	14.5
Quartile 3	107	15.9	92	10.9	92	12.0	64	57.8	63	17.5
Quartile 4	106	18.9	101	13.9	101	16.8	70	67.1	69	29.0
Cigarette use ^b										
Never	147	10.9	131	7.6	131	9.2	97	54.6	97	18.6
Tertile 1	95	17.9	89	10.1	89	11.2	59	59.3	57	19.3
Tertile 2	96	16.7	84	11.9	84	4.8	61	63.9	61	21.3
Tertile 3	91	16.5	81	9.9	81	17.3	66	43.9	66	19.7
NSAID use										
Never	176	17.0	157	10.2	157	12.7	119	53.8	117	26.5
Former	59	10.2	54	11.1	54	3.7	41	53.7	41	22.0
Current	193	14.5	173	8.7	173	10.4	122	56.6	122	12.3

^a Percentage with abnormality.

^b Classified into tertiles of pack-years among ever-smokers.

sternum and above the iliac crest. Waist measurement was made at the level of the iliac crest. The hips were measured at the largest circumference around the buttocks. Thigh circumference was at the level of the gluteal fold on the right thigh.

Statistical Analyses. Unconditional logistic regression was used to calculate adjusted ORs and 95% CIs for each intermediate marker. Analyses controlled for age in five categories (30–44, 45–54, 55–64, 65–74, and 75+ years), gender, and education in three categories (high school graduate or less, some college, and college graduate). BMI was calculated from baseline measurements as weight divided by the square of height and categorized as <25.0, 25.0–27.4, 27.5–29.9, and ≥30.0 kg/m². Ratios of waist:hip, waist:thigh, abdomen:hip, and abdomen:thigh were calculated from baseline measurements and classified into quartiles based on all participants. History of cigarette use was analyzed in four categories (never, and tertiles based on pack-years of use among ever users). NSAID users were classified into those who never used them regularly, former users (used regularly >1 year before baseline interview), and current users (used regularly within the past year). Tests for trend reported in the tables were based on the likelihood ratio test associated with the addition of the variable of interest (e.g., BMI or waist:hip ratio) in grouped linear form. Additional trend tests were carried out using transformed and

untransformed continuous measures of BMI and waist:hip ratio. Statistical analyses were carried out using the STATA (release 6) statistical package (56).

Results

The status of the biomarker measurements by selected characteristics of the study subjects are described in Table 1. In general, higher frequencies of histological, flow cytometric, and genetic abnormalities were observed among those who were older, male, and had attained fewer years of education. One exception was observed for 17p LOH, which was more common among females. No clear pattern in prevalence of the intermediate markers by BMI was noted. In contrast, histological, flow cytometric, and genetic abnormalities were observed more frequently among those with higher waist:hip ratio. Histological and flow cytometric abnormalities were more common among those who had ever regularly smoked cigarettes. Participants who had never regularly used NSAIDs also were more likely to have marker abnormalities, particularly 17p LOH. Adjusted ORs for categories of BMI and waist:hip ratio are given in Table 2. There was no evidence of increasing risk of any intermediate marker with increasing BMI; in fact, most ORs associated with BMI greater than 30 kg/m² were less than

Table 2 ORs for intermediate markers associated with anthropometric measures

	BMI (kg/m ²)				<i>P</i> (trend)	Waist:hip ratio				<i>P</i> (trend)
	OR ^a (95% CI)					OR ^a (95% CI)				
	<25	25.0–27.4	27.5–29.9	30+		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Histology										
HGD	1.0	0.6 (0.2–1.4)	0.7 (0.3–1.7)	0.7 (0.3–1.5)	0.55	1.0	0.9 (0.4–2.4)	1.2 (0.5–3.1)	1.5 (0.6–3.7)	0.27
Flow cytometry										
Increased 4N	1.0	0.6 (0.2–2.1)	1.3 (0.4–3.7)	0.9 (0.3–2.6)	0.82	1.0	1.6 (0.4–5.7)	2.1 (0.6–7.5)	2.9 (0.8–10.3)	0.07
Aneuploidy	1.0	1.2 (0.4–3.6)	1.3 (0.4–4.1)	0.9 (0.3–2.9)	0.85	1.0	1.8 (0.5–6.7)	2.8 (0.8–10.3)	4.3 (1.2–15.6)	0.01
Allelic loss										
9p LOH	1.0	0.9 (0.4–2.0)	1.3 (0.6–2.9)	1.1 (0.5–2.4)	0.52	1.0	1.0 (0.5–2.2)	1.5 (0.7–3.3)	2.7 (1.2–6.3)	0.007
17p LOH	1.0	1.5 (0.6–4.0)	0.8 (0.3–2.2)	0.9 (0.3–2.3)	0.44	1.0	1.2 (0.4–3.3)	1.6 (0.5–4.8)	3.9 (1.3–11.4)	0.005

^a ORs and 95% CIs adjusted for age, gender, education, cigarette use, and NSAID use.

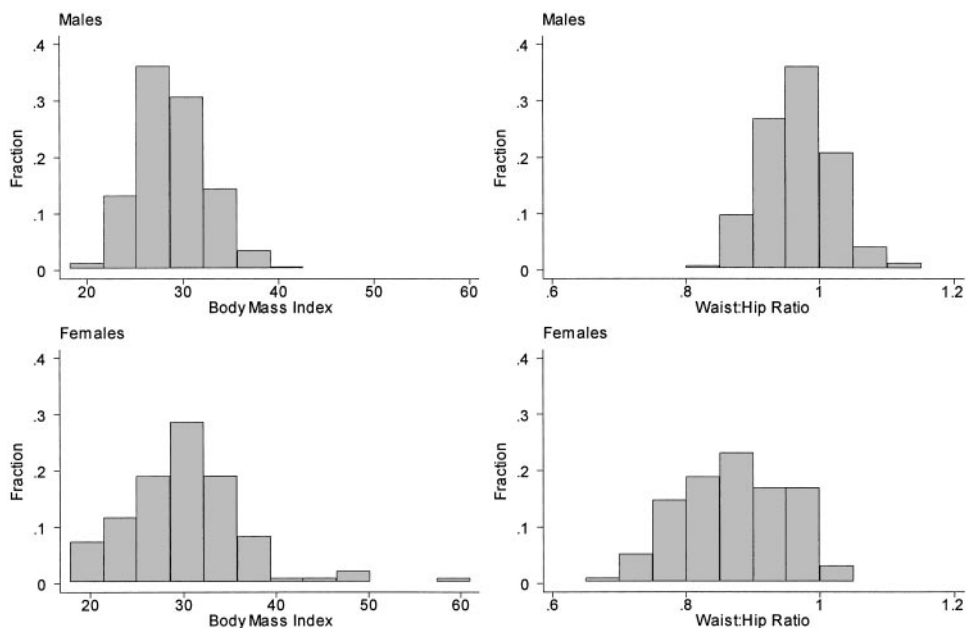


Fig. 1. Distribution of BMI (kg/m²) and waist:hip ratio by gender among 429 persons with BE.

1. In contrast, there was consistent evidence for increasing risk of genetic and cell cycle abnormalities with increasing waist:hip ratio. The strongest associations were for aneuploidy, for which the OR comparing extreme quartiles was 4.3 [95% CI, 1.2–15.6; *P* (trend) = 0.010], and for 17p LOH, for which the corresponding OR was 3.9 [95% CI, 1.3–11.4; *P* (trend) = 0.005]. Similar patterns of elevated risk were also observed for the other anthropometric variables examined (data not shown).

The results in Table 2 reflect adjustment for age, gender, education, cigarette use, and NSAID use. The same patterns of relative risk were obtained for BMI and waist:hip ratio after additional control for the other anthropometric variable, although individual ORs tended to be somewhat further from unity (*i.e.*, higher for waist:hip ratio and lower for BMI). For example, after control for BMI, the ORs comparing extreme quartiles of waist:hip ratio for aneuploidy, 9p LOH, and 17p LOH were 5.0 (95% CI, 1.3–19.8), 2.8 (95% CI, 1.1–6.9), and 5.5 (95% CI, 1.7–18.0), respectively.

Gender differences were noted in the distribution of BMI and waist:hip ratio (Fig. 1). The mean BMI was higher in females (30.3 kg/m²; SD = 6.60) than in males (28.9 kg/m²; SD = 3.77), whereas the mean waist:hip ratio was lower in

females (0.87; SD = 0.078) than in males (0.97; SD = 0.052). The small number of females in the cohort, their lower prevalence of abnormalities in most markers, and the scarcity of females with high waist:hip ratios made it difficult to investigate with precision gender differences in the associations with anthropometric measures. In females, as was observed in males, ORs corresponding to the two highest categories of BMI were less than 1 for most markers. An exception was 9p LOH, for which the ORs were 3.9 (95% CI, 0.6–28) and 2.5 (95% CI, 0.5–12.6) for the third and fourth quartiles, respectively [*P* (trend) = 0.19]. For waist:hip ratio, similar patterns of increased risk with increasing quartile of waist:hip ratio were observed in females and males, although the ORs for females were quite imprecise. None of the gender differences was statistically significant. Additional analyses of gender differences were carried out using a dichotomized waist:hip ratio variable, using the median as cutoff. For males and females, respectively, the ORs were 1.3 (95% CI, 0.7–2.5) and 4.0 (95% CI, 0.8–2.1) for HGD, 1.7 (95% CI, 0.8–3.9) and 4.6 (95% CI, 0.5–40) for increased 4N, 3.2 (95% CI, 1.2–8.3) and 1.2 (95% CI, 0.1–15) for aneuploidy, 2.0 (95% CI, 1.2–3.6) and 1.1 (95%

Table 3 ORs for intermediate markers associated with cigarette and NSAID use

	Cigarette use				NSAID use			
	OR ^a (95% CI)				OR ^a (95% CI)			
	Never	Tertile 1	Tertile 2	Tertile 3	Never	Ever	Former	Current
Histology								
HGD	1.0	1.5 (0.7–3.3)	1.4 (0.7–3.2)	1.1 (0.5–2.6)	1.0	0.6 (0.4–1.1)	0.5 (0.2–1.5)	0.7 (0.4–1.2)
Flow cytometry								
Increased 4N	1.0	1.4 (0.5–3.7)	1.4 (0.5–3.6)	1.1 (0.4–3.2)	1.0	0.7 (0.4–1.5)	1.1 (0.4–3.1)	0.6 (0.3–1.4)
Aneuploidy	1.0	1.2 (0.4–3.1)	0.4 (0.1–1.4)	1.2 (0.5–3.2)	1.0	0.5 (0.2–1.0)	0.2 (0.0–1.1)	0.6 (0.3–1.3)
Allelic loss								
9p LOH	1.0	1.1 (0.6–2.3)	1.3 (0.6–2.6)	0.5 (0.2–1.0)	1.0	1.1 (0.6–1.8)	0.9 (0.4–2.0)	1.1 (0.6–2.0)
17p LOH	1.0	0.9 (0.4–2.4)	1.4 (0.6–3.5)	0.9 (0.4–2.3)	1.0	0.4 (0.2–0.8)	0.7 (0.3–1.7)	0.3 (0.1–0.7)

^a ORs and 95% CIs adjusted for age, gender, education, waist:hip ratio, BMI, cigarette use, and NSAID use.

CI, 0.2–5.3) for 9p LOH, and 2.0 (95% CI, 0.9–4.4) and 12.2 (95% CI, 0.9–160) for 17p LOH.

Table 3 describes ORs associated with cigarette and NSAID use for each of the markers. Although most point estimates comparing ever-smokers to nonsmokers according to tertile of pack-years were above 1, none was statistically significant, nor was there evidence of a trend with increasing cumulative cigarette use.

Persons who had ever regularly used NSAIDs tended to be at lower risk for each of the biomarkers, with the exception of 9p LOH. The strongest inverse association was with 17p LOH, for which the OR for ever use was 0.4 (95% CI, 0.2–0.8), and the OR for current use was 0.3 (95% CI, 0.1–0.7). The inverse associations with ever use of NSAIDs tended to be stronger among women than men, particularly for HGD, for which the OR among women was 0.2 (95% CI, 0.0–0.7), aneuploidy (OR, 0.1; 95% CI, 0.0–0.7), and 17p LOH (OR, 0.1; 95% CI, 0.0–0.3). The corresponding ORs for men were 0.9 (95% CI, 0.5–1.7), 0.8 (95% CI, 0.3–1.7), and 0.7 (95% CI, 0.3–1.4), respectively. These gender differences were statistically significant for HGD, aneuploidy, and 17p LOH [*P* (homogeneity) = 0.028, 0.048, and 0.010, respectively].

There is evidence that the presence of aneuploid populations with ploidy > 2.7 is associated with higher risk of progression to cancer than the presence of those with ploidy ≤ 2.7 (49). Analyses using a definition of aneuploidy that excludes those with ploidy ≤ 2.7 (thus excluding 22.5% of all persons with aneuploidy) did not reveal substantially different point estimates.

To investigate whether the associations noted in Tables 2 and 3 varied according to whether the subjects were new enrollees versus ongoing participants, we carried out additional analyses that were limited to new enrollees or included interaction terms between enrollee status and NSAID use and anthropometric measures. Among new enrollees only, most point estimates associated with higher BMI remained below unity. For example, the ORs associated with the third and fourth quartiles, respectively, were 0.6 (95% CI, 0.2–2.0) and 0.7 (95% CI, 0.3–1.6) for HGD, 1.0 (95% CI, 0.3–5.3) and 0.6 (95% CI, 0.2–1.9) for increased 4N, and 0.9 (95% CI, 0.4–2.7) and 0.5 (95% CI, 0.2–1.7) for 17p LOH. The patterns of increased risk with increasing waist:hip ratio were little changed when analyses were limited to new enrollees only. For example, the ORs associated with the third and fourth quartiles, respectively, were 1.7 (95% CI, 0.4–6.6) and 3.7 (95% CI, 1.0–14.6 [*P* (trend) = 0.016] for increased 4N, 2.3 (95% CI, 0.6–9.3) and 4.1 (95% CI, 1.0–16.7 [*P* (trend) = 0.018] for aneuploidy, and 1.3 (95% CI, 0.3–4.6) and 4.1 (95% CI, 1.1–

15.5 [*P* (trend) = 0.029] for 17p LOH. For NSAIDs, the ORs associated with current use were 0.7 (95% CI, 0.3–1.6) for HGD, 0.8 (95% CI, 0.3–2.0) for aneuploidy, and 0.4 (95% CI, 0.2–1.1) for 17p LOH. Similarly, there was little change in the point estimates associated with cigarette use. None of the differences in ORs between ongoing subjects and new enrollees approached statistical significance [all *P*s (homogeneity) ≥ 0.30].

Discussion

In this cross-sectional analysis of persons with BE, we found evidence of increasing risk of histological, flow cytometric, and genetic abnormalities with an increasing abdominal fat distribution, but not with increasing BMI. In addition, persons who regularly took NSAIDs were at reduced risk of each of the intermediate markers except 9p LOH, with the strongest associations observed in women. No evidence was found that cigarette smoking substantially increased risk of any of the abnormalities studied.

There are several notable strengths to this study. It included a relatively large number of persons with histologically proven BE who were well characterized with regard to exposures and host factors potentially related to risk of progressing to EA. Analyses therefore could take into account the major known or suspected risk factors for EA, decreasing the likelihood of confounding by unmeasured factors. The study included as outcomes several carefully measured biomarkers demonstrated to be predictive of subsequent risk of developing EA, including HGD, aneuploidy, and increased 4N fraction (36, 49). 17p LOH targeting *p53* is associated with a relative risk of 16 (95% CI, 6.2–39) for development of EA, and preliminary evidence suggests that 9p LOH targeting *p16* is an early event that may be a useful predictor of EA risk as well (37, 38).

Several limitations should also be noted. In particular, the cross-sectional nature of the analyses, in which exposure histories and host factors are compared in persons with and without prevalent abnormalities, necessarily limits the conclusions that can be drawn. Also, we examined the associations between a number of potential risk factors and five different intermediate markers; these multiple comparisons should be taken into account in interpreting the findings. This is particularly important with regard to the apparent modification by gender of the associations between the intermediate markers and NSAID use, which were not hypothesized *a priori*.

The lack of association between BMI and any of the biomarkers studied contrasts with the strong dose-response relationships that have been reported for BMI in case-control

studies of EA. The large United States collaborative study found a 2.9-fold (95% CI, 1.8–4.7) increase in risk comparing extreme quartiles (with highest quartile defined as ≥ 27.32 kg/m² in males and ≥ 27.44 kg/m² in females), consistent with other studies in the United States (15, 16, 57). A similar study in Sweden found even stronger associations, whether comparing extreme quartiles (OR, 7.6; 95% CI, 3.8–15.2) or focusing on those with a BMI above 30.0 kg/m² [OR, 16.2; 95% CI, 6.3–41.4 (17)]. None of the previous studies was able to take into account anthropometric characteristics because most cases of EA are diagnosed at an advanced stage with attendant weight loss.

The present cross-sectional results support the concept that the distribution of adipose tissue may be more important than overall BMI among persons who have already developed BE. It is possible that the effects of increased BMI are largely manifested early in the pathogenesis of EA, that is, in the development of the specialized intestinal metaplasia that characterizes BE, possibly by increasing risk of developing a hiatal hernia, with resulting increased frequency and severity of reflux (19). In contrast, central adiposity may be more important later in the pathogenesis, that is, in the development of cell cycle and genetic abnormalities that mark the progression of BE toward cancer. This might occur through alteration of growth factors, hormones, or other metabolic factors that affect regulation of cell growth. Abdominal obesity is strongly associated with decreased insulin sensitivity, lower levels of sex hormone-binding globulin, and higher levels of insulin, leptin, and free fatty acids (58). Insulin affects the levels of IGFs and their binding proteins (IGF-binding proteins) in complex ways. High insulin levels suppress hepatic synthesis of IGF-binding protein 1 (59). This, in turn, increases the bioavailability of IGFs, including IGF-I, which has been associated with increased risk of cancers of the breast, prostate, lung, colon, and rectum (60). Although highly speculative, it is possible that the metabolic consequences of central body obesity also include accelerated rates of division and proliferation of Barrett's epithelium.

The results of several studies suggest that regular use of NSAIDs may protect against the development of esophageal cancer, despite the irritating effect of these drugs on the upper GI tract. In the United States collaborative case-control study, a strong inverse association was found between NSAID use and both esophageal and gastric cancers (34). For EA in particular, ORs of 0.4 (95% CI, 0.2–0.6) for current users of aspirin and 0.6 (95% CI, 0.3–1.0) for current users of other NSAIDs were observed. In a case-control study conducted using the General Practice Research Database covering four million United Kingdom residents, a protective association was found for cancer of the esophagus (OR, 0.6; 95% CI, 0.4–1.0), along with cancer of the stomach, colon, and rectum (61). In a hospital-based case-control study, Coogan *et al.* (62) observed little overall association between current NSAID use and esophageal cancer (OR, 0.8; 95% CI, 0.5–1.4), although among current users for at least 5 years, the association was strengthened (OR, 0.4; 95% CI, 0.2–1.1). The results from two cohort studies are also consistent with a protective effect of NSAIDs in the upper GI tract. The American Cancer Society cohort study reported a relative risk of 0.6 (95% CI, 0.3–1.0) for esophageal cancer and 0.5 (95% CI, 0.3–0.8) for gastric cancer (25), whereas the National Health and Nutrition Examination Survey (NHANES I) cohort study reported a relative risk of 0.1 (95% CI, 0.0–0.8) and 0.9 (95% CI, 0.5–1.7) for esophageal and gastric cancers, respectively (29, 35). With the exception of the United States collaborative case-control study, none of the others were able to separate out esophageal cancers by histological type.

The major anti-inflammatory effect of NSAIDs occurs through inhibition of the COX enzymes, particularly the inducible COX-2 enzyme, which controls the rate-limiting step in prostaglandin synthesis (20, 21). Most mechanisms proposed to explain a protective effect of NSAIDs in the lower GI tract focus on possible direct or indirect effects of COX-2 inhibition because high levels of COX-2 expression are observed in both malignant and premalignant tissues in the colon. Mouse experiments in which a knockout mutation of the *COX-2* gene was introduced into a line with a heterozygous mutation in the *APC* gene, resulting in substantial reductions in both the number and size of intestinal polyps in both homozygous and heterozygous COX-2 knockouts (63), suggest a key role of COX-2 in early colonic neoplasia, particularly in polyp growth (20, 21). By inhibiting mitogen- or growth factor-induced prostaglandin production and inflammation, NSAIDs may act by reducing cellular proliferation and oxidative damage from free radical production. Another line of evidence suggests that NSAIDs promote apoptosis, possibly through reduction in prostaglandin E₂ production, elevation of the prostaglandin precursor arachidonic acid, or other pathways (64–67). Support for this line of study in relation to EA comes from several lines of evidence: COX-2 is overexpressed in BE and EA (68–70); COX-2 inhibitors reduce proliferation in Barrett's cell lines (71); and COX-2 inhibitors can reduce risk of EA induced by reflux in the rat model (72).

Whereas cigarette smoking is a well-established and strong risk factor for esophageal squamous cell carcinoma, for EA heavy smokers experience only a modest, 2- to 3-fold increase in risk (16, 73, 74). Interestingly, it appears that the effect of smoking cessation does not manifest until 20–30 years after cessation, in contrast to esophageal squamous cell carcinoma and respiratory cancers, in which cessation has a measurable impact within 5 years (16, 73, 74). This suggests that the major effect of cigarettes must be at a relatively early stage of neoplastic progression in EA. Our findings of little association between measures of cigarette smoking duration and intensity and intermediate markers among persons with BE are consistent with the effects of cigarettes occurring early in the neoplastic process, possibly before BE even develops.

We conclude that an abdominal distribution of body fat, which is more common in men and is termed male-pattern obesity, may be a strong predictor of risk of neoplastic progression among persons with BE and may account in part for the male predominance of BE and EA. We also conclude that NSAID use may reduce the risk of progression to cancer in this population. Whereas prospective observational and intervention studies are needed to confirm these results, they do suggest at least two preventive approaches by which the rising incidence of EA eventually may be controlled.

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