Published in final edited form as:

Cancer Causes Control. 2018 January ; 29(1): 115–123. doi:10.1007/s10552-017-0990-z.

Plasma lipopolysaccharide-binding protein and colorectal cancer risk: a nested case-control study in the Multiethnic Cohort

Jessica S Citronberg^{1,2}, Lynne R Wilkens³, Loic Le Marchand³, Unhee Lim³, Kristine R Monroe⁴, Meredith AJ Hullar², Emily White^{1,2}, Polly A Newcomb^{1,2}, and Johanna W Lampe^{1,2}

¹Department of Epidemiology, University of Washington, Seattle, WA

²Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA

³Epidemiology Program, University of Hawaii Cancer Center, Honolulu, HI

⁴Keck School of Medicine of University of Southern California, Los Angeles, CA

Abstract

BACKGROUND—Lipopolysaccharide (LPS), an endotoxin found on the outer cell wall of gram-negative bacteria, increases inflammatory response signaling and may play a role in the pathogenesis of several adverse outcomes, including inflammatory bowel diseases, cardiovascular disease, and cancer. While LPS is hypothesized to be associated with colorectal carcinogenesis, there are relatively few human studies which have examined this association.

METHODS—We examined the association between colorectal cancer (CRC) and plasma lipopolysaccharide-binding protein (LBP), a marker of LPS, in 1,638 participants (819 CRC cases and 819 controls matched on multiple factors, including age, sex, and race/ethnicity, from the Multiethnic Cohort study (MEC). Conditional logistic regression models were used to estimate the multivariable-adjusted odds ratios (OR) and 95% confidence intervals (95% CI).

RESULTS—Compared to individuals whose LBP concentrations were in the lowest quartile, the ORs associated with second, third, and fourth quartiles were 1.23 (95% CI = 0.91–1.67), 1.36 (95% CI = 1.01–1.83), and 1.01 (95% CI = 0.73–1.39), respectively, ($P_{\text{trend}} = 0.66$). No differences were observed by BMI, fiber intake, saturated fat intake, cancer site, or cancer stage.

CONCLUSIONS—This study did not find an overall statistically significant association between LBP (as a marker of LPS exposure) and CRC. Further prospective studies with multiple LBP measurements are needed to validate current findings.

Corresponding Author: Jessica Citronberg, Fred Hutchinson Cancer Research Center, PO Box 19024, M4-B402, Seattle, WA 98102 (Phone: 206-667-4068, jcitronb@fredhutch.org).

Conflicts of interest:

The authors acknowledge they have no conflicts of interest.

Introduction

Colorectal cancer (CRC) is the fourth most common incident cancer in the United States, with an estimated 135,430 new cases expected to occur in 2017 [1]. Recent research has pointed to a possible key role of gut microbial communities (GMC) in understanding the link between obesity, chronic inflammation, and the development of CRC [2]. Cani et al. identified lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, as an underlying factor of obesity-driven low-grade inflammation [3]. High fat, high caloric diets or high carbohydrate diets have been shown to increase serum LPS concentrations [4,5] while increasing age and obesity have also been shown to be positively associated with LBP [6,7].

LPS increases inflammatory response signaling, alters gut barrier function [8] and may play a role in the pathogenesis of several adverse outcomes, including diabetes [9,10], inflammatory bowel disease [11], cardiovascular disease [12,13], and cancer [14,15]. LPS and lipopolysaccharide-binding protein (LBP), a marker of LPS exposure, have been shown to be associated with reduced apoptosis [16,17] and increased proliferation in metastatic tumor cells [18,19] in both *in vitro* and *in vivo* experimental studies. Several studies have shown that LBP polymorphisms (LBP SNPs rs2232613 and rs2232571) were associated with increased plasma LBP concentrations [20–22], suggesting that the increased risk of CRC associated with certain polymorphisms may be caused by increased levels of circulating LBP.

In vitro studies have shown that LPS promotes cancer-cell survival and proliferation, angiogenesis, vascular permeability, and tumor cell adhesion [16,19,23,24], however, to our knowledge, no epidemiologic studies have prospectively examined the association between LBP and CRC risk. Thus, the goal of this study was to examine whether pre-diagnostic plasma LBP concentrations were associated with CRC incidence in a case-control study nested within a prospective cohort study.

Methods

Study Population and Overview

The Multiethnic Cohort (MEC) is a longitudinal study designed to investigate the association of dietary, lifestyle, and genetic factors with the incidence of cancer and other chronic diseases [25]. The MEC includes 215,251 men and women primarily from five different racial-ethnic groups (African Americans, Japanese Americans, Latinos, Native Hawaiians and whites in Hawaii and California [mostly in Los Angeles county]) aged 45–75 years at recruitment. The cohort was assembled in 1993–1996 by mailing a self-administered, 26-page questionnaire to individuals identified primarily through the driver's license files from Hawaii and California. The baseline questionnaire obtained information on demographics, medical and reproductive histories, cigarette smoking, medication use, family history of various cancers, physical activity, and a quantitative food frequency questionnaire (FFQ). In Year 5 of the follow-up (1999–2001), a short questionnaire updated information on medical conditions and history, including screening tests for cancer. A ten-year update of the dietary and other exposure data was completed between 2003 and 2008 by re-

administration of the full baseline questionnaire. The Institutional Review Boards at the University of Hawaii and the University of Southern California approved the study protocol.

Ascertainment of Case Status, Site and Stage

Identification of incident cancer cases was achieved by regular linkage with the Hawaii Tumor Registry, the Los Angeles County Cancer Surveillance Program, and the State of California Cancer Registry, all of which are members of the NCI Surveillance, Epidemiology, and End Results (SEER) program [26]. Deaths in the cohort were identified by linkage to the state death-certificate files in CA and HI, and to the National Death Index for deaths occurring in other states. Colon cancer cases included those with ICD-O-3 codes of C18.0-C18.9 and rectal cancer cases were those with ICD-O-3 codes of C19.9 and C20.9. Histologies >9000 (large intestinal neoplasms with hematopoietic lineages) were excluded. SEER summary stage was used, which defines localized cancer as cancer that is limited to the organ of origin, regional as cancer spread beyond the original site to nearby lymph nodes or organs and tissues, and distant as cancer that has spread to distant organs or distant lymph nodes.

Individuals with a history of CRC prior to their phlebotomy date, based on the baseline questionnaire and/or linkage to the SEER registries, were excluded. A total of 846 colorectal cases whose diagnoses occurred after blood collection and before the censor date (10/2013) were initially identified for LBP assessment. Two cases who were ultimately discovered to have *in situ* tumors, as well as 25 cases with unspecified tumor stage, were excluded (as we could not ascertain tumor stage) leaving 819 cases with invasive cancers of the colon and rectum.

For this analysis, a nested case-control design was used, matching controls to CRC cases on a 1–1 ratio. For each case, a control pool was created consisting of cohort members who had donated specimens and who were alive and have never been diagnosed with CRC at the age of the case's diagnosis. Controls were matched to cases on birth year, location (Hawaii or Los Angeles), sex, ethnicity, age at phlebotomy (within ± 1 year), date of specimen collection (± 1 month), time of blood collection (± 2 hours) and time since last meal (± 2 hours).

LBP Exposure Assessment

Between 2001 and 2006, biospecimens, which included fasting blood samples, as well as a short questionnaire on current medication and dietary supplement use were collected from surviving members of the MEC who reside in the catchment areas of the research centers in Hawaii and Los Angeles. This biorepository includes approximately 70,000 subjects who are broadly representative of all cohort members [27]. The majority of these participants (72%) also provided responses to the follow-up questionnaires as described above.

For this study, the circulating concentration of LBP was measured in heparinized plasma using a commercial ELISA kit (Cell Sciences Inc) designed for either heparinized plasma or serum samples. Samples were diluted 1:1000 and the assay was conducted according to the kit protocol with a standard curve of 1.5–100 ng/mL. Ten percent of samples were blinded quality control duplicates, and laboratory personnel were blinded to the case-control status

of the participants. Samples for matched cases and controls were positioned in random order next to each other in the same batch in order to reduce bias due to laboratory variation. Based on the QC duplicate samples, the intra- and inter- batch coefficient of variation percent (CV%) was 4.3 and 11.3, respectively.

Statistical Analysis

LBP was categorized into quartiles with the cutoff for each quartile determined using control values. Conditional logistic regression with matched sets as strata was used to compute odds ratios (ORs) and 95% confidence intervals (CIs) for the relationship between LBP and CRC in the minimally-adjusted model. Additional adjustment for covariates not matched on was performed in the multivariable model.

Covariates included in multivariable analyses were selected *a priori* (see Table 1), and included factors associated with LBP, LPS and/or CRC. The multivariable model included the following covariates evaluated at baseline: education (less than high school, high school graduation, some college, college graduate or higher), history of intestinal polyps (yes or no), diabetes (yes or no), history of CRC among first-degree relatives (yes or no), BMI (kg/m^2 , categorized according to federal guidelines [28]: underweight (<18.5), normal weight ($18.5\text{--}24.9$), overweight ($25\text{--}29.9$), and obese (≥ 30), with normal weight serving as the reference group), aspirin-use status (never, yes currently, yes formerly), alcohol consumption (g/day, categorized based on one standard drink equaling 14g alcohol and the Department of Agriculture and Department of Health & Human Services recommendation of no more than 1 drink/day for women and 2 drinks/day for men [29], women: non-drinker [0 g/day], $0.1\text{--}<14$ g/day, and ≥ 14 g/day; men: non-drinker [0 g/day], $0.1\text{--}<28$ g/day, and ≥ 28 g/day), relative density of dietary fiber (g/1,000 kcal/day) and saturated fat (% of energy, calculated as $9 \times \text{g saturated fat/kcal intake}$), and physical activity (METs/day). Dietary fiber intake, saturated fat intake, and physical activity were categorized into tertiles based on the distribution among controls.

Additionally, previous colonoscopy or sigmoidoscopy (yes or no) reported at the 5-year follow-up questionnaire, as well as non-aspirin NSAID use (yes or no), hormone replacement therapy use (yes or no/refused/don't know), and smoking status (never, current, former) at the time of blood draw were included in the multivariable model. We created a "missing" category for covariates with missing data in order to reduce the number of participants who would be dropped from the analysis.

Stratified analyses were performed to examine the association between LBP and CRC risk by BMI, dietary fiber intake, and saturated fat intake at baseline, dichotomized at the median values of controls. P-values for interaction were calculated by including a single cross-product term between LBP concentration, modeled as an ordinal variable, and the binary variable (BMI, saturated fat, or dietary fiber) in a single model, adjusted for the covariates listed above.

We also evaluated heterogeneity of the LBP-CRC association by cancer site (colon vs. rectum), and cancer stage (local vs. regional/distal) at time of diagnosis. In the case of synchronous colorectal cancers ($n=18$), the tumor with the higher stage was used. Four

individuals were excluded from the analysis of LBP-CRC by tumor site as they had diagnosed tumors in both the colon and the rectum that were at the same stage. Conditional logistic regression restricting cases to specific sites/stages was used to determine point estimates and corresponding 95% CI while unconditional logistic regression limited to cases (where regional or colon cancers were coded as cases and local or rectal cases were coded as controls) was used to determine statistical significance (P for difference) of subsite- and stage- specific differences. These regression models were adjusted for all covariates listed above as well as the matching variables sex, age, study site, and time since last meal.

A sensitivity analysis, which excluded all cases diagnosed within one year of follow-up, was performed to address the possibility that preclinical CRC may influence circulating LBP concentrations. Further sensitivity analyses were performed by replacing baseline covariates with responses from follow-up questionnaire to examine any potential bias that may have occurred from the temporal discordance in blood and questionnaire collection - as the blood was drawn several years after the baseline questionnaire was administered. Additionally, we also reanalyzed the association between LBP and CRC including only those individuals with complete data to explore whether missing covariate data may have influenced results. A final sensitivity analysis dropping individuals with implausible dietary values (calories: <700 or >4200 kcal/day (n=126), alcohol: >100g/day (n=61) and weight measures (BMI: <15 or >50 (n=3), total dropped=144) was performed to examine bias introduced from individuals with extreme measurements.

All reported P-values are two-sided, and a P-value < 0.05 was considered statistically significant. All analyses were carried out using STATA 14 (StataCorp, College Station, TX).

Results

Table 1 presents the distributions and means of the study covariates by matched case-control status (819 controls and 819 cases). Compared to controls, a history of diabetes was more prevalent among cases, while controls were more likely to be current smokers. Japanese represented the largest ethnicity among cases (n=305), accounting for more than a third of total cases. Cases were more likely to be obese compared to controls, and a smaller percentage of cases had previously undergone sigmoidoscopy/colonoscopy compared to controls. The mean ($\pm$ SD) LBP concentration was 34.3 ± 14.3 $\mu\text{g/mL}$ in cases and 34.6 ± 15.4 $\mu\text{g/mL}$ in controls. Additionally, among controls, mean concentrations differed slightly by ethnicity (Black: 37.6 ± 17.9 , Hawaiian: 31.9 ± 11.4 $\mu\text{g/mL}$, Japanese: 32.8 ± 13.5 $\mu\text{g/mL}$, Latino: 36.8 ± 15.8 $\mu\text{g/mL}$, white: 32.4 ± 12.6 $\mu\text{g/mL}$), by not by sex (men: 34.4 ± 15.0 $\mu\text{g/mL}$, women: 34.4 ± 14.7 $\mu\text{g/mL}$).

Associations of LBP concentrations and CRC risk are presented in Table 2. Overall LBP concentrations were not statistically significantly associated with CRC risk in either the minimally adjusted or the multivariable adjusted models. In the fully adjusted model, the OR among those in the third quartile was statistically significantly higher than that in the lowest quartile (OR = 1.36; 95% CI = 1.01–1.83). However, there was no observed increased risk among those in the highest LBP quartile (OR = 1.01; 95% CI = 0.73–1.39) and the test for trend was not statistically significant ($P_{\text{trend}} = 0.66$).

Tables 3 presents analyses of the findings related to LBP concentrations, stratified by BMI, dietary fiber intake, and saturated fat intake, respectively. Among individuals in the high-BMI ($> 26.12 \text{ kg/m}^2$, median at blood draw) group, there was a statistically significantly increased risk for those in the third quartile vs. first quartile (Q3: OR = 1.35; 95% CI = 1.01–1.81). Associations were slightly attenuated, albeit similar, in the low-BMI ($\leq 26.12 \text{ kg/m}^2$) group, and there was no evidence of heterogeneity between the groups ($P_{\text{interaction}} = 0.24$). The association of higher LBP concentration and CRC risk was more apparent among individuals in the lower fiber category (vs. higher fiber), with a non-statistically significant trend observed across quartiles ($P_{\text{trend}} = 0.10$); however, there was no apparent heterogeneity between fiber groups ($P_{\text{interaction}} = 0.12$). In both the high- and low-saturated fat groups, the positive association between LBP and CRC was most pronounced in the third tertile, but there were no apparent trends in CRC risk by LBP quartile in either group and no interaction between saturated fat intake and LBP on CRC risk ($P_{\text{interaction}} = 0.36$). Table 4 presents analyses of LBP concentration and CRC by anatomic subsite, and cancer stage at diagnosis. While ORs across quartiles varied by cancer site (colon vs. rectum), these differences were not statistically significant (all $P_{\text{difference}} = 0.45$). The association between LBP concentration and CRC risk was similar for local and regional/distant disease, with no evidence of heterogeneity (all $P_{\text{difference}} = 0.52$).

In a sensitivity analyses, the associations between LBP concentration and CRC risk remained virtually unchanged after excluding cases diagnosed within one year of follow-up, after excluding individuals with extreme dietary and weight measurements, and after excluding those with missing covariate data (data not shown).

Discussion

Results from this study do not provide clear evidence of an association between plasma LBP concentrations and CRC risk. Additionally, there was no evidence of differential effects by BMI, dietary fiber intake, saturated fat intake, cancer site, or cancer stage. However, the stratified results in Table 3 led to small numbers in some subgroups and limited power to detect significant main effects or interactions.

LPS is hypothesized to be associated with colorectal carcinogenesis, but relatively few human studies have examined this association, and no studies have examined the association between circulating LBP concentrations and CRC specifically. A recent cross-sectional study of individuals undergoing colonoscopy found that those with higher plasma LPS concentrations were more likely to have adenomas compared to those with low LPS concentrations (odds ratio (OR) 1.4, 95% CI 1.0–2.1), and this excess risk was independent of cytokine levels (TNF- α , IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, and IFN- γ) [14]. Additionally, a recent prospective study showed that polymorphisms in the LBP gene (GA and GG genotypes of LBP rs2232596) correlated with increased CRC risk (OR = 1.51, 95% confidence interval (CI): 1.15–1.99, $P = 0.003$; OR = 2.49, 95% CI: 1.16–5.38, $P = 0.016$, respectively) [30]. Differences in results may be due to study design and use of adenomas, rather than CRC, in the first study, and the use of LBP genes (not plasma LBP) in the second study [14].

Use of an endogenously produced biomarker such LBP, which reflects the host's response to microbial products, can be influenced by various inflammatory factors; LPS transfer into the bloodstream may be caused by factors such as tissue damage, infection, or other medical conditions which may induce systemic inflammation. As a result, in the cross-sectional study of Kang et al [14], the higher concentrations observed in the adenoma cases may be a byproduct, not a cause, of the adenoma. The MEC participants in the current study were healthy at the time of blood draw, which, in turn, may have reduced the likelihood that associations would be confounded by illness or medical conditions, such as colorectal adenomas. Thus, differences in study results may be due to timing at which LBP concentrations were ascertained relative to the neoplasm diagnosis.

While LBP binds to LPS, the protein also recognizes the lipoteichoic acid of gram-positive bacteria, as well as structurally similar spirochetal glycolipids [31,32]. Nonetheless, it should be noted that the LBP assay is more reliable than the one used for LPS, given the large amount of interference in the Limulus Amebocyte Lysate (LAL) assay [33]; moreover, in addition to difficulties with the assay, the need to collect samples under LPS-free conditions and high variability of LPS throughout the day may make LBP a more stable marker [34,35]. As such, inconsistencies between current results and previous studies may have been due to inherent differences between LPS and LBP, as well as the assays used to measure them.

Human studies have shown that LPS concentrations are higher in participants who consume a high-fat diet [6], and that postprandial LPS concentrations rise significantly after a high-fat meal [4,5,36,37]. While our study obtained fasting blood samples from healthy individuals to minimize the influence of short-term factors that may affect LBP concentrations, such as diet or illness, residual confounding may have persisted, as, at least in sepsis patients, LBP remains elevated for 12–24 hours following exposure – thus a high-calorie, high-fat meal the night before the blood draw may have led to a higher LBP concentration.

Additionally, it is important to note that the range in LBP concentrations in the current population was outside of normal healthy population limits. Based on previous literature, LBP concentrations in plasma range from 1 µg/mL to 24 µg/mL among healthy individuals [38,39]. However, in the current population, LBP ranged from 1 µg/mL –107.5 µg/mL (Cases range: 2 µg/mL –107.5 µg/mL; Control range: 4 µg/mL –100.4 µg/mL). LBP in the current population was normally distributed in both cases and controls (data not shown). It is possible that the wide ranges may have been a consequence of processing times or long storage periods (samples were stored at –180 C for roughly 10 years), although there are currently no studies examining how LBP measurements change in stored samples over time. The possibility that a delay in processing times led to artificially higher LBP concentrations in some of the samples may help to explain why a positive trend was seen between LBP concentration and CRC risk in the second and third quartile, but not the fourth (highest, which would have been erroneously high). However, this scenario is unlikely given that all blood samples were drawn and processed within 4 hours of collection by centrifugation, in accordance with the study protocol. As such, additional research is needed to explain how LBP concentrations may change in stored frozen samples over time.

Strengths of the study include its prospective design which allowed for LBP concentration levels prior to diagnosis of CRC to be measured, thus reducing bias resulting from reverse causality. Additionally, excluding cancer cases identified during the first year of follow up did not impact results, a finding that supports the likelihood that reverse causality did not influence point estimates. Lastly, our findings for LBP concentration and CRC by stage suggest that the associations were not stronger for those with more advanced stage, as would be expected if disease stage affected LBP.

Study limitations include the use of single measure of LBP. LBP is an acute phase reactant and is influenced by a range of factors; one measure may not capture average long-term LBP concentrations within an individual. A previous study of the reliability of LBP showed that the intraclass correlation coefficient over a three month period ranged from 0.49-0.6, with shorter follow-up times corresponding to a higher ICC [40]. Therefore, multiple measures would be optimal for precise LBP measurement, especially for longer periods of exposure.

In summary, we did not find an association between LBP and CRC, nor did we find an association by BMI, saturated fat intake, dietary fat intake, cancer site or cancer state. However, given that this was the first study to examine this association, future prospective studies are needed to validate findings and development of complementary biomarkers of this process may be warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the National Institutes of Health grants, P01 CA168530 (National Cancer Institute), K05 CA154337 (National Cancer Institute and Office of Dietary Supplements) and R25CA094880 (National Cancer Institute)

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* [Internet]. 66:7–30. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26742998>.
2. Davis CD, Milner JA. Gastrointestinal microflora, food components and colon cancer prevention. *J Nutr Biochem* [Internet]. 2009; 20:743–52. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19716282>.
3. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* [Internet]. 2007; 56:1761–72. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17456850>.
4. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am J Clin Nutr* [Internet]. 2007; 86:1286–92. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17991637>.
5. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, et al. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care* [Internet]. 2009; 32:2281–7. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19755625>.

6. Amar J, Burcelin R, Ruidavets JB, Cani PD, Fauvel J, Alessi MC, et al. Energy intake is associated with endotoxemia in apparently healthy men. *Am J Clin Nutr* [Internet]. 2008; 87:1219–23. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18469242>.
7. Stehle JR, Leng X, Kitzman DW, Nicklas BJ, Kritchevsky SB, High KP. Lipopolysaccharide-binding protein, a surrogate marker of microbial translocation, is associated with physical function in healthy older adults. *J Gerontol A Biol Sci Med Sci* [Internet]. 2012; 67:1212–8. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22960476>.
8. Kong SY, Tran HQ, Gewirtz AT, McKeown-Eyssen G, Fedirko V, Romieu I, et al. Serum Endotoxins and Flagellin and Risk of Colorectal Cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC) Cohort. *Cancer Epidemiol Biomarkers Prev* [Internet]. 2016; 25:291–301. [cited 2017 Jun 26]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/26823475>.
9. Sun L, Yu Z, Ye X, Zou S, Li H, Yu D, et al. A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. *Diabetes Care* [Internet]. 2010; 33:1925–32. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20530747>.
10. Creely SJ, McTernan PG, Kusminski CM, Fisherff M, Da Silva NF, Khanolkar M, et al. Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. *Am J Physiol Endocrinol Metab* [Internet]. 2007; 292:E740–7. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17090751>.
11. Pastor Rojo O, López San Román A, Albéniz Arbizu E, de la Hera Martínez A, Ripoll Sevillano E, Albillos Martínez A. Serum lipopolysaccharide-binding protein in endotoxemic patients with inflammatory bowel disease. *Inflamm Bowel Dis* [Internet]. 2007; 13:269–77. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17206721>.
12. Lepper PM, Schumann C, Triantafilou K, Rasche FM, Schuster T, Frank H, et al. Association of lipopolysaccharide-binding protein and coronary artery disease in men. *J Am Coll Cardiol* [Internet]. 2007; 50:25–31. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/17601541>.
13. Blomkalns AL, Stoll LL, Shaheen W, Romig-Martin SA, Dickson EW, Weintraub NL, et al. Low level bacterial endotoxin activates two distinct signaling pathways in human peripheral blood mononuclear cells. *J Inflamm (Lond)* [Internet]. 2011; 8:4. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21352551>.
14. Kang M, Edmundson P, Araujo-Perez F, McCoy AN, Galanko J, Keku TO. Association of plasma endotoxin, inflammatory cytokines and risk of colorectal adenomas. *BMC Cancer* [Internet]. 2013; 13:91. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23442743>.
15. Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. *Gastroenterology* [Internet]. 2007; 133:1869–81. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18054559>.
16. Wang JH, Manning BJ, Di Wu Q, Blankson S, Bouchier-Hayes D, Redmond HP. Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. *J Immunol* [Internet]. 2003; 170:795–804. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12517943>.
17. Andrews EJ, Wang JH, Winter DC, Laug WE, Redmond HP. Tumor cell adhesion to endothelial cells is increased by endotoxin via an upregulation of beta-1 integrin expression. *J Surg Res* [Internet]. 2001; 97:14–9. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/11319874>.
18. Coffey JC, Wang JH, Bouchier-Hayes D, Cotter TG, Redmond HP. The targeting of phosphoinositide-3 kinase attenuates pulmonary metastatic tumor growth following laparotomy. *Ann Surg* [Internet]. 2006; 243:250–6. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16432359>.
19. Wang L, Zhu R, Huang Z, Li H, Zhu H. Lipopolysaccharide-induced toll-like receptor 4 signaling in cancer cells promotes cell survival and proliferation in hepatocellular carcinoma. *Dig Dis Sci* [Internet]. 2013; 58:2223–36. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23828139>.

20. LeVan, TD., Slager, RE., Romberger, DJ., Von Essen, SG. Proc Am Thorac Soc. American Thoracic Society; 2012. Lipopolysaccharide-Binding Protein (LBP) Polymorphisms Are Associated with Serum Levels of LBP in Agricultural Workers.
21. Guinan, EC., Palmer, CD., Mancuso, CJ., Brennan, L., Stoler-Barak, L., Kalish, LA., et al. Innate Immun [Internet]. Vol. 20. NIH Public Access; 2014. Identification of single nucleotide polymorphisms in hematopoietic cell transplant patients affecting early recognition of, and response to, endotoxin; p. 697-711.[cited 2016 Jul 26]Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24107515>
22. Chien, JW., Boeckh, MJ., Hansen, JA., Clark, JG. Blood [Internet]. Vol. 111. American Society of Hematology; 2008. Lipopolysaccharide binding protein promoter variants influence the risk for Gram-negative bacteremia and mortality after allogeneic hematopoietic cell transplantation; p. 2462-9.[cited 2016 Aug 19]Available from: <http://www.bloodjournal.org/cgi/doi/10.1182/blood-2007-09-101709>
23. Ikebe M, Kitaura Y, Nakamura M, Tanaka H, Yamasaki A, Nagai S, et al. Lipopolysaccharide (LPS) increases the invasive ability of pancreatic cancer cells through the TLR4/MyD88 signaling pathway. J Surg Oncol [Internet]. 2009; 100:725–31. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19722233>.
24. Harmey JH, Bucana CD, Lu W, Byrne AM, McDonnell S, Lynch C, et al. Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. Int J cancer [Internet]. 2002; 101:415–22. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12216068>.
25. Kolonel LN, Henderson BE, Hankin JH, Nomura AM, Wilkens LR, Pike MC, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol [Internet]. 2000; 151:346–57. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10695593>.
26. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, et al. Cancer treatment and survivorship statistics. CA Cancer J Clin [Internet]. 2014; 64:252–71. [cited 2016 Aug 19]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24890451>.
27. Park, S-Y., Wilkens, LR., Henning, SM., Le Marchand, L., Gao, K., Goodman, MT., et al. Cancer Causes Control [Internet]. Vol. 20. Springer Netherlands; 2009. Circulating fatty acids and prostate cancer risk in a nested case control study: the Multiethnic Cohort; p. 211-23.[cited 2016 Aug 19]Available from: <http://link.springer.com/10.1007/s10552-008-9236-4>
28. NHLBI Obesity Education Initiative Expert Panel on the Identification E T of O in A (US). National Heart, Lung, and Blood Institute. 1998. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults.
29. U.S. Department of Health and Human Services and U.S. Department of Agriculture. 2015–2020 Dietary Guidelines for Americans [Internet]. Washington DC: 2015. Available from: <http://health.gov/dietaryguidelines/2015/>
30. Chen R, Luo F-K, Wang Y-L, Tang J-L, Liu Y-S. LBP and CD14 polymorphisms correlate with increased colorectal carcinoma risk in Han Chinese. World J Gastroenterol [Internet]. 2011; 17:2326–31. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21633598>.
31. Schröder NWJ, Morath S, Alexander C, Hamann L, Hartung T, Zähringer U, et al. Lipoteichoic acid (LTA) of Streptococcus pneumoniae and Staphylococcus aureus activates immune cells via Toll-like receptor (TLR)-2, lipopolysaccharide-binding protein (LBP), and CD14, whereas TLR-4 and MD-2 are not involved. J Biol Chem [Internet]. 2003; 278:15587–94. [cited 2016 Jun 28]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12594207>.
32. Schröder NW, Opitz B, Lamping N, Michelsen KS, Zähringer U, Göbel UB, et al. Involvement of lipopolysaccharide binding protein, CD14, and Toll-like receptors in the initiation of innate immune responses by Treponema glycolipids. J Immunol [Internet]. 2000; 165:2683–93. [cited 2016 Jun 28]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/10946299>.
33. Dobrovolskaia MA, Neun BW, Clogston JD, Ding H, Ljubimova J, McNeil SE. Ambiguities in applying traditional Limulus amebocyte lysate tests to quantify endotoxin in nanoparticle formulations. Nanomedicine (Lond) [Internet]. 2010; 5:555–62. [cited 2016 Jun 28]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/20528451>.

34. Novitsky TJ. Limitations of the Limulus amebocyte lysate test in demonstrating circulating lipopolysaccharides. *Ann N Y Acad Sci* [Internet]. 1998; 851:416–21. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/9668634>.
35. Munford RS. Detoxifying endotoxin: time, place and person. *J Endotoxin Res* [Internet]. 2005; 11:69–84. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/15949133>.
36. Laugerette F, Vors C, Gélouën A, Chauvin MA, Soulage C, Lambert-Porcheron S, Peretti N, Alligier M, Burcelin R, Laville M, Vidal HMM. Emulsified lipids increase endotoxemia: possible role in early postprandial low-grade inflammation. *J Nutr Biochem*. 2011; 22:53–9. [PubMed: 20303729]
37. Clemente-Postigo M, Queipo-Ortuño MI, Murri M, Boto-Ordoñez M, Perez-Martinez P, Andres-Lacueva C, et al. Endotoxin increase after fat overload is related to postprandial hypertriglyceridemia in morbidly obese patients. *J Lipid Res* [Internet]. 2012; 53:973–8. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/22394503>.
38. Blairon L, Wittebole X, Laterre P-F. Lipopolysaccharide-binding protein serum levels in patients with severe sepsis due to gram-positive and fungal infections. *J Infect Dis* [Internet]. 2003; 187:287–91. [cited 2016 Jun 27]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12552453>.
39. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, Gude F. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. *PLoS One* [Internet]. 2013; 8:e54600. [cited 2016 Jun 24]. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23349936>.
40. Citronberg, JS., Wilkens, LR., Lim, U., Hullar, MAJ., White, E., Newcomb, PA., et al. Cancer Causes Control [Internet]. Springer International Publishing; 2016. Reliability of plasma lipopolysaccharide-binding protein (LBP) from repeated measures in healthy adults; p. 1-4.[cited 2016 Jul 16]Available from: <http://link.springer.com/10.1007/s10552-016-0783-9>

Table 1

Baseline Characteristics of Colorectal Cancer Cases and Controls, the Multiethnic Cohort

	Cancer Cases (n=819) Mean (SD) or N (%)	Controls (n=819) Mean (SD) or N (%)
<u>Matched Factors</u>		
Sex		
Male	453 (55.3%)	453 (55.3%)
Female	366 (44.7%)	366 (44.7%)
Age at blood draw, y	61.2 (8.3)	60.3 (8.2)
Ethnicity		
Black	160 (19.5%)	160 (19.5%)
Native Hawaiian	50 (6.1%)	50 (6.1%)
Japanese	305 (37.2%)	305 (37.2%)
Latino	172 (21.9%)	172 (21.9%)
White	132 (16.1%)	132 (16.1%)
<u>Unmatched Factors</u>		
Family History of CRC	85 (10.4%)	75 (9.1%)
LBP (µg/mL)	34.3 (14.3)	34.6 (15.4)
History of Colorectal Polyps Removed	54 (6.6%)	64 (7.8%)
Previous Colonoscopy or Sigmoidoscopy (at 5 year follow-up)	292 (35.6%)	326 (39.8%)
Alcohol Consumption (g/day)		
None	359 (45.1%)	365 (45.5%)
< 14 g/day	276 (34.7%)	287 (35.7%)
14–28 g/day	64 (8.0%)	67 (8.3%)
>28 g/day	97 (12.2%)	84 (10.5%)
Caloric Intake (kcal/day)	2,179 (1,043)	2,180 (976)
Saturated Fat Intake (% calories)	9.8 (2.9)	10.0 (2.9)
0 – 8.56	280 (35.2%)	265 (33.1%)
8.57 – 11.2	286 (34.9%)	271 (33.7%)
> 11.2	238 (29.9%)	267 (33.2%)
Dietary Fiber Intake (g/1,000kcal/day)		
0 – 9.39	284 (35.7%)	265 (33.1%)
9.40 – 12.72	220 (27.6%)	271 (33.7%)
> 12.72	292 (36.7%)	267 (33.2%)
Physical Activity (METs/day)		
0–1.50	270 (34.6%)	260 (33.6%)
1.51 – 1.75	297 (37.8%)	265 (34.1%)
>1.75	216 (27.5%)	249 (32.2%)
History of Diabetes	97 (11.5%)	61 (7.5%)
BMI (kg/m ²)		
< 18.5	8 (1.1%)	9 (1.2%)
18.5 – 24.9	257 (31.2%)	322 (39.4%)

	Cancer Cases (n=819)	Controls (n=819)
	Mean (SD) or N (%)	Mean (SD) or N (%)
25.0 – 30.0	338 (41.1%)	332 (40.8%)
> 30.0	212 (26.2%)	150 (18.6%)
Smoking Status at blood draw		
Never	258 (41.6%)	216 (35.5%)
Former	332 (53.5%)	251 (41.3%)
Current	31 (5.0%)	141 (23.2%)
Years of School Completed		
< High School (11 years or less)	118 (14.7%)	117 (14.3%)
High School (12 years)	210 (25.3%)	191 (23.7%)
Some University (13–15 years)	258 (32.3%)	266 (32.5%)
University Graduate or higher (16+ years)	228 (27.6%)	239 (29.4%)
Aspirin Use		
Current Use	153 (19.5%)	124 (15.9%)
Former Use	158 (19.6%)	159 (20.5%)
No Use	499 (60.6%)	514 (63.5%)
Non-aspirin NSAID Use at blood draw	132 (16.2%)	138 (16.9%)
Estrogen Use at blood draw (Women only)	50 (6.0%)	76 (9.1%)

Table 2

Estimated Odds Ratios (OR) of Colorectal Cancer associated with LBP concentrations

LBP (quartiles)	Cases		Controls		Minimally adjusted*	Multivariate adjusted**
	N	N	N	N		
1	0–24.0 µg/mL	183	205	Ref.	Ref.	Ref.
2	24.1 – 31.7 µg/mL	211	205	1.15 (0.87–1.50)	1.23 (0.91–1.67)	
3	31.8 – 42.6 µg/mL	238	204	1.28 (0.99–1.67)	1.36 (1.01–1.83)	
4	42.7 – 107.5 µg/mL	187	205	1.02 (0.76–1.36)	1.01(0.73–1.39)	
P _{trend}				0.63		0.66

* Matched variables: age, location (Hawaii or California), sex, ethnicity, age at phlebotomy, date of specimen collection, time of blood collection, and time since last meal

** Additional covariates: education, BMI (kg/m²), physical activity, smoking status at time of blood draw, history of intestinal polyps, previous colonoscopy or sigmoidoscopy, diabetes, history of CRC among 1st degree relatives, NSAID use at blood draw, aspirin use, alcohol consumption, dietary fiber density intake, saturated fat density intake and hormone replacement therapy use among women at blood draw.

Table 3
Odds Ratios (OR) of Colorectal Cancer Associated with LBP Concentrations, Stratified by Potential Effect Modifiers

High BMI (> 26.12 kg/m ² , N=883					Low BMI (26.12 kg/m ² , N=755					
BMI	Cases	Controls	OR ^a	(95% CI)	Cases	Controls	OR ^a	(95% CI)	P for difference	
LBP (quartiles)										
1	98	91	1.00	Ref	85	114	1.00	Ref	0.24	
2	115	106	1.25	(0.92–1.70)	96	99	1.17	(0.86–1.58)		
3	143	103	1.35	(1.01–1.81)	95	102	1.26	(0.92–1.72)		
4	114	113	0.98	(0.71–1.34)	73	91	0.91	(0.63–1.31)		
P _{trend}	0.33				0.87					
High Fiber (> 10.89 g/1,000kcal/day), N=836										Low Fiber(10.89 g/1,000kcal/day), N=802
Fiber	Cases	Controls	OR ^a	(95% CI)	Cases	Controls	OR ^a	(95% CI)	P for difference	
LBP (quartiles)										
1	92	103	1.00	Ref	91	102	1.00	Ref	0.12	
2	117	96	1.10	(0.80–1.53)	94	109	1.30	(0.95–1.78)		
3	110	97	1.36	(1.01–1.83)	128	108	1.60	(1.12–2.28)		
4	100	121	1.13	(0.80–1.59)	87	83	1.32	(0.84–2.09)		
P _{trend}	0.41				0.10					
High Saturated Fat (Relative % > 8.94), N=820										Low Saturated Fat (Relative % 8.94), N=818
Saturated Fat	Cases	Controls	OR ^a	(95% CI)	Cases	Controls	OR ^a	(95% CI)	P for difference	
LBP (quartiles)										
1	78	85	1.00	Ref	105	120	1.00	Ref	0.36	
2	102	103	1.15	(0.83–1.59)	109	102	1.26	(0.92–1.73)		
3	126	108	1.35	(1.01–1.82)	112	97	1.49	(1.06–2.09)		
4	97	121	1.06	(0.76–1.46)	90	83	1.16	(0.76–1.79)		
P _{trend}	0.66				0.26					

^aMatched on: age, location (Hawaii or California), sex, ethnicity, age at phlebotomy, date of specimen collection, time of blood collection, and time since last meal

Adjusted for education, BMI (kg/m2), physical activity, smoking status at time of blood draw, history of intestinal polyps, previous colonoscopy or sigmoidoscopy, diabetes, history of CRC among 1st degree relatives, intake, saturated fat intake and hormone replacement therapy use among women

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 4

Odds Ratios (OR) of Colorectal Cancer Associated with LBP Concentrations, by Cancer Site and Stage

Site	Colon Cancer, N=651			Rectal Cancer, N=165		
	Cases	OR ^a	(95% CI)	Cases	OR ^a	(95% CI)
LBP (quartiles)						
1	146	1.00	Ref	35	1.00	Ref
2	162	1.26	(0.89–1.79)	48	0.98	(0.46–2.10)
3	186	1.42	(1.00–2.00)	52	1.16	(0.56–2.43)
4	157	1.06	(0.74–1.52)	29	0.82	(0.33–2.01)
P _{trend}	0.553			0.88		
Local, N=407						
Regional/Distant, N=412						
Stage	Cases	OR ^a	(95% CI)	Cases	OR ^a	(95% CI)
LBP (quartiles)						
1	95	1.00	Ref	88	1.00	Ref
2	91	1.12	(0.70–1.80)	120	1.45	(0.94–2.22)
3	123	1.37	(0.90–2.08)	115	1.41	(0.90–2.20)
4	98	1.14	(0.72–1.80)	89	1.06	(0.64–1.71)
P _{trend}	0.36			0.78		
P for difference						
0.45						
0.52						

^aMatched on: age, location (Hawaii or California), sex, ethnicity, age at phlebotomy, date of specimen collection, time of blood collection, and time since last mealAdjusted for education, BMI (kg/m²), physical activity, smoking status at time of blood draw, history of intestinal polyps, previous colonoscopy or sigmoidoscopy, diabetes, history of CRC among 1st degree relatives, NSAID use, aspirin use, alcohol consumption, dietary fiber intake, saturated fat intake and hormone replacement therapy use among women