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Impact of Adiposity Status on the Association of Regular Statin Use and Increased Number of Aberrant Crypt Foci

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ABSTRACT

INTRODUCTION: While animal studies consistently show a preventive role of statin use in colon cancer development, evidence from human studies is conflicting with recent reports suggesting an increased risk of aberrant crypt foci and adenoma. We hypothesize that insufficient control of confounding due to indication bias for prescribing of statins, particularly obesity-induced physiologic dysregulation, might partly explain discrepant results. **METHODS:** We analyzed data from patients receiving standard colonoscopy at the Colon Cancer Prevention Program of the University of Connecticut Health Center, which was followed by chromoendoscopy for ACF detection. ACF number was categorized at low (<10) or high (≥10). We calculated Univariate, Age-Adjusted and Multivariate Logistic Regression to estimate Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for high ACF number in relation to regular statin use (i.e., ≥ 1 pill per week for the past 12 months). Adiposity measures of Body Mass Index (BMI), Waist-Hip-Ratio (WHR) and Waist Circumference (WC) were added separately in the multivariate model as surrogates to assess indication bias. A composite variable was created to assess the impact of individual compared to joint regular use of statins and aspirin (baby and/or full dose) on ACF number. **RESULTS:** Participants who reported regular use of statins had a higher mean ACF number than participants who reported taking <1 pill per week (17.20 vs. 9.02, respectively, p=0.001). The mean age for the high ACF group was greater than the mean age for the low ACF group (59.40 versus 54.17 years, respectively, p=0.002). Never and past smokers were more likely to have low ACF than current smokers (58.9% vs. 60.0% vs. 18.2%, respectively, p=0.037). Univariate logistic regression showed that patients who regularly took statins were 3.9 (95%CI=1.43-10.74) times more likely to have a high ACF count than those not taking statins regularly, which was reduced to 2.67 (95%CI=0.89-7.97) in the age-adjusted analysis. In the basic multivariate model, the OR for high ACF among regular statin users was 1.47 (95%CI=0.28-7.74) in comparison to patients who consumed 1 or fewer pills per week. When adiposity measures were added into the basic multivariate model, the ORs for high ACF were: 1.24 for BMI (95%CI=0.21-7.47; p=0.017); 0.68 for WHR (95%CI=0.10-5.40; p=0.702); and 1.20 for WC (95%CI=0.20-1.20; p=0.842). Compared to taking neither aspirin nor statins (referent group), univariate ORs were: 15.0 (95%CI=1.55-145.23) for taking statins only on a regular basis; 1.83 (95%CI=0.59-5.68) for regular use of aspirin only; and 4.29 (95%CI=1.24-14.83) for regular use of both statins and aspirin. OR estimates were slightly attenuated in age-adjusted analyses and were substantially reduced in multivariate analyses, and no longer statistically significant. **CONCLUSIONS:** While regular statin use in our study population was associated with a statistically significant higher ACF count in univariate analyses, this effect did not remain after controlling for key risk factors for colon cancer. It is possible that prior evidence of an adverse role of statin use in colorectal neoplasia in human studies may be explained in part by confounding. Our results must be interpreted with caution due to small numbers in study groups.

1.0 INTRODUCTION

1.1 Established Risk Factors for Colorectal Cancer

Colorectal cancer (CRC) is the second leading cause of cancer-related mortality for both men and women in the United States with over 50,000 deaths reported in 2007 alone (CDC, 2012). Incidence rates of CRC in the United States, however, have been decreasing recently in both males and females (Jemal et al., 2011), over the past two decades, largely due to detection and removal of precancerous lesions through CRC screening.

Regular use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) has been reported to reduce the primary occurrence of adenomas and contribute to their regression (Stevens et al., 2006). Regular aspirin use has been associated with a reduced risk of CRC as well (Potter, 1999). Two proposed mechanisms linking CRC pathogenesis are insulin resistance and chronic inflammation (Bruce et al., 2000; Kaaks et al., 2000; Potter, 1999; Stevens et al., 2006). Adiposity-induced insulin resistance is thought to be the pathway linking surplus body fat with colon cancer risk (Swede et al., 2009). A study by Swede et al. (2009) found that a relatively high level of visceral fat was associated with increased ACF. Together, this, and the chronic inflammation model provide a framework from which a large portion of CRC etiology and epidemiology can be interpreted (Stevens et al., 2006).

Modifiable risk factors for CRC also include smoking, physical inactivity, and dietary intake (Jemal et al., 2011). Diet is thought to influence 70-90% of CRC (Lipkin et al., 1999). While the mechanism through which diet influences CRC is not well understood, it is thought that the increased consumption of red meat, and possibility the low intake of fiber, including fruits and vegetables, increases CRC risk (Stevens et al., 2006). Additionally, lack of physical activity and obesity are associated with increased risk of CRC (Stevens et al., 2006).

1.2 Statins and Colorectal Cancer: Unclear Evidence

As noted above, anti-inflammatory agents, such as aspirin, are thought to be preventive for CRC and possibly ACF. Another class of drugs, statins, has been examined for a possible preventive role in CRC but human evidence remains inconsistent. Statins, which are HMG-CoA inhibitors, are among the most commonly prescribed drugs worldwide for their cholesterol-lowering properties (Boudreau et al, 2010). Their use has dramatically risen in the past decade and is likely to continue rising (Boudreau et al., 2010). It is estimated that 1 out of every 10 adults in the U.S. population is on statins to reduce circulating lipoprotein levels and to consequently decrease their risk for cardiovascular disease (Ahnen & Byers, 2009). There are currently six statins on the U.S. market: lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin, and rosuvastatin.

Statins inhibit HMG-CoA reductase, a major rate-limiting enzyme of the mevalonate pathway, thereby preventing the conversion of HMG-CoA to mevalonate (Boudreau et al., 2010). This results in decreased levels of mevalonate and its subsequent products (Boudreau et al., 2010). Many products of the mevalonate pathway are vital for proper cellular functions, including membrane integrity, cell signaling, protein synthesis, and cell cycle progression (Boudreau et al., 2010). Inhibition of the mevalonate pathway may disrupt these processes in neoplastic cells, resulting in control of tumor initiation, growth, and metastasis (Boudreau et al., 2010). Statins have many biologically plausible effects on cancer risk (Ahnen & Byers, 2009) from affecting protein synthesis to angiogenesis, apoptosis, cellular immunity, and cancer metastasis (Ahnen & Byers, 2009). Many studies demonstrate statin-induced programmed cell death, or apoptosis, in several cell lines, including mammary carcinoma, lung, colorectal, pancreatic, and prostrate carcinoma (Boudreau et al., 2010).

The initial evidence of a role in CRC came from animal studies, which suggested that statins could have chemopreventive properties (Agarwal et al., 1999). Epidemiological studies have also suggested that statins play a role in CRC. For example, two large randomized control trials showed decreases in the risk for CRC among those randomized to take statins by 19% and 43% (Ahnen & Byers, 2009). These reductions were not statistically significant, however, and were secondary endpoints in trials originally conducted for other

purposes (Ahnen & Byers, 2009). Nevertheless, this paved way for a large number of observational case-control and cohort studies examining the relationship between statins and CRC (Ahnen & Byers, 2009).

Several case-control and cohort studies have found no association between statin use and CRC risk, while two large observational studies reported a 35-43% reduction in CRC among statin users compared to non-users (Boudreau et al., 2010). In a population-based cohort study, Singh et al. assessed the effect of the long-term regular use of statins on the risk of CRC (2009). They reported no statistically significant reduction in CRC risk with regular use of statins, irrespective of the duration or dose (Singh et al., 2008). Singh et al. also found a small increased risk of CRC associated with low dose statin use (Singh et al., 2008). Statins have been shown to have a stimulatory effect on some CRC cell lines (Singh et al., 2008).

In a cohort of male veterans undergoing surveillance colonoscopy, Parker-Ray et al. (2010) reported that statin use did not decrease the risk for or number of adenomatous polyps. The number of polyps that were found on the index colonoscopy was the only factor that was associated with adenoma recurrence (Parker-Ray et al., 2010). Age, BMI, and number of polyps detected at index colonoscopy were found to be positively associated with the number of polyps detected at follow-up colonoscopy (Parker-Ray et al., 2010). Diabetes and non-Hispanic white race was found to be inversely associated with the number of polyps detected at follow-up colonoscopy (Parker-Ray et

al., 2010). The use of statins was not protective against the recurrence of adenomatous polyps (Parker-Ray et al., 2010). Currently, there is no convincing evidence to support prescribing statins for the chemoprevention of colorectal neoplasia.

1.3 Aberrant Crypt Foci

Colonic intestinal epithelium is rapidly renewing tissue, due to the loss of cells during the final stage of the digestive process, which is marked by homeostatic equilibrium between cellular proliferation and abatement (Lopez-Ceron & Pellise, 2011). Aberrant crypt foci (ACF) are pre-polyp abnormalities identified in single crypts detected by high magnification chromendoscopy (Stevens et al., 2006) using dye (e.g., indigo carmine). ACF consist of large, thick crypts in the colon, first identified in mice treated with azoxymethane (Bird, 1987). ACF were also reported in the colonic mucosa of humans (Takayama et al., 1998).

Histologically, ACF can be classified as hyperplastic or dysplastic (Lopez-Ceron & Pellise, 2011). Hyperplastic ACF are the most common type and consist of larger and longer crypts with apical branching and serration (Lopez-Ceron & Pellise, 2011). Dysplastic ACF are less common, but are more frequent in Eastern than Western populations and are composed of smaller, non-serrated crypts (Lopez-Ceron & Pellise, 2011). In most instances, ACF are defined as a cluster of crypts that stain darker than their surrounding

mucosa in addition to one of the following characteristics: crypts with larger diameters than surrounding mucosa, thicker epithelial linking, dilated crypt lumen, or a slightly raised appearance (Lopez-Ceron & Pellise, 2011).

1.4 Clinical Utility of ACF

Data on ACF prevalence range from 15% to 77% in healthy individuals and 80% to 100% in patients with colorectal neoplasms (Lopez-Ceron & Pellise, 2011). ACF are thought to increase in size by a dynamic process known as crypt fission, although this mechanism is not well understood (Lopez-Ceron & Pellise, 2011).

Colonic ACF may be predictive markers of future risk of CRC (Stevens et al., 2006). ACF are the earliest identifiable morphological change in the pathway to CRC, according to Kinzler and Vogelstein (1996). Both morphologically and genetically distinct, ACF may be precursors of adenoma and cancer. It has been shown, however, that ACF are a heterogeneous group of lesions, and while some may be important in CRC development, others are not (Stevens et al., 2006).

The number, size, and lumen morphology of ACF have been correlated with CRC risk (Lopez-Ceron & Pellise, 2011). The total number and density (number/cm^2) of ACF in the rectum tend to be representative of the amount of ACF present elsewhere in the colon (Lopez-Ceron & Pellise, 2011).

Accordingly, most endoscopic studies focus on rectal ACF (Lopez-Ceron & Pellise, 2011). Crypt multiplicity, the number of crypts per focus, is correlated with ACF size (Lopez-Ceron & Pellise, 2011). Studies have shown that as age increases, ACF size increases (Lopez-Ceron & Pellise, 2011). Still, ACF size and risk of CRC are not clear (Lopez-Ceron & Pellise, 2011). A recent study showed that small ACF were associated with distal adenomas (Lopez-Ceron & Pellise, 2011). While some reports have shown an association between the shape of the lumen and dysplasia, other studies have found no correlation between the two (Lopez-Ceron & Pellise, 2011).

The progression of CRC is a very lengthy process that can span up to two decades (Alrawi et al., 2006). Intervention by polyp removal has now become a standard of clinical care (Alrawi et al., 2006). Understanding the pathology of the colonic crypt can provide insight into the mechanisms of malignant transformation and could be key to identifying populations at increased risk of CRC and providing them with better preventative care measures (Alrawi et al., 2006).

In a study by Sakai et al. (2011), significant stepwise increments in both prevalence and number of ACF were observed from normal to adenoma to CRC cases. Mean number of ACF was also found to be significantly higher in the subject group with advanced adenoma than in the subject group with non-advanced adenoma (Sakai et al., 2011). These results indicate that ACF may

serve as a reliable biomarker of human colorectal carcinogenesis (Sakai et al., 2011).

ACF also harbor genetic and epigenetic alterations that can be detected in early neoplastic lesions and CRC (Lopez-Ceron & Pellise, 2011). For example, mutations in the adenomatous polyposis coli (APC) gene have been strongly associated with dysplastic changes in ACF (Lopez-Ceron & Pellise, 2011). As a result, patients with familial adenomatous polyposis present with a high occurrence of dysplastic ACF (Lopez-Ceron & Pellise, 2011). Conversely, a small proportion of dysplastic ACF show APC mutations in sporadic CRC while there is no evidence that hyperplastic ACF have this mutation (Lopez-Ceron & Pellise, 2011).

CRC carcinogenesis can also occur through microsatellite instability (MSI), a condition that results from defects in the normal DNA repair process due to the inactivation of certain genes, such as MLH1, MSH2, MSH6, or PMS2 (Lopez-Ceron & Pellise, 2011). This inactivation can be either inherited (e.g., Lynch Syndrome) or acquired (e.g., MLH1 Promoter methylation) (Lopez-Ceron & Pellise, 2011). Fifteen percent of sporadic CRC is associated with MSI, and this frequency increases from ACF to adenoma to carcinoma (Lopez-Ceron & Pellise, 2011). Almost all patients with Lynch Syndrome present with ACF that show MSI (Lopez-Ceron & Pellise, 2011). One study reported that a high concentration of MSI is found in adenomas of patients with Lynch

Syndrome, and 100% of ACF in these patients exhibited MSI (Lopez-Ceron & Pellise, 2011).

An alternative pathway to the development of CRC, known as the serrated pathway, suggests the development of cancer without classic adenoma, but rather from serrated lesions related to hyperplastic polyps (Lopez-Ceron & Pellise, 2011). Serrated lesions are thought to occur as a result of DNA modifications not related to modifying the basic structure of genes (Lopez-Ceron & Pellise, 2011). The best-known epigenetic mechanism of CRC results from the inactivation of the hypermethylation of the promoter region of tumor suppressor genes (Lopez-Ceron & Pellise, 2011).

ACF, while relatively common, in normal individuals, continue to be investigated as the earliest precursors of adenomas and cancers (Alrawi et al., 2006). While most ACF do not progress on to become cancerous, with some even regressing or disappearing, it is likely that certain factors induce them to become malignant (Alrawi et al., 2006). The histomorphologic and/or genetic changes in ACF that lead to their potential role in neoplastic progression are still not well understood. Studies on murine models have demonstrated that genetic cluster analyses are useful in identifying which murine ACF are high risk to go on to undergo malignant transformation (Alrawi et al., 2006).

Alrawi et al. (2006) examined whether genomic instabilities exist in ACF from the normal, non-inflammatory setting. Inter-(simple sequence repeat) PCR was used to quantify the genomic damage present in each ACF sample.

Even single base pair insertions or deletions can be identified through this technique (Alrawi et al., 2006). Generally, however, detected genetic anomalies are found to be larger (Alrawi et al., 2006). ACF from the same patient were observed to each have unique genomic fingerprints (Alrawi et al., 2006). Additionally, one-fourth of the thirty-two ACF examined revealed moderate instability after inter-PCR (Alrawi et al., 2006). The results of this study indicate that ACF characterization could become a valuable clinical screening tool to identify those individuals that are more likely to develop colorectal carcinoma from instable ACF.

1.5 Risk Factors of ACF

There are many factors thought to influence the natural history of ACF. Takayama et al. (1998) found that the prevalence and number of ACF increased abruptly between the ages of 40 and 50, and patients with cancer had a consistently higher prevalence and number of ACF, regardless of age. Patients on low dose aspirin have been shown to have lower prevalence of ACF, and lower prevalence of ACF have been reported in patients taking NSAIDs as well (Lopez-Ceron & Pellise, 2011). These results have not been consistent, however. Tobacco has been shown to be one agent that increases number of ACF in most studies (Lopez-Ceron & Pellise, 2011) and is an important risk factor for colorectal neoplasia (Anderson et al., 2010).

Alcohol is thought to induce ACF as well (Lopez-Ceron & Pellise, 2011), but the effects of different types of alcoholic beverages (e.g. beer, liquor, wine) are not well understood (Lopez-Ceron & Pellise, 2011).

1.6 Conclusion

There is growing evidence that statins may increase ACF number in humans, and that ACF may be precursor lesions of CRC, but much remains to be understood. ACF are an efficient way to study this issue, but the role of statins in CRC development, with ACF as an intermediate, is still uncertain. Indication bias due to adiposity could be at play here since statins could be prescribed to people who are more likely to be overweight and obese. As such, these people may be at increased risk for high ACF count and subsequent CRC development. This research project will incorporate adiposity measures and a wider range of circulating biomarkers in order to use ACF to provide insight into the role of statins in CRC. It is thought that patients on statins will have a higher number of ACF than those not on statins, but this association will lessen once controlling for adiposity. Using data from a pool of patients who received a standard colonoscopy at the Colon Cancer Prevention Program at the University of Connecticut Health Center, this investigation aims to:

1. Determine the association between statin use and ACF number, controlling for key risk factors for CRC, and

2. Assess if the relationship between statin use and ACF count may be modified by controlling for different measures of adiposity (body mass index [BMI], waist circumference [WC], and waist-hip-ratio [WHR]).

2.0 METHODS

2.1 Study Population

We obtained data from 101 patients receiving standard colonoscopy at the Colon Cancer Prevention Program of the University of Connecticut Health Center from February 2010 to November 2011. Patients attended the clinic for routine screening or surveillance following the detection of a pathological finding at a previous exam. The goal of the clinic-based ACF study is to identify and describe the clinical importance of ACF and the epidemiologic risk factors for and molecular features of ACF and other early colonic neoplasia. A total of 14 people were excluded because they were underweight (BMI < 18.5) or were missing adiposity measurements.

2.2 Patient Data

An Advanced Practice Nurse Practitioner (APRN) took measurement of height, weight, hip (i.e., widest torso circumference), and waist (i.e., narrowest torso circumference as defined in Gram et al. (2006). A fasting blood sample (three heparinized vials at 10 ml) was drawn from the intravenous line at the colonoscopy exam and transported to the research laboratory. Samples were separated into 0.5 ml aliquots and frozen at -80℃. Patients were also

questioned about medication use, family history, personal history, and lifestyle-behavior questions (e.g., tobacco and alcohol use).

2.3 Colonoscopy Procedures and ACF Detection

All patients in the study underwent routine colonoscopy for clinical purposes. Prior to the clinic visit, colon preparation was performed with a magnesium citrate based prep. ACF detection, performed after the colonoscopy, lasted for up to 30 minutes. High definition colonoscopes (Olympus PCF-H180AL and CFH-180AL) were utilized for examination purposes. Close focus properties allowed for clear, detailed observations.

The distal 20 cm-section of colon, including the rectum, were washed. This was followed by water wash to remove mucous, after which examination was performed. In order to visualize the colon, a fresh solution of indigo carmine 0.8% was prepared and applied for contrast staining using spray catheters. The dye was allowed to absorb for two minutes before endoscopy was performed. A finding of an ACF is accepted if two or more crypts are darkly stained and have lumen diameters that are 1.5-2.0 times those of surrounding crypt lumens under close magnification.

ACF were also required to be raised above the mucosal level. The requirements of being round, dilated, slit, or having a star-shaped lumen or thick crypt wall with compressed lumen were necessary to confirm

identification. The colon was divided into four quadrants or the scope was withdrawn in a clockwise fashion as techniques to ensure that the ACF were not double-counted or missed. Narrow-band imaging (NBI), which is a non-dye imaging technique that enhances mucosal and vascular detail, was used to detect ACF in some patients.

The variability of the raters was addressed by including the requirement that the raters be trained in ACF determination and confer and reach an agreement on the determination of an ACF finding at the time of chromoendoscopy. In the 20-cm distal region of the colon, ACF were counted and digital images were captured. A maximum of 10 biopsy specimens of ACF were taken within the distal 20 cm using forceps (Precisor EXL, CR Bard). A GI pathologist (TVR), who was unbiased concerning the clinical findings, analyzed coded specimens of frozen section of ACF that had been stained with H&E (hematoxylin and eosin stain). Histologic analysis was performed using light microscopy.

2.4 Variables

The **number of ACF** was categorized into two groups (<10, ≥10), termed low ACF and high ACF. The median number of ACF was 9 and therefore, our outcome was having <10 or ≥10 ACF.

Statins were divided into <1 pill per week or ≥1 pill per week (regular use) in past 12 months. Use of medications other than statins, including **baby aspirin, aspirin, and other NSAIDs**, were divided into never, <1 pill per week, or ≥ 1 pill per week. In the logistic regression, these variables were split into two categories: <1 pill per week and ≥1 pill per week. This was done to ensure no cell counts were too small.

Biomarkers were analyzed as continuous variables. **BMI, WHR, WC**, and age were analyzed as continuous variables as well as categorical. The following groups defined categories of BMI for use in descriptive analysis: Normal (18.5–24.9), Overweight (25.0–29.9), Obese I (30.0–34.9), Obese II (35.0–39.9), and Obese III (≥40.0). BMI was defined as weight divided by height (kg/m^2). As previously mentioned, underweight (BMI <18.5) participants were excluded from the study.

WC and WHR were used as measures of central adiposity. Cut-off levels for elevated WHR matched those defined by WHO criteria (Balkau et al., 2002), namely >0.90 for males and >0.85 for females. Elevated WC based on sex was defined as >40 in (102 cm) waist for males and >35 in (88 cm) for females (Swede et al., 2009).

History of a first-degree relative with CRC was defined as having a parent, sibling, or child with a history of CRC. **Personal history of polyps** was categorized as yes (a personal history of one or more polyp(s) in the past) or no (no personal history of polyps to date).

Smoking status was divided into three categories: never smokers, past smokers, and current smokers. The following groups of categories defined **alcohol** consumption in analyses: red wine intake, white wine intake, beer intake, and liquor intake. This alcohol consumption variable was further divided into the following classification scheme: never drinkers, individuals who consumed 0 to 3 drinks per month, and individuals who consumed ≥2 drinks per week.

Finally, since there is a chance that people taking statins are on other anti-inflammatory drugs, we created a **composite variable** that was split into the following categories: no statin or aspirin use; statin use only; aspirin (baby or full-dose) use only; and statin and aspirin (baby or full-dose) use to assess the impact of individual vs. joint statin and aspirin use on ACF number.

2.5 Enzyme-Linked Immunosorbent Assays (ELISAs)

Blood specimens of 41 participants were available for ELISA analysis. The following biomarkers from serum samples were measured: hsCRP (mg/L); IL-6 (pg/mL); TNF-a (pg/mL); Insulin (uIU/mL); Glucose; Triglyceride; Cholesterol, Total; Cholesterol, HDL; IGF-1 (ng/mL); IGF-2 (ng/mL); IGFBP3 (ug/mL).

2.6 Statistical Analyses

Descriptive analyses were performed for: age, sex, BMI, WHR, WC, medication (baby aspirin, full-dose aspirin, other NSAIDs), smoking, alcohol, personal history of polyps, and biomarkers as correlates of statin use and in relation to low versus high ACF number, using t-tests for continuous data and chi-square tests for proportions.

Univariate, age-adjusted and various multivariate logistic regressions were conducted to calculate odds ratios (ORs) and 95% confidence intervals (95% CI) to identify the link between ACF number (i.e., outcome) and statin use (exposure.) Statin use was assessed as regular statin use as well as the composite variable (i.e., joint use of aspirin and statins) in models. To identify possible confounders of the relationship between statin use and ACF number, the univariate and age-adjusted logistic regressions were conducted for ACF number in relation to the following variables: age, sex, BMI, WHR, WC, medication use (baby aspirin, aspirin, other NSAIDs), smoking, alcohol consumption, personal polyp history, family history of CRC), and biomarkers

A base multivariate model was developed to include variables that were significant in univariate analyses ($p < 0.20$) or were judged to be clinically important variables. The base model included the following variables: statins, age, sex, medication use (baby aspirin, other NSAIDs), family history of CRC (first degree relative), personal polyp history, and smoking habits. To

understand the effects of adiposity on ACF number, the base model was re-run to include, separately, BMI, WHR, and WC. Also, since age is a key factor in most disease outcomes, including CRC development and progression, all multivariate logistic regressions were stratified by age (<50 years of age vs. ≥50 years).

Results for all tests were considered statistically significant if $p<0.05$ or results within the confidence intervals (95%) did not include 1.0. SPSS version 20.0 was used, and tests were two-sided in all the analyses.

3.0 RESULTS

3.1 Participant Characteristics

Demographics and adiposity measures of all eligible participants (n=86) are shown in Table 1. In regards to statin use, 68.8% of the study population took <1 pill per week and 31.3% took 1 or more pills per week. The mean age was 56.79 years (SD 8.58). Males made up 65% of the study sample while females accounted for 35%. The majority of participants were White (90.7%), followed by Black (8.1%) and Asian (1.2%). A slight majority (59.4%) of the participants had some college education, a 2-year degree, or a college degree. About 21% of the study population had completed vocational or trade school, and 17.5% of the population had a high school education or less. Nearly 1/5 (21%) of participants had a first degree relative (parent, sibling, or child) with a history of CRC, and about 18% of participants had a personal history of polyps.

Regarding measures of excess body weight, the majority of participants were overweight or obese (72.1%). Specifically, 27.9% of participants had a normal BMI (18.5-24.9), while 41.9% were overweight (BMI of 25.0-29.9) and 30.2% were Obese (BMI of 30+). The mean waist-hip-ratio (WHR) for males was 0.93 (SD 0.06), while the mean for females was 0.87 (SD 0.09). When categorizing by WHO risk level, 28.6% of males had a normal WHR-risk (≤0.90), while 57.1% of males had an elevated WHR-risk. In females, 33.3%

had a normal WHR-risk (≤0.85), while 56.7% had an elevated WHR-risk. The mean waist circumference for males and females, respectively, was 38.05 (SD 4.61) and 36.41 (SD 5.96). With respect to waist circumference risk, 21.2% of males had an elevated risk (<40.0), compared to 48.1% of females (>35.0).

3.2 Correlates of Regular Statin Use

In order to identify potential confounders in the relationship between statin use and ACF number, we explored correlates of statin use and ACF number (see below.) As seen in Table 2, participants who consumed at least 1 statin pill per week (i.e., regular use of statins), had a higher mean ACF number than participants who reported taking less than 1 pill per week (17.20 vs. 9.02, respectively, p=0.001). We also categorized ACF number into low (<10) and high levels (≥10). Fewer participants with low ACF number reported taking statins on a regular basis compared to those with high ACF level (18.6% versus 47.2, respectively, p=0.006), As expected, the mean age for patients taking 1 or more statin pill per week was higher than the mean age for participants on less than 1 statin pill per week (62.40 (SD 7.69) vs. 54.51 (SD 8.24), p<0.001). In addition, patients aged 50 years and older were more likely to be on statin medication (1 or more pills per week) than those younger than 50 years old (36.8% vs. 0, p=0.014). There was no significant difference in

percentage of males or females on statin medication (33.3% vs. 26.9%, p=0.562).

Regarding obesity measures, the mean BMI of participants taking statins on a regular basis was higher among those taking less than one pill per week (30.26 (SD 5.61) vs. 27.25 (SD 4.38), p=0.011). Additionally, participants on a regular dosage of statins were more likely to be obese than those on <1 pill per week (60.9% vs. 39.1%, p=0.001). People taking statins regularly were also more likely to take baby aspirin on a weekly basis compared to those taking less than 1 statin pill per week (59.3% vs. 40.7%, respectively, p=0.001).

3.3 Correlates of ACF Number

To understand the relationship between the correlates of ACF number, t-tests for continuous data and chi-square tests for proportions were done with the following variables: medication use (statins, baby aspirin, aspirin, other NSAIDs), demographics (age, sex, race, education), personal polyp history, and family history of CRC. Participants on a regular statin regimen were more likely to have a high ACF count than participants taking <1 statin pill per week (66.7% vs 37.1%, p=0.010). As anticipated, the mean age for participants with a high ACF count (59.40) was higher than the mean age for participants with a low ACF count (54.17 years, p=0.002). There were no significant differences between low and high ACF groups for participants based on sex, race,

education, personal polyp history, or a first degree relative with a history of CRC (Table 4).

Adiposity was explored as a correlate of ACF number (high vs. low) by analyzing BMI, WHR, WHR-risk, and WC. As predicted, the mean BMI for the low ACF group was less than that of the high ACF group (27.21 vs. 28.99, respectively, p=0.152). There were no significant differences between the low and high ACF groups in waist-hip-ratio, waist-hip ratio risk level, or waist circumference. There was a marked difference, however, when smoking was analyzed as a correlate of ACF (Table 6). Never and past smokers were more likely to be in the low ACF group than current smokers (58.9% vs. 60.0% vs. 18.2%, p=0.037). Eighty-one percent of current smokers fell into the high ACF group vs. 40.0% and 41.1% of past and never smokers, respectively. There were no significant differences between beer, red wine, and white wine consumption and ACF count. Almost 82% of participants that consumed 2 or more drinks of liquor per week, however, had high ACF, compared to 42.9 and 40.4% of participants who had 0-3 drinks per month or did not drink on a regular basis (p=0.039). When serum biomarkers were analyzed as correlates of ACF, there were no significant differences between the measured biomarkers and ACF count, with the exception of insulin levels (Table 7). The mean level of insulin (ulU/mL) in the high ACF group compared to the low ACF group was 5.84 vs. 3.44 (p=0.035).

3.4 Univariate and Age-Adjusted Logistic Regression Assessing the Relationship between Regular Statin Use and ACF Number

We calculated univariate and age-adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) in order to identify variables that might have an association between statin use and ACF number (Table 8). Compared to people taking <1 statin pill per week, people with regular statin use were 3.91 times more likely to have a high ACF count (95%CI=1.43-10.74, p=0.008). Age, current smoking, and frequent liquor intake were also shown to increase the risk for having a high ACF count. Participants age 50 years and older were 3 times more likely to have high ACF than those younger than age 50 (95%CI=0.77-12.32), and current smokers had a 4.90 times greater odds of having high ACF compared to never smokers (95%CI=0.92-26.11). Additionally, the age-adjusted OR for high ACF was 6.21 (95%CI= 1.22-31.78) when comparing users of high level of liquor (≥3 drinks per week) with never users of liquor. No significant differences in the logistic regression were noted for the following variables: sex, BMI, WHR, WC, baby aspirin, aspirin, other NSAIDs, red white, white wine, beer, personal history of polyps, and family history of CRC.

Age-adjusted ORs for the logistic regression are shown in Table 8. After adjusting for age, the OR for high ACF of taking ≥1 statin pill per week vs. taking <1 pill per week decreased to 2.67 (95%CI=0.89-7.97) and was no long

statistically significant. The OR for high ACF and being a current smoker compared to being a non-smoker, on the other hand, increased to 7.17 (95%CI=1.22-42.17) and became statically significant (p=0.029). The OR for high ACF of having a high intake of liquor vs. no liquor intake increased to 7.13 (95%CI=1.33-38.35) and remained statistically significant (p=0.022) after age adjustment.

Univariate and age-adjusted ORs and 95% CIs were additionally measured in order to identify serum inflammatory or adiposity-related biomarkers that might have an association between statin use and ACF number (Table 9). Insulin was the only biomarker that showed statistical significance in the crude and age adjusted logistic regressions (Crude OR: 1.31 (95%CI=1.01-1.70), p=0.045; Age-adjusted OR: 1.33 (95%CI=1.01-1.75) p=0.039). The following serum biomarkers were not significantly associated with ACF number in both the crude- and age-adjusted logistic regressions: IGF-2, hsCRP, IGFBP3, IGF1, IL-6, TNF-a, glucose, total cholesterol, and HDL cholesterol.

3.5 Base Multivariate Logistic Regression

Variables that were related to ACF in the Univariate model or were judged to have clinical value were placed in the multivariate model. The results for the multivariate regression base model can be found in Table 10. The OR for high

ACF in relation to regular statin use compared to no statin use was 1.47 (95%CI=0.28-7.74). Unlike the univariate estimate, this was no longer statistically significant (p=0.652). The OR for high ACF of age was 1.10 (95%CI=0.99-1.22; p=0.065), and the OR for high ACF of being a current smoker in comparison to having never smoked was 4.80 (95%CI=0.61-37.68; p=0.136) in the multivariate logistic regression. There was no statistical significance of any variables after stratifying the base model by age (≥50 years vs. <50 years old) (Table 14).

3.6 Adding Obesity Measures into the Base Multivariate Model

In order to understand if adiposity influences the association between ACF number and statin use, BMI, WHR, and WC were added into the base model individually. The results for the multivariate logistic regression BMI model can be found in Table 11. The OR for high ACF of regular statin use compared to no statin use was 1.24 (95%CI=0.21-7.47; p=0.017). The OR for high ACF of age was 1.11 (95%CI=1.00-1.23; p=0.063). Current smokers had more than 5 times greater odds of having high ACF than non-smokers (OR: 5.43; 95%CI=0.64-46.32; p-0.122). After stratifying for age (≥50 years vs. <50 years old), there was no longer statistical significance of any of the variables measured in the BMI model.

The results for the multivariate logistic regression WHR model can be found in Table 12. The OR for high ACF and regular statin use compared to no regular statin intake was 0.68 and no longer statistically significant (95%CI=0.10-5.40; p=0.702). Age, however, was significantly associated with high ACF number in the WHR model. The OR for age was 1.15 (95%CI=1.01-1.32; p=0.035), and the OR for high ACF of being a current smoker compared to non-smokers was 7.62 (95%CI=0.79-73.37; p=0.079). After stratifying for age (≥50 years vs. <50 years old) (Table 16), none of the variables showed statistical significance any longer.

The results for the multivariate logistic regression WC model can be found in Table 13. The OR for high ACF of regular statin use vs. no statin use was 1.20 (95%CI=0.20-1.20; p=0.842). The OR for high ACF of age was 1.12 (95%CI=0.99-1.26; p=0.62) and the OR for high ACF of being a current smoker vs. non-smoker was 2.58 (95%CI=0.54-38.54; p=0.162). After age stratification (≥50 years vs. <50 years old) (Table 17), there was still no statistical significance for statin use. The OR for high ACF of age was 1.19 (95%CI=1.00-1.41; p=0.054), and the OR for high ACF of currently smoking vs. non-smoking was 3.30 (95%CI=0.25-43.07; p=0.363). No variables showed statistical significant in either of the WC models.

3.7 Composite Variable of Joint Aspirin and Statin Use

In assessing the impact of joint statin and aspirin use, compared to individual use of these medications, we found that the crude OR for high ACF of taking statins compared to no statins or aspirin was 15 (p=0.019), compared to 1.83 (p=0.293) for aspirin only, and 4.29 (0.022) for statins and aspirin. After adjusting for age, they reduced to 11.03 (p=0.042), 1.35 (p=0.622), and 2.26 (p=0.265), respectively. After controlling for age, sex, NSAID use, family history of CRC, personal polyp history, and smoking in the multivariate model, the OR for high ACF of taking statins compared to no statin or aspirin use was 2.06 (p=0.620), compared to 0.45 (p=0.369) for aspirins only, and 0.45 (p=0.535) for statins and aspirins. Results for the composite statin and aspirin variable can be found in Table 17.

4.0 DISCUSSION

4.1 Key Findings

About 30% of our study population took statins on a regular basis (i.e., ≥1 pill per week for the past 12 months). Participants on a regular statin regimen had almost twice the number of mean ACF than those not taking statins regularly, which was statistically significant. Additionally, two-thirds of participants with regular statin use were categorized as having high ACF in comparison to a little over a third of participants who reported little or no statin use. Univariate logistic regression analyses showed that patients regularly taking statins had close to four times the likelihood of high levels of ACF than those not taking statins regularly. After adjustment for age, the OR reduced to just below 3.0 but did not remain statistically significant. When potential confounders were placed in the multivariate base model, this effect lessened, more so, with statin users having an approximately 1.5 times greater odds of having high ACF than non-statin users. OR estimates from neither the base model nor each of the three models with the adiposity measures were statically significant.

When examining joint use of statins and aspirin, univariate logistic regression suggested that participants who consumed statins (only) on a regular basis were 15 times more likely to have a high ACF count compared to the reference group of participants who consumed neither aspirin nor statins

regularly. In the age-adjusted and multivariate models, however, this effect was attenuated substantially and became non-significant. Of note, though not statistically significant, the odds of having a high ACF count among statin-only users was 2 times greater than odds among users of neither statin nor aspirin.

4.2 Consistency with Prior Studies on Statins and Colorectal Neoplasia

Our univariate logistic regression analyses of a positive association with ACF number are inconsistent with animal studies, which have suggested uniformly that statins lower the risk of colonic neoplasia (NCI, 2005). As predicted, virtually all of our multivariate analyses reduced the OR estimates to null. One such animal study, Narisawa et al. (1994), examined the effects of pravastatin and simvastatin on mice that received injections of 1,2-dimethylhydrazine (DMH) to induce colon cancer development and found that the incidence of colon tumors examined at weeks 25 or 30 was reduced by 67% in the pravastatin group and by 30% in the simvastatin group (Narisawa et al., 1994). While these results did not reach statistical significance, there was a considerable reduction in the number of tumors per mouse in both groups (Narisawa et al., 1994).

Evidence from human studies has been quite mixed. We believe, in part, this is due to all reports on statins and colonic neoplasia in humans having been derived from secondary analyses, with the recent exception of a

multicenter, phase II trial of statins for CRC chemoprevention by Limburg et al. (2011). Using ACF as the intermediate endpoint, Limburg (2011) reported a lack of convincing evidence that six months of statin use (atorvastatin or sulindac) reduced ACF number.

Singh et al. (2009) did not find any statistically significant reductions in CRC risk with regular use of statins, irrespective of duration and dose. Oddly, however, they did find a slightly increased risk with low dose statin intake among regular users compared to nonusers of statins. Low doses of statins have been shown to have a stimulatory effect on CRC cell lines (Kodach et al., 2007). Poynter et al. (2005) reported a 47% reduction in CRC risk among individuals using statins for 5 or more years. Hence, ascertaining dose may be in critical in human studies.

A recent study by Bertagnolli et al. (2010) suggests that for patients at high risk of CRC (e.g., patients with multiple adenomas or patients who have had a single adenoma ≥6 mm in diameter removed), statins do not protect against colorectal neoplasms and may even increase the risk of developing adenomas. These findings are consistent with early clinical trials that suggest that statin use might increase cancer risk (Alsheikh & Karas, 2009; Oliver, 1991; Rossebo et al., 2008; Shepherd et al., 2002). In the Bertagnolli (2010) study, participants who used statins for more than 3 years in the placebo group of the Adenoma Prevention with Celecoxib (APC) trial showed a 40% increase in adenoma detection over 5 years of surveillance.

On the other hand, Siddiqui et al. (2009) found that long-term statin use was associated with a 29% reduction in the incidence of new and advanced adenomatous colon polyps (APs) in patients that had previously had APs removed colonoscopically. This association remained, even after controlling for other known risk factors (Siddiqui et al., 2009). These data suggest that statins may decrease the development of CRC by reducing the development of new APs (Siddiqui et al., 2009), yet it is unknown what the incidence of ACF was.

Regarding evidence of indication bias related to adiposity, our study is inconclusive in that OR estimates were reduced in the base model prior to the introduction of adiposity measures into the model. While our adiposity results are not statistically significant, our data do show similar tends to Swede et al. (2009) in that higher adiposity levels seem to be associated with high ACF count. In terms of the association between adiposity and ACF number, Swede et al. (2009) found that high ACF count was significantly associated with higher BMI, WHR, and WC compared to people with low ACF. However, high ACF was defined as ≥5 whereas the cut point for the current study count was 10 ACF.

Our descriptive findings on the relationship between elevated BMI, WHR, and WC and increased ACF number are also consistent with a report by Takahashi et al. (2007), who found that a high level of visceral fat was associated with elevated ACF number. While our findings do not show a

statistically significant relationship between high ACF number and obesity, high WHR, and high WC, we did observe similar general trends. Individuals who were obese (BMI ≥30) were more likely to have a higher ACF count than individuals with normal BMI (18.5-24.9). Additionally, both males and females with high ACF had slightly higher mean WHR values. Our findings are consistent with findings reported by Swede et al. (2009), in terms of this relationship being stronger in females than males. Furthermore, over half of participants with elevated WHR-risk were more likely to fall into the high ACF group.

In relation to WC, both males and females in the high ACF group tended to have a higher mean WC. Females in the high ACF group had a slightly higher mean WC than that of those in the low ACF group. In males, this association was slightly stronger. While our findings reported WC to be associated with high ACF more strongly in males, Swede et al. (2009) found the opposite. Females with a higher WC were more strongly associated with high ACF than males ($p=0.06$ for females; $p=0.17$ for males) (Swede et al., 2009).

In reference to other lifestyle behaviors that affect CRC risk, smoking use and alcohol consumption are thought to induce ACF (Lopez-Ceron & Pellise, 2011). However, the effects of different types of alcoholic beverages (e.g. beer, liquor, wine) are not well understood (Lopez-Ceron & Pellise, 2011). In our study, over 80% of current smokers had high ACF. Current smokers

had an almost 5 times greater odds of having high ACF as opposed to non-smokers in the multivariate model. Additionally, frequent liquor consumption was significantly associated with high ACF count.

In regards to family history of CRC, Stevens et al. (2007) reported a significantly higher mean ACF number in patients with a positive family history of CRC in a first degree relative or a personal history of CRC ($p<0.01$ and $p<0.05$, respectively). Surprisingly, having a first-degree relative with CRC did not make participants more likely to have a higher ACF number in our study, nor did we see a link with polyps.

In terms of conventional anti-inflammatory medications, many observational studies have noted a lower CRC risk in association with regular intake of aspirin or NSAIDs (Simon, 2012). This was not quite as clear in our study, but, as described below, our findings about ACF were consistent with other observational studies in that there was a lower percentage of participants with high ACF in regular users of these anti-inflammatory medications in comparison to non-regular users.

Regarding baby aspirin use, we found that about two-thirds of participants taking low levels (i.e., <1 pill per week for past 12 months) had high ACF, compared to about half of regular aspirin users. Among regular aspirin users, 30% had a high ACF count compared to almost 62% of participants who took <1 pill per week.

While many studies have found that the sustained use of aspirin and other NSAIDs may decrease the incidence of APs and CRC and may decrease the mortality rate from CRC (Siddiqui et al., 2009), the gastrointestinal and other serious toxicities of NSAIDs limit their utility for the chemoprevention of CRC, especially in the older populations in which CRC is most prevalent (Siddiqui et al., 2009).

Age was significantly associated with high ACF count in our study. Participants on regular statins had a mean age 5 years greater than those taking <1 pill per week. People older than 50 that took statins did have slightly increased odds of high ACF in comparison to those younger than 50 in the multivariate model. Swede et al. (2009), however, did not find age to be significantly associated with high ACF. The mean age of individuals in the high ACF group (≥5 ACF) was slightly higher than the low ACF group (<5 ACF).

4.3 Strengths and Limitations

A major advantage of our study was the creation of a composite variable in which we assessed joint statin and aspirin use. Since, it is likely that patients on statins could be on other anti-inflammatory drugs (i.e., aspirin) for the long-term, we were able to examine the effects of statin use alone. Our results should be approached with caution, however, as we had a very small sample size in one of our study groups (e.g., n=7 for statins only). Understanding the

impact of a combination of different medications, and taking into account confounding between medications, should be a goal of future studies. Another strength of our study was that several inflammatory, adiposity, and lipid-associated biomarkers were assessed in relation to ACF number. Perhaps because of the small number of patients with these values (n=41), results were not conclusive. Also, the link between alcohol use and colonic neoplasia is not well understood, but we were able to broadly examine it in this study by comparing beer, liquor and wine intake to ACF count.

There are several limitations of this study. First, dietary intake and physical activity were not taken into account in our multivariate model. Both of these have shown to have an impact on ACF number and CRC risk and could have affected our results. Due to several methodical issues in measuring and evaluating physical activity, we chose not to include it in our study. A second limitation of this study is that biomarker levels were measured as one point in time. It is conceivable that serum levels of some biomarkers (cholesterol, insulin, glucose, etc.) can vary from baseline to reassessment. ACF number also shows dynamic variability (Schoen et al., 2008), but was measured at only a single point in time

Another key limitation of this study is the small size of the study population. The number of people taking ≥1 statin pill per week (i.e., regular statin use) was very low (n=25, 31.3%). This could potentially have resulted in unreliable estimates. Finally, type of statin use may influence tissue response

(Bertagnolli et al., 2010). While hydrophilic statins (e.g., fluvastin, rosuvastatin, pravastatin) are hepatoselective, lipophilic statins (e.g., lovastatin, simvastatin, atorvastin) tend to achieve higher drug levels in nonhepatic tissues and are thought to alter colorectal mucosa more (Bertagnolli et al., 2010). In an analysis using the Women's Health Initiative, Simon et al. (2012) found no overall protective effect of statins for CRC. A significant reduction was observed in CRC risk, however, for lovastatin users and a modest, but insignificant reduction for overall statin use of ≥3 years.

4.4 Conclusions

While statin use was associated with a significantly higher ACF count in our study population in univariate analyses, this effect did not remain after controlling for confounders. After adding measures of adiposity (BMI, WHR, WC) to the multivariate base model, only increased age and current smoking status remained associated with ACF number. Since CRC can take up to 20 years to develop, insufficient drug exposure and follow-up could be the culprit to a lack of consensus on the topic (Bertagnolli et al., 2010).

Future studies should focus more on length of time of statin use, perhaps by breaking it down into more discrete categories, as well as type of statin and dosage. Additionally, regular use of statins needs to be standardized. Most studies define regular statin use differently, making it

difficult to compare results. Finally, there is a need for studies to address indication bias, especially as adiposity continues to garner interest as a modifiable risk factor in CRC. It could be that statins are prescribed more often to people who are at high risk of acquiring CRC. Thus, it could appear that statins are causing high ACF number, when they are in fact being prescribed more often to those at high risk for CRC. Further exploration of these topics may provide a greater understanding of the inter-relationships of statins, adiposity, and CRC, leading to effective preventive interventions that will improve the public health.

References

Agarwal, B., Rao, C. V., Bhendwal, S., Ramey, W. R., Shirin, H., Reddy, B. S., et al. (1999). Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. *Gastroenterology, 117*(4), 838-47.

Ahnen, D. J., & Byers, T. (2009). Editorial: Colorectal cancer and statins: Reflections from the end of the road. *Am J Gastroenterol, 104*(12), 3024-6.

Alrawi, S. J., Carroll, R. E., Hill, H. C., Gibbs, J. F., Tan, D., Brenner, B. M., et al. (2006). Genomic instability of human aberrant crypt foci measured by inter-(simple sequence repeat) PCR and array-CGH. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 601*(1–2), 30-8.

Alsheikh, A., & Karas, R. H. (2009). The relationship of statins to rhabdomyolysis, malignancy, and hepatic toxicity: Evidence from clinical trials. *Current Atherosclerosis Reports, 11*(2), 100-4.

Anderson, J. C., Pleau, D. C., Rajan, T. V., Protiva, P., Swede, H., Brenner, B., et al. (2010). Increased frequency of serrated aberrant crypt foci among smokers. *American Journal of Gastroenterology, 105*(7), 1648-1654.

Balkau, B., Charles, M. A., Drivsholm, T., Borch-Johnsen, K., Wareham, N., Yudkin, J. S., et al. (2002). Frequency of the WHO metabolic syndrome in european cohorts, and an alternative definition of an insulin resistance syndrome. *Diabetes & Metabolism, 28*(5), 364-76.

Bertagnolli, M. M., Hsu, M., Hawk, E. T., Eagle, C. J., & Zauber, A. G. (2010). Statin use and colorectal adenoma risk: Results from the adenoma prevention with celecoxib trial. *Cancer Prev Res, 3*(5), 588-96.

Bird, R. P. (1987). Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: Preliminary findings. *Cancer Letters, 37*(2), 147-151.

Boudreau, D. M., Yu, O., & Johnson, J. (2010). Statin use and cancer risk: A comprehensive review. *Expert Opinion on Drug Safety, 9*(4), 603-21.

Bruce, W. R., Giacca, A., & Medline, A. (2000). Possible mechanisms relating diet and risk of colon cancer. *Cancer Epidemiology Biomarkers & Prevention, 9*(12), 1271-1279.

Centers for Disease Control and Prevention [CDC]. (2010). Colorectal cancer statistics. Retrieved from www.cdc.gov/cancer/colorectal/statistics/index.htm

Gram, I. T., Norat, T., Rinaldi, S., Dossus, L., Lukanova, A., Téhard, B., et al. (2006). Body mass index, waist circumference and waist-hip ratio and serum levels of IGF-I and IGFBP-3 in european women. *International Journal of Obesity, 30*(11), 1623-31.

Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E., & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians, 61*(2), 69-90.

Kaaks, R., Toniolo, P., Akhmedkhanov, A., Lukanova, A., Biessy, C., Dechaud, H., et al. (2000). Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins, and colorectal cancer risk in women. *Journal of the National Cancer Institute, 92*(19), 1592-1600.

Kinzler, K. W., & Vogelstein, B. (1996). Lessons from hereditary colorecal cancer. *Cell, 87*(2), 159-170.

Kodach, L. L., Bleuming, S. A., Peppelenbosch, M. P., Hommes, D. W., van den Brink, G. R., & Hardwick, J. C. (2007). The effect of statins in colorectal cancer is mediated through the bone morphogenetic protein pathway. *Gastroenterology, 133*(4), 1272-81.

Limburg, P.J., Mahoney, M.R., Ziegler, K.L., Sontag, S.J., Schoen, R.E., Benya, R. et al. (2011). Randomized phase II trial of sundilic, atorvastatin, and prebiotic dietary fiber for colorectal cancer chemoprevention. *Cancer Prev Res, 4*(2), 259-69.

Lipkin, M., Reddy, B., Newmark, H., & Lamprecht, S. A. (1999). Dietary factors in human colorectal cancer. *Annual Review of Nutrition, 19*(1), 545-586.

Lopez-Ceron, M., & Pellise, M. (2011). Review article: Biology and diagnosis of aberrant crypt foci. *Colorectal Disease,* no-no.

Narisawa, T., Fukaura, Y., Terada, K., Umezawa, A., Tanida, N., Yazawa, K., et al. (1994). Prevention of 1,2-dimethylhydrazine-induced colon tumorigenesis by HMG-CoA reductase inhibitors, pravastatin and simvastatin, in ICR mice. *Carcinogenesis, 15*(9), 2045-8.

National Cancer Institute [NCI]. (2005). Statins and cancer prevention: Fact sheet. Retrieve from www.cancer.gov/cancertopics/factsheet/prevention/statins

Oliver, M. F. (1991). Might treatment of hypercholesterolaemia increase non-cardiac mortality? *Lancet, 337*(8756), 1529-31.

Parker-Ray, N., Barakat, J., Roy, P., White, R. E., Schrader, R. M., & Hoffman, R. M. (2010). Statin use does not prevent recurrent adenomatous polyp formation in a VA population. *Indian J Gastroenterol, 29*(3), 106-111.

Potter, J. D. (1999). Colorectal cancer: Molecules and populations. *Journal of the National Cancer Institute, 91*(11), 916-932.

Poynter, J. N., Gruber, S. B., Higgins, P. D., Almog, R., Bonner, J. D., Rennert, H. S., et al. (2005). Statins and the risk of colorectal cancer. *N Engl J Med, 352*(21), 2184-92.

Rossebo, A. B., Pedersen, T. R., Boman, K., Brudi, P., Chambers, J. B., Egstrup, K., et al. (2008). Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *The New England Journal of Medicine, 359*(13), 1343-56.

Sakai, E., Takahashi, H., Kato, S., Uchiyama, T., Hosono, K., Endo, H., et al. (2011). Investigation of the prevalence and number of aberrant crypt foci associated with human colorectal neoplasm. *Cancer Epidemiology Biomarkers & Prevention, 20*(9), 1918-1924.

Schoen, R. E., Mutch, M., Rall, C., Dry, S. M., Seligson, D., Umar, A., et al. (2008). The natural history of aberrant crypt foci. *Gastrointestinal Endoscopy,67*(7), 1097-102.

Shepherd, J., Blauw, G. J., Murphy, M. B., Bollen, E. L., Buckley, B. M., Cobbe, S. M., et al. (2002). Pravastatin in elderly individuals at risk of vascular disease (PROSPER): A randomized controlled trial. *Lancet, 360*(9346), 623-30.

Siddiqui, A., Nazario, H. E., Patel, M., Mahgoub, A., & Spechler, S. J. (2009). Reduction in low-density lipoprotein cholesterol levels during statin therapy is associated with a reduced incidence of advanced colon polyps. *Am J Med Sci, 338*(5), 378-81.

Simon, M. S., Rosenberg, C. A., Rodabough, R. A., Greenland, P., Ockene, I. S., Hemant, R. K. et al. (2010). Prospective analysis of the association between use of statins or other lipid-lowering agents and colorectal cancer risk [Abstract]. *Cancer Prevention Research, 22*(1) 17-27.

Singh, H., Mahmud, S. M., Turner, D., Xue, L., Demers, A. A., & Bernstein, C. N. (2008). Long-term use of statins and risk of colorectal cancer: A population-based study. *The American Journal of Gastroenterology, 104*(12), 3015-3023.

Stevens, R. G., Swede, H., Heinen, C. D., Jablonski, M., Grupka, M., Ross, B., et al. (2006). Aberrant crypt foci in patients with a positive family history of sporadic colorectal cancer. *Cancer Letters, 248*(2007), 262-268.

Stevens, R. G., Swede, H., & Rosenberg, D. W. (2006). Epidemiology of colonic aberrant crypt foci: Review and analysis of existing studies. *Cancer Letters, 252*(2007), 171-183.

Swede, H., Rohan, T., Yu, H., Anderson, J., Stevens, R., Brokaw, J., et al. (2009). Number of aberrant crypt foci associated with adiposity and IGF1 bioavailability. *Cancer Causes and Control, 20*(5), 653-661.

Takahashi, H., Yoneda, K., Tomimoto, A., et al. (2007). Life style-related diseases of the digestive system: Colorectal cancer as a life style-related disease: From carcinogenesis to medical treatment. *J Pharmacol Sci, 105*(2), 129-132.

Takayama, T., Katsuki, S., Takahashi, Y., Ohi, M., Nojiri, S., Sakamaki, S., et al. (1998). Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med, 339*(18), 1277-1284.

Table 1: Description of Study Population (n=86)

Statin Use in Past 12 months	<1 pill per week		55 (68.8%)
	≥1 pill per week		25 (31.3%)
Age (mean, SD)			56.79 (8.58)
Age	<50 yrs		12 (14%)
	≥50 yrs		74 (86%)
Sex	Male		56 (65.1%)
	Female		30 (34.9%)
Race	White		78 (90.7%)
	Black		7 (8.1%)
	Asian		1 (1.2%)
Education	Less than High School		5 (5.9%)
	High School or GED		10(11.6%)
	Vocational or Trade School		18 (20.9%)
	Some College		20 (23.3%)
	2-year Degree		17 (19.8%)
	At Least College Degree		14 (16.3%)
1st Degree Relative with CRC		Yes	18 (20.9%)
		No	61 (70.9%)
Personal History of Polyps		Yes	14 (17.7%)
		No	65 (82.3%)
BMI	Normal (18.5-24.9)		24 (27.9%)
	Overweight (25.0-29.9)		36 (41.9%)
	Obese (30+)		26 (30.2%)
Waist-hip-ratio (mean, SD)	Male		0.93 (0.06)
	Female		0.87 (0.09)
Waist-Hip-Ratio Risk	Male	Normal (≤0.90)	16 (28.6%)
		High (>0.90)	32 (57.1%)
	Female	Normal (≤0.85)	10 (33.3%)
		High (>0.85)	17 (56.7%)
Waist Circumference (mean, SD)	Male		38.05 (4.61)
	Female		36.41 (5.96)
Waist Circumference Risk	Male	Normal (≤40.0)	41 (78.8%)
		High (>40.0)	11 (21.2%)
	Female	Normal (≤35.0)	14 (51.9%)
		High (>35.0)	13 (48.1%)

Table 2: Correlates of Regular Statin Use (n=86)

			Weekly Statin Use Past 12 Months		
			<1 pill	**≥1 pill**	***p*-value[1]**
ACF number			9.02	17.20	0.001
ACF Number		Low (<10)	35 (81.4%)	8 (18.6%)	0.006
		High (≥10)	19 (52.8%)	17 (47.2%)	
Age (mean, SD)			54.51 (8.24)	62.40 (7.69)	<0.001
Age		<50 years	12 (100%)	0	
		≥ 50 years	43 (63.2%)	25 (36.8%)	0.014
Sex		Male	36 (66.7%)	18 (33.3%)	0.562
		Female	19 (73.1%)	7 (26.9%)	
1st Degree Relative with CRC		Yes	12 (66.7%)	6 (33.3%)	0.969
		No	38 (69.1%)	17 (30.9%)	
Personal History of Polyps		Yes	13 (65.0%)	7 (35.0%)	0.418
		No	30 (75.0%)	10 (25.0%)	
Body Mass Index (mean, SD)			27.25 (4.38)	30.26 (5.61)	0.011
Body Mass Index		Normal [18.5-24.9]	18 (75.0%)	6 (25.0%)	0.001
		Overweight [25.0-29.9]	28 (84.8%)	5 (15.2%)	
		Obese [30+]	9 (39.1%)	14 (60.9%)	
Sex-specific Waist-Hip-Ratio Risk		Normal	21 (80.8%)	5 (19.2%)	0.091
		Elevated	27 (61.4%)	17 (38.6%)	
Waist Circumference (mean, SD)			36.44 (4.87)	39.28 (5.10)	0.022
Baby Aspirin		Never	41 (82.0%)	9 (18.0%)	0.001
		<1 pill/week	2 (100%)	0	
		≥1 pill/week	11 (40.7%)	16 (59.3%)	
Regular Aspirin		Never	39 (68.4%)	18 (31.6%)	0.683
		<1 pill/week	10 (76.9%)	3 (23.1%)	
		≥1 pill/week	6 (60.0%)	4 (40.0%)	
Other NSAIDS		Never	24 (77.4%)	7 (22.6%)	0.080
		<1 pill/week	20 (74.1%)	7 (25.9%)	
		≥1 pill/week	11 (50.0%)	11 (50.0%)	
Smoking		Never	34 (70.8%)	14 (29.2%)	0.865
		Past	16 (66.7%)	8 (33.3%)	
		Current	5 (62.5%)	3 (37.5%)	
Alcohol	Red Wine	Never	32 (71.1%)	13 (28.9%)	0.813
		0-3 drinks /month	12 (63.2%)	7 (36.8%)	
		≥2 drinks per week	10 (66.7%)	5 (33.3%)	
	White Wine	Never	39 (67.2%)	19 (32.8%)	0.939
		0-3 drinks /month	5 (71.4%)	2 (28.6%)	
		≥2 drinks per week	10 (71.4%)	4 (28.6%)	
	Beer	Never	28 (62.2%)	17 (37.8%)	0.211
		0-3 drinks /month	13 (68.4%)	6 (31.6%)	
		≥2 drinks per week	13 (86.7%)	2 (13.3%)	
	Liquor	Never	36(72.0%)	14 (28.0%)	0.522
		0-3 drinks /month	12 (66.7%)	6 (33.3%)	
		≥2 drinks per week	6 (54.5%)	5 (45.5%)	

[1] T-tests for independent samples for continuous data and chi-square test for proportions

Table 3: Correlates of Statin Use: *Biomarkers Related to Inflammation, Insulin Resistance, and Lipid Levels* (n=41)

	Weekly Statin Use Past 12 Months		
	Never	**≥1 pill**	***p*-value[1]**
hsCRP, mg/L	4.22	4.94	0.734
IL-6, pg/mL	62.86	31.10	0.282
TNF-a, pg/mL	5.78	4.17	0.234
Insulin, ulU/mL	4.41	4.38	0.980
Glucose	88.17	89.29	0.913
Triglyceride	105.83	88.07	0.245
Cholesterol, Total	185.72	189.86	0.705
Cholesterol, HDL	49.94	58.43	0.166
IGF-1, ng/mL	122.80	110.64	0.235
IGF-2, ng/mL	758.16	685.07	0.064
IGFBP3, ug/mL	5.07	4.44	0.045

Table 4: Correlates of ACF Number: ***Demographics, Medication Use, Personal Polyp History, and 1st Degree relative with Colorectal Cancer (n=86)***

		ACF Number		
		Low (<10)	**High (≥10)**	***p*-value[1]**
Statin Use (Past 12 months)	<1 pill/week	39 (62.9%)	23 (37.1%)	0.010
	≥1 pill/week	9 (33.3%)	18 (66.7%)	
Baby Aspirin	None	30 (60.0%)	20 (40%)	0.390
	<1 pill/week	1 (33.3%)	2 (66.7%)	
	≥1 pill/week	12 (46.2%)	14 (53.8%)	
Aspirin	None	31 (54.4%)	26 (45.6%)	0.381
	<1 pill/week	5 (38.5%)	(8) 61.5%	
	≥1 pill/week	7 (70.0%)	3 (30.0%)	
Other NSAIDs	None	19 (61.3%)	12 (38.7%)	0.556
	<1 pill/week	13 (48.1%)	51.9% (14)	
	≥1 pill/week	11 (50%)	11 (50%)	
Age (years)	Mean	54.17	59.40	0.002
	Range	51.82-56.52	57.03-61.78	
Sex	Male	33 (52%)	29 (46.8%)	0.685
	Female	19 (57.6%)	14 (42.4%)	
Race	White	42 (54.5%)	35 (45.5%)	0.268
	Black	2 (28.6%)	5 (71.4%)	
	Asian	1 (100%)	0	
Education	Less than High School	1 (20%)	4 (80%)	0.520
	High School or GED	6 (60%)	4 (40%)	
	Vocational/Trade School	12 (66.7%)	6 (33.3%)	
	Some College	9 (45%)	11 (55%)	
	2 Year Degree	8 (50%)	8 (50%)	
	4 or 5 Year Degree	9 (64.3%)	5 (35.7%)	
Personal Polyp History	Yes	9 (42.9%)	12 (57.1%)	0.165
	No	24 (61.5%)	15 (38.5%)	
1st Degree Relative with CRC	Yes	11 (61.1%)	7 (38.9%)	0.668
	No	31 (51.7%)	29 (48.8%)	

[1]T-tests for independent samples for continuous data and chi-square test for proportions

Table 5: Correlates of ACF Number: *Adiposity*

		ACF Number		
		Low (<10)	**High (≥10)**	***p*-value[1]**
Body Mass Index (kg/m^2)		27.21 (SD)[2]	28.99 (SD)	0.152
Body Mass Index	Normal [18.5-24.9]	14 (58.3%)	10 (41.7%)	0.634
	Overweight [25.0-29.9]	20 (57.1%)	15 (42.9%)	
	Obese [≥30]	11 (42.3%)	15 (57.5%)	
Waist-Hip-Ratio	Male	0.92 (0.07)	0.93 (0.06)	0.788
	Female	0.86 (0.07)	0.89 (0.11)	0.405
Waist-Hip-Ratio Risk	Normal	18 (62.1%)	11 (37.9%)	0.196
	Elevated	24 (47.1%)	27 (52.9%)	
Waist Circumference	Male	37.14 (5.23)	38.89 (3.87)	0.174
	Female	36.22 (5.85)	36.59 (6.59)	0.883

[1] T-tests for independent samples for continuous data and chi-square test for proportions

[2] Mean (SD)

Table 6: Correlates of ACF Number: *Smoking and Alcohol*

			ACF Number		
			Low (<10)	**High (≥10)**	***p*-value**[1]
Smoking	Never		33 (58.9%)	23 (41.1%)	0.037
	Past		15 (60.0%)	10 (40.0%)	
	Current		2 (18.2%)	9 (81.8%)	
Alcohol	Beer	Never	27 (50.9%)	26 (49.1%)	0.694
		0-3 drinks/month	13 (61.9%)	8 (38.1%)	
		≥2 drinks/week	8 (53.3%)	7 (46.7%)	
	Liquor	Never	34 (59.6%)	23 (40.4%)	0.039
		0-3 drinks/month	12 (57.1%)	9 (42.9%)	
		≥2 drinks/week	2 (18.2%)	9 (81.8%)	
	Red Wine	Never	28 (53.8%)	24 (46.2%)	0.667
		0-3 drinks/month	10 (47.6%)	11 (52.4%)	
		≥2 drinks/week	10 (62.5%)	6 (37.5%)	
	White Wine	Never	37 (56.1%)	29 (43.9%)	0.783
		0-3 drinks/month	4 (50.0%)	4 (50.0%)	
		≥2 drinks/week	7 (46.7%)	8 (53.3%)	

[1] T-tests for independent samples for continuous data and chi-square test for proportions

Table 7: Correlates of ACF Number: *Biomarkers of Inflammation, Insulin Resistance, and Lipid Levels* (n=41)

	ACF Number		
	Low (<10)	**High (≥10)**	***p*-value[2]**
hsCRP (mg/L)	4.09 (4.08)[1]	5.39 (7.23)[1]	0.507
IL-6 (pg/mL)	87.79 (215.97)	57.90 (99.82)	0.614
TNF-a (pg/mL)	5.45 (3.66)	4.51 (3.24)	0.473
Insulin (uIU/mL)	3.44 (2.78)	5.84 (3.16)	0.035
Glucose	86.72 (27.93)	90.47 (28.20)	0.705
Triglyceride	97.33 (48.20)	98.47 (33.87)	0.939
Cholesterol, Total	185.78 (28.02)	186.33 (34.72)	0.960
Cholesterol, HDL	52.00 (12.09)	54.27 (22.16)	0.712
IGF-1 (ng/mL)	116.54 (21.38)	126.07 (37.05)	0.348
IGF-2 (ng/mL)	710.96 (110.43)	772.05 (116.38)	0.121
IGFBP3 (ug/mL)	4.80 (0.95)	5.05 (1.04)	0.457

[1] Standard deviation for continuous variables
[2] T-tests for independent samples for continuous data

Table 8: Univariate and Age-Adjusted Odds Ratios and 95% Confidence Intervals for ACF Number in relation to Key CRC Risk Factors

		Crude		Age-Adjusted	
		OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
Statins	<1 pill per week	1.00		1.00	
	≥ 1 pill per week	3.91 (1.43-10.74)	0.008	2.67 (0.89-7.97)	0.079
Age		1.08 (1.02-1.14)	0.009	-	-
Age	< 50 years	1.00		1.00	
	≥ 50 years	3.08 (0.77-12.32)	0.111	1.10 (0.21-1.15)	0.910
Sex		0.71 (0.29-1.75)	0.451	0.65 (0.25-1.71)	0.385
BMI		1.06 (0.98-1.16)	0.159	1.06 (0.97-1.14)	0.207
BMI	Normal	1.00		1.00	
	Overweight	0.95 (0.33-2.73)	0.928	0.95 (0.31-2.91)	0.927
	Obese	1.82 (0.65-5.08)	0.254	1.68 (0.58-4.81)	0.337
Sex-specific Waist-Hip-Ratio Risk	Normal	1.00		1.00	
	Elevated	1.89 (0.72-5.00)	0.199	1.50 (0.53-4.21)	0.443
Waist Circumference		1.06 (0.96-1.15)	0.243	1.04 (0.95-1.14)	0.431
Baby Aspirin		1.64 (0.64-4.23)	0.303	0.89 (0.30-2.66)	0.833
Aspirin		0.45 (0.11-1.90)	0.279	0.44 (0.10-1.91)	0.272
Other NSAIDs	<1 pill per week	1.00		1.00	
	≥ 1 pill per week	1.23 (0.46-3.29)	0.679	1.15 (0.42-3.19)	0.784
Smoking	Never	1.00		1.00	
	Past	1.00 (0.37-2.70)	1.000	1.02 (0.35-2.97)	0.969
	Current	4.90 (0.92-26.11)	0.063	7.17 (1.22-42.17)	0.029
Red Wine	None	1.00		1.00	
	0-3 drinks per month	1.27 (0.43-3.72)	0.663	1.23 (0.40-3.85)	0.719
	≥ 3 drinks per week	0.76 (0.23-2.50)	0.654	0.69 (0.20-1.14)	0.554
White Wine	None	1.00		1.00	
	0-3 drinks per month	1.76 (0.36-8.58)	0.484	1.67 (0.32-8.64)	0.540
	≥ 3 drinks/week	1.76 (0.54-5.72)	0.347	1.67 (0.49-5.76)	0.414
Beer	None	1.00		1.00	
	0-3 drinks per month	0.76 (0.26-2.24)	0.620	0.93 (0.29-2.92)	0.897
	≥ 3 drinks per week	0.92 (0.28-2.95)	0.881	1.32 (0.38-4.61)	0.663
Liquor	None	1.00		1.00	
	0-3 drinks per month	0.88 (0.29-2.64)	0.818	0.90 (0.28-2.89)	0.855
	≥ 3 drinks per week	6.21 (1.22-31.78)	0.028	7.13 (1.33-38.35)	0.022
Personal Polyp History	Yes	1.00			
	No	0.47 (0.16-1.38)	0.169	0.64 (0.20-2.02)	0.446
1st Degree Relative with CRC	Yes	1.00			
	No	1.89 (0.62-5.77)	0.261	1.78 (0.55-5.88)	0.337

Table 9: Univariate and Age-Adjusted Odds Ratios and 95% Confidence Intervals for ACF Number in relation to Biomarkers of Inflammation, Insulin Resistance and Lipid Levels

	Crude		Age Adjusted	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
IGF-2	1.01 (0.10-1.01)	0.112	1.01 (1.00-1.01)	0.117
hsCRP	1.03 (0.91-1.16)	0.686	1.04 (.91-1.18)	0.605
IGFBP3	1.40 (0.62-3.14)	0.421	1.46 (.62-3.42)	0.388
IFG1	1.01 (0.99-1.04)	0.362	1.02 (.99-1.05)	0.201
Insulin	1.31 (1.01-1.70)	0.045	1.33 (1.01-1.75)	0.039
IL-6	1.03 (0.99-1.01)	0.561	1.00 (1.00-1.01)	0.418
TNF-a	0.92 (0.73-1.16)	0.463	0.93 (0.73-1.18)	0.547
Glucose	1.01 (0.98-1.03)	0.696	1.01 (0.98-1.04)	0.530
Triglyceride	1.00 (0.98-1.02)	0.937	1.00 (0.99-1.02)	0.863
Cholesterol, total	1.00 (0.98-1.02)	0.958	1.00 (0.98-1.02)	0.926
Cholesterol, HDL	1.01 (0.97-1.05)	0.702	1.00 (0.96-1.04)	0.950

Table 10: Multivariate ORs and 95% CIs in relation to ACF Number- *Base Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.47	0.28-7.74	0.652
Age		1.10	0.99-1.22	0.065
Sex	Male	1.00		
	Female	0.96	0.26-3.51	0.952
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.79	0.17-3.64	0.762
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.67	0.15-3.05	0.608
1st Degree Relative with CRC	Yes	1.00		
	No	1.25	0.26-6.00	0.783
Personal History of Polyps	Yes	1.00		
	No	1.08	0.26-4.43	0.915
Smoking	Never	1.00		
	Past	0.76	0.19-3.05	0.704
	Current	4.80	0.61-37.68	0.136

Table 11: Multivariate ORs and 95% CIs in relation to ACF Number: - *BMI added to Base Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.24	0.21-7.47	0.817
Age		1.11	1.00-1.23	0.063
Sex	Male	1.00		
	Female	1.03	0.27-3.89	0.967
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.73	0.15-3.55	0.694
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.69	0.15-3.14	0.627
1st Degree Relative with CRC	Yes	1.00		
	No	1.15	0.24-5.62	0.862
Personal History of Polyps	Yes	1.00		
	No	1.11	0.27-4.60	0.890
Smoking	Never	1.00		
	Past	0.85	0.20-3.54	0.817
	Current	5.43	0.64-46.32	0.122
BMI		1.04	0.89-1.22	0.637

Table 12: Multivariate ORs and 95% CIs in relation to ACF Number: *WHR added to Base Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	0.68	0.10-5.40	0.702
Age		1.15	1.01-1.32	0.035
Sex	Male	1.00		
	Female	1.03	0.24-4.43	0.969
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.98	0.18-5.40	0.984
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.93	0.19-4.58	0.931
1st Degree Relative with CRC	Yes	1.00		
	No	1.10	0.21-5.63	0.914
Personal History of Polyps	Yes	1.00		
	No	1.30	0.25-6.80	0.754
Smoking	Never	1.00		
	Past	0.66	0.11-3.92	0.648
	Current	7.62	0.79-73.37	0.079
Waist-Hip-Ratio		0.03	0.00-412.07	0.473

Table 13: Multivariate ORs and 95% CIs in relation to ACF Number: *WC added to Base Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.20	0.20-7.36	0.842
Age		1.12	0.99-1.26	0.062
Sex	Male	1.00		
	Female	0.91	0.23-3.59	0.893
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.62	0.17-3.26	0.569
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.61	0.13-2.89	0.536
1st Degree Relative with CRC	Yes	1.00		
	No	1.24	0.25-6.22	0.793
Personal History of Polyps	Yes	1.00		
	No	0.95	0.20-4.55	0.949
Smoking	Never	1.00		
	Past	1.00	0.19-5.17	0.999
	Current	2.58	0.54-38.54	0.162
Waist Circumference		1.02	0.88-1.17	0.829

Table 14: Multivariate ORs and 95% CIs in relation to ACF Number: *Age-stratified (≥50 yrs) Base Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.48	0.27-8.14	0.650
Age		1.09	0.96-1.24	0.175
Sex	Male	1.00		
	Female	1.04	0.23-4.73	0.959
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.99	0.19-5.01	0.986
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.64	0.13-3.06	0.638
1st Degree Relative with CRC	Yes	1.00		
	No	1.84	0.31-10.89	0.500
Personal History of Polyps	Yes	1.00		
	No	1.00	0.22-4.57	0.997
Smoking	Never	1.00		
	Past	0.69	0.17-2.90	0.615
	Current	1.67	0.17-16.40	0.662

Table 15: Multivariate ORs and 95% CIs in relation to ACF Number: *Age-stratified (≥50 yrs) BMI Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.44	0.22-9.63	0.704
Age		1.09	0.96-1.25	0.196
Sex	Male	1.00		
	Female	1.05	0.23-4.82	0.953
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.97	0.17-5.40	0.971
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.64	0.13-3.12	0.582
1st Degree Relative with CRC	Yes	1.00		
	No	1.82	0.30-11.18	0.518
Personal History of Polyps	Yes	1.00		
	No	1.00	0.22-4.66	0.997
Smoking	Never	1.00		
	Past	0.71	0.15-3.31	0.659
	Current	1.70	0.16-18.07	0.661
BMI		1.01	0.84-1.20	0.950

Table 16: Multivariate ORs and 95% CIs in relation to ACF Number: *Age-stratified (≥50 yrs) WHR Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	0.55	0.06-4.80	0.591
Age		1.19	1.00-1.41	0.054
Sex	Male	1.00		
	Female	1.03	0.19-5.53	0.970
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	1.08	0.17-6.77	0.935
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	1.03	0.19-5.64	0.976
1st Degree Relative with CRC	Yes	1.00		
	No	1.76	0.27-11.5	0.554
Personal History of Polyps	Yes	1.00		
	No	1.33	0.21-8.21	0.762
Smoking	Never	1.00		
	Past	0.66	0.10-4.44	0.670
	Current	3.30	0.25-43.07	0.363
Waist-Hip-Ratio		0.07	.00-1760.30	0.600

Table 17: Multivariate ORs and 95% CIs in relation to ACF Number: *Age-stratified (≥50 yrs) WC Model*

		Adjusted OR	95% CI	*p*-value
Statins	<1 pill per week	1.00		
	≥ 1 pill per week	1.18	0.17-8.00	0.869
Age		1.13	0.96-1.32	0.133
Sex	Male	1.00		
	Female	0.87	0.18-4.29	0.865
Baby Aspirin	<1 pill per week	1.00		
	≥ 1 pill per week	0.72	0.12-4.56	0.731
All Other NSAIDs	<1 pill per week	1.00		
	≥ 1 pill per week	0.63	0.12-3.20	0.577
1st Degree Relative with CRC	Yes	1.00		
	No	1.87	0.30-11.75	0.507
Personal History of Polyps	Yes	1.00		
	No	0.89	0.15-5.25	0.894
Smoking	Never	1.00		
	Past	0.96	0.16-5.66	0.967
	Current	1.63	0.15-17.40	0.685
Waist Circumference		1.00	0.87-1.18	0.874

Table 18: Univariate, Age-adjusted, and Multivariate ORs and 95% CIs in relation to ACF Number: *Joint Statin & Aspirin Use*

			Univariate		Age-Adjusted[1]		Multivariate	
		n	**Crude OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**	**OR (95% CI)**	***p*-value**
Statins & Aspirin Use	No Statins or Aspirin	28	1.00		1.00		1.00	
	Statins Only	7	15.00 (1.55-145.23)	0.019	11.03 (1.10-111.01)	0.042	2.06 (0.12-36.25)	0.620
	Aspirin Only (baby or regular)	26	1.83 (0.59-5.68)	0.293	1.35 (0.41-4.47)	0.622	0.45 (0.08-2.57)	0.369
	Statins and Aspirin (baby or regular)	19	4.29 (1.24-14.83)	0.022	2.26 (0.54-9.48)	0.265	0.45 (0.04-5.53)	0.535
Age		80	-	-	1.06 (0.99-1.13)	0.09	1.14 (1.01-1.29)	0.035
Sex	Male	39	-	-	-	-	1.00	
	Female	18	-	-	-	-	0.82 (0.21-3.17)	0.775
NSAIDs[2]	<1 pill per week	45	-	-	-	-	1.00	
	≥1 pill per week	12	-	-	-	-	0.69 (0.15-3.11)	0.627
1st Degree Relative CRC	Yes	12	-	-	-	-	1.00	
	No	45	-	-	-	-	1.35 (0.27-6.72)	0.715
Personal Polyp History	Yes	21	-	-	-	-	1.00	
	No	36	-	-	-	-	1.28 (0.30-5.51)	0.737
Smoking	Never	33	-	-	-	-	1.00	
	Past	16	-	-	-	-	0.69 (0.16-2.94)	0.616
	Current	8	-	-	-	-	7.30 (0.87-61.65)	0.068

[1] See Table 10 for age-adjusted analyses for sex, NSAIDs, 1st Degree Relative with CRC, Personal Polyp History, and Smoking
[2] Removing NSAIDs from the analysis did not significantly affect results.