Obesity and bowel cancer: from molecular mechanisms to interventions

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18 **Abbreviations:** NSAID, non-steroidal anti-inflammatory drug(s); CRC, colorectal
19 cancer; HNPCC, hereditary non-polyposis colon cancer; BMI, body mass index; WC,
20 waist circumference; IARC, International Agency for Research on Cancer; RONS,
21 reactive oxygen and nitrogen species; CRP, C-reactive protein; ESR, erythrocyte
22 sedimentation rate; CVD, cardiovascular disease; CAPP2, Colorectal
23 Adenoma/carcinoma Prevention Programme; RCTs, randomised controlled trials;
24 RYGB, Roux-en-Y gastric bypass; LS, Lynch Syndrome; MRC, Medical Research
25 Council; BBSRC, Biotechnology and Biological Sciences Research Council.
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Abstract

The purpose of this review article is to examine the evidence of the mechanisms linking obesity with bowel cancer risk and to comment on the development of interventions which that may lower risk of this common age-related disease. The choice of topics for inclusion reflect my personal research on the aetiology and prevention of bowel cancer over the past 3 decades. I have cited literature which addresses each of these topics but, because of the breadth of the review article, I have not attempted to do this systematically. The accumulation of genomic damage which leads to colorectal (bowel) cancer (CRC) is influenced by the balance between damage acquisition through environmental exposures and endogenous factors/ processes and the effectiveness of genomic repair mechanisms. There is now convincing evidence that obesity is associated with increased bowel cancer risk and this increased risk is apparent even among those with Lynch Syndrome who are genetically predisposed to the disease. It seems likely that genomic damage resulting from obesity-related inflammation is a key driver of colorectal tumorigenesis but mechanisms may also include altered insulin/ IGF1 signalling which may give tumour cells a competitive advantage. The importance of inflammation as a mediator of the effects of obesity is supported by the fall in CRC risk which follows sustained exposure to NSAID such as aspirin and the abrogation of the excess CRC risk in obese patients with Lynch Syndrome who were randomised to aspirin. Since the available evidence about the effects of weight loss in the obese on CRC risk is limited, this should be a high priority for future research. In the meantime, to lower the global CRC burden, it would be prudent to institute effective public measures to

51 reduce the development of obesity and to enable those who are obese to lose
52 weight.

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54 **Key words:** Obesity; weight loss; colorectal neoplasms; inflammation;
55 carcinogenesis

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1. Introduction: Aetiology of colorectal (bowel) cancer – role of environmental exposures and endogenous factors

All cancers are due to genomic damage in stem cells which causes silencing of tumour suppressor genes and/ or activation of oncogenes. The accumulation of genomic damage is influenced by the balance between damage acquisition through environmental exposures and endogenous factors/ processes and the effectiveness of genomic repair mechanisms. The latter is influenced by both genotype (which establishes the capacity for repair) and the environment (which influences the expression/ activity of repair-related proteins). Because of the important role of lifestyle factors and other environmental exposures in influencing cancer risk, it has been estimated that, globally, 30 – 50% of all cancers are preventable [1]. Colorectal cancer (CRC) risk increases with age and with exposure to risk factors including, importantly, lifestyle factors. CRC is one of the most preventable cancers and it has been estimated that 54% of bowel cancer cases in the UK are potentially preventable [2].

Hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch Syndrome, is a cancer predisposition syndrome that results in early onset CRC as well as cancers at other sites including the endometrium, stomach, and upper urinary tract. Lynch Syndrome is inherited in an autosomal dominant fashion, is caused by germ-line mutation in one of the genes encoding the DNA mismatch repair system and is characterised by a type of genomic instability known as microsatellite instability. *MLH1* is one of the commonest genes mutated in Lynch Syndrome and, in addition, this gene is silenced epigenetically by hypermethylation of the promoter

region in about one sixth of sporadic CRC [3]. For these reasons, Lynch Syndrome is an informative model for investigating effects of dietary and other factors in the aetiology and prevention of bowel cancer as discussed below.

1.1 Purpose of, and processes used in, this review article

This narrative review is based on an invited presentation at the conference marking the 50th Anniversary of Korean Nutrition Society. The topics for inclusion reflect my personal research on the aetiology and prevention of bowel cancer over the past 3 decades. I have cited literature which addresses each of these topics but, because of the breadth of the review article, I have not attempted to do this systematically.

2. Obesity and bowel cancer risk

There is strong evidence that obesity increases CRC risk. A systematic review and meta-analysis of 56 observational studies involving more than 7 million individuals and 93,812 CRC cases, confirmed that higher BMI was associated with increased bowel cancer risk in both prospective and retrospective studies [4]. In this analysis, for every 5 units (kg.m^{-2}) increase in BMI, CRC risk increased by 18%. This association with adiposity was stronger for colon than for rectal cancer and for men than for women [4]. In addition, increased adiposity is a significant risk factor for colorectal adenomas [5] which are premalignant precursors of bowel tumours. This suggests that increased body fatness may act at an early stage in colorectal tumorigenesis. In particular, there is a linear dose-response relationship between abdominal fatness (visceral adipose tissue) and colorectal adenoma risk which suggests that fat accumulation around, and within, the visceral organs may mediate

the observed positive associations between BMI and waist circumference (WC) with both adenomas and bowel cancer [6, 7]. It appears that greater body fatness at any stage in the adult life-course is a CRC risk factor. For example, among Swedish conscripts to military service, higher BMI at ages 16 – 20 years was associated with significantly greater CRC risk 35 years later [8]. Further, in a meta-analysis of seven prospective cohort studies including 18 668 men and 24 751 women (mean ages 62 and 63 years, respectively), who were followed up for median 12 years, all measures of adiposity (including BMI, WC, hip circumference and waist-hip ratio) showed positive associations with colorectal cancer risk in these older adults [9]. In addition, a meta-analysis of data from prospective studies has shown that weight gain in adulthood is associated with significantly greater CRC risk in men but not in women [10]. The number of studies, especially those focussing on women, included in this review was small and the possibility of a sex-specific effect of body weight gain on CRC risk remains equivocal. Whilst the evidence for excess body weight as an aetiological factor for bowel cancer is strong, the reported effect of obesity on CRC-specific survival differs between studies [11, 12] and this evidence gap should be an important focus for future research.

2.1 Mechanisms linking obesity with bowel cancer

The fact that increased body fatness is a risk factor for a least 13 cancer types/ sites [13] suggests that there may be common mechanisms through which greater adiposity drives the accumulation of genomic damage, reduces the efficacy of DNA repair and/ or enhances the competitiveness of tumour cells. The International Agency for Research on Cancer (IARC) Working Group concluded that there was

strong evidence for a role of altered sex hormone metabolism and of chronic inflammation in mediating the link between obesity and cancer and that there was moderate evidence for a role of abnormalities in insulin and insulin-like growth factor (IGF) signalling [13]. In the Darwinian competition for survival and growth, the combination of systemic metabolic dysregulation, pro-inflammatory cytokines and adipokines may tip the balance in favor of reduced apoptosis and increased proliferation of cancer cells [14].

2.2 Obesity, inflammation and bowel cancer

It is well established that sites of chronic inflammation within the gut are associated with greater cancer risk e.g. chronic *Helicobacter pylori* infection leads to gastric cancer and inflammatory bowel disease leads to CRC [15]. The tumour-promoting effects of inflammation appear to be due to the proliferation enhancing effects of inflammation-related cytokines which potentiate failure to repair completely the genomic damage resulting from inflammation-related reactive oxygen and nitrogen species (RONS) [16]. In addition, there is evidence that obesity is associated with epigenetic dysregulation and altered gene expression [17] which may contribute to the epigenetic defects seen in CRC [18]. There is growing evidence that the metabolic activities of gut microorganisms may contribute to CRC risk [19, 20] but it is unclear whether this is a direct or an indirect effect given the critical role of dietary factors in modulating the composition and activities of the gut microbiota [21].

Obesity is associated with chronic low level systemic inflammation observed e.g. as elevated concentrations C-reactive protein (CRP) and other pro-inflammatory

cytokines such as IL-1 β , IL-6 and TNF α which are produced in greater amounts as adipocytes fill with triacylglycerols [14]. This association between increased BMI and CRP appears to be stronger in women than in men and in North Americans/Europeans than in Asians [22]. In the analysis of follow-up of adolescent Swedish military conscripts, higher erythrocyte sedimentation rate (ESR) – a non-specific marker of inflammation – at baseline was associated with greater CRC risk 35 years later [8]. This positive relationship remained when those young people with recorded illness at baseline were excluded from the analysis [8] which is consistent with the hypothesis that chronic inflammation drives colorectal tumorigenesis.

3. Aspirin and bowel cancer risk

Acetylsalicylic acid (aspirin) is a non-steroidal anti-inflammatory drug (NSAID) that is used to manage a wide range of conditions including pain, fever and inflammatory diseases. In addition, aspirin is effective in reducing risk of cardiovascular disease (CVD). Using data from two large UK-based randomised trials which tested the effects of aspirin on CVD endpoints and which had reliable post-trial follow-up for more than 20 years, Flossman and colleagues observed that consumption of ≥ 300 mg aspirin daily for ≈ 5 years is effective in primary prevention of CRC and that this effect becomes evident after about 10 years [23]. This finding was consistent with meta-analysis of data from 19 case-control studies and 11 cohort studies which showed that regular use of aspirin, or other NSAIDs, was associated consistently with lower CRC risk, especially after use for ≥ 10 years [23]. This supports the idea that suppressing systemic inflammation reduces CRC risk.

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183 In the CAPP2 Study, we recruited more than 1000 individuals with Lynch Syndrome
184 and randomised them to aspirin (600mg/d) and/or to resistant starch (30g/d) for 2+
185 years and determined effects on colorectal neoplasia [24]. At the end of the
186 intervention period there was no effect of either intervention agent on colorectal
187 neoplasia [24]. However, in anticipation that the anti-cancer effects of aspirin might
188 take more time to become evident [23], we consented the CAPP2 Study participants
189 to longer-term follow-up. After mean follow-up of 55.7 months, we observed that
190 those randomised to aspirin, especially if they had taken the NSAID for at least 2
191 years, had significantly lower incidence of CRC and of other Lynch Syndrome-
192 related cancers [25]. The relative risk of CRC was reduced by about 50% in those
193 who had taken the anti-inflammatory agent for ≥ 2 years [25]. In addition to the likely
194 reduction in genomic damage following suppression of inflammation by aspirin [16],
195 there is evidence that aspirin may have particular beneficial effects in those with
196 DNA mismatch repair defects by “selecting” for stem cells that show greater
197 microsatellite stability [26].

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199 Given the role of obesity in enhancing CRC risk in the general population, we
200 hypothesised that obesity would influence bowel cancer risk in those with the cancer
201 predisposition Lynch Syndrome. Using data from the CAPP2 Study, we observed
202 that CRC risk was significantly greater in obese Lynch Syndrome patients [27]. For
203 reasons that are not yet evident, the excess CRC risk associated with obesity
204 appeared to be restricted to those participants with mutations in *MLH1* [27].
205 However, this greater CRC risk associated with obesity was abrogated when the
206 Lynch Syndrome patients were randomised to aspirin [27]. These observations

support the hypothesis that obesity drives CRC development, even in those at high genetic risk of the disease, and that inflammation may be one of the damaging processes contributing to carcinogenesis.

4. Weight loss and colorectal cancer risk

When compared with the wealth of evidence demonstrating links between overweight or obesity and risk of a wide range of common non-communicable diseases [28], there is limited information about the effects of weight loss in the obese on incidence of, or mortality from, obesity-related diseases. A recent systematic review and meta-analysis of data from 54 RCTs of lifestyle-based weight loss interventions reported significantly lower all-cause mortality over a median trial duration of two years [29]. There were no detectable effects on specific causes of mortality including cardiovascular disease and cancer [29].

In contrast with behavioural interventions, bariatric (obesity) surgery produces much bigger and more sustained losses of body weight and substantial improvements in many health outcomes including lower risk of cancer after 10 years of follow-up [30, 31]. However, the effects of bariatric surgery on bowel cancer risk remain unclear and, because of the changes in gut physiology resulting from such surgery, it should not be assumed that any benefits for cancer overall will also apply to CRC. For example, a Swedish observational study reported apparently greater CRC risk after bariatric surgery [32] whereas a recent retrospective cohort study of patients undergoing bariatric surgery in the USA reported lower colon cancer ($P = 0.04$) at mean 3.5 years follow up [31]. To address this issue, we undertook a systematic

review and meta-analysis in which we found 4 studies which met our inclusion criteria and that showed, on average, 27% lower CRC risk in obese individuals who had undergone bariatric surgery [33]. Very recently, Aravani and colleagues used data from a cohort of >1 million obese individuals in England to investigate links between bariatric surgery and CRC risk [34]. Almost 40,000 of these initially obese individuals underwent bariatric surgery among whom there were 43 CRC cases in the period of follow-up and no effect on CRC incidence was detected [34]. Overall, current evidence suggests that commonly-used types of bariatric surgery do not increase CRC risk and, indeed, may decrease risk. However, given the relatively small number of studies and the small number of CRC patients investigated, this conclusion is tentative.

4.1 Mechanisms through which weight loss in obese individuals may reduce CRC risk

In an attempt to understand how weight loss in obese people may alter bowel cancer risk, Steinbach et al. (1994) enrolled 8 obese patients in a behaviour modification weight-reduction programme [35]. The primary outcome measure was the number and distribution of dividing cells in crypts obtained from colorectal mucosal biopsies (36, 37, 38]. After 12 weeks of dietary energy restriction, the participants had lost on average 8.6% body weight and showed a 39% reduction in whole-crypt labelling index ($P < 0.001$) and a 57% reduction in upper crypt labelling index ($P < 0.05$) [35]. This reduction in the total number of dividing cells within the crypt and a reduction in mitotic cells in the upper part of the crypt is consistent with the hypothesis that the weight reduction intervention lowered bowel cancer risk. Similarly, Pendyala et al.

(2011) investigated effects of behaviour-induced weight loss on mucosal inflammation markers in 10 healthy, pre-menopausal women. After the intervention, the women had lost $10.1 \pm 1\%$ of their initial weight and this was accompanied by a significant reduction in several mucosal inflammatory biomarkers including TNF- α , IL-1 β , IL-8, and monocyte chemotactic protein 1 concentrations [39]. In addition, data from gene expression arrays revealed dramatic down-regulation of pro-inflammatory cytokine and chemokine pathways, prostaglandin metabolism, and of proto-oncogenes *JUN* and *FOS* [39]. The authors concluded that diet-induced weight loss in obese individuals reduces colorectal mucosal inflammation and modulates inflammatory and cancer-related pathways [39].

In contrast with the (albeit limited) evidence of likely benefit of diet-induced weight loss on biomarkers of CRC risk, evidence of the effects of the much larger amounts of weight loss induced by bariatric surgery on CRC biomarkers is more mixed. In initially morbidly obese individuals whose BMI fell by 12.6 units at 26 weeks after bariatric surgery, rates of mitosis increased whilst apoptosis decreased in colorectal mucosal crypts and there was elevated mucosal expression of pro-inflammatory genes including *COX-1* and *COX-2* [40]. The elevated crypt cell proliferation was sustained at 3 years after surgery [41]. However, in a further study by the same research team, weight loss following bariatric surgery had no effect on crypt cell proliferation at 6 months and circulating CRP concentration fell [41]. Kant and colleagues hypothesised that the differences between the two studies could be attributed to differences in the type of bariatric surgery used viz. Roux-en-Y gastric bypass (RYGB) in the study by Sainsbury and colleagues (2008) and sleeve gastrectomy in the study by Kant et al. (2014) [42]. More recently, we have reported

changes in inflammatory and other CRC biomarkers before, and 6.5 months after, RYBG surgery in initially obese individuals [43]. Following surgery, participants lost on average 28.5 kg body mass and showed improvements in systemic markers of inflammation (CRP) and of glycaemic control (fasting glucose, HbA_{1c}, insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)) [43]. In addition, at 6.5 months post-surgery, there was a significant fall in expression of the pro-inflammatory gene *COX-1* and reduced total mitoses and mitoses in the top half of the crypt in the colorectal mucosa [43]. These findings are consistent with the hypothesis that weight loss induced by bariatric surgery can lower CRC risk [33].

5. Future research

A recent systematic review reported very low quality evidence for an effect of weight loss interventions on cancer mortality (n=8 trials, 34 events; risk ratio 0.58, 95% confidence interval 0.30 to 1.11 [44]. Given the paucity of available evidence, further research on the effects of weight loss in obese individuals on future CRC risk is warranted. Weight loss could be induced by lifestyle changes (reduced dietary energy intake and/or increased energy expenditure in physical activity), use of pharmaceutical agents or bariatric surgery [45] but lifestyle-based interventions are likely to minimise adverse effects. Given the evidence that certain nutritional risk factors including alcoholic beverages, red or processed meat, and overweight/obesity increase CRC risk in those with a family history of the disease [46], it will be especially important to investigate such factors in those with LS and in others at higher CRC risk for familial reasons. A feasibility study by Anderson and colleagues suggests that lifestyle-based interventions may be feasible and acceptable in those with a family history of CRC such as LS patients [47]. The

evidence that aspirin reduces CRC risk suggests that inflammation may be an important driver of the genomic damage that is responsible for the development of bowel cancer but this putative mechanism needs further investigation. In addition, a recent meta-analysis of data from primary prevention trials testing efficacy of aspirin suggests that there may be significant interactions between aspirin dose and body mass on both cardiovascular and cancer outcomes [48]. This analysis showed that low-dose aspirin (75-100mg/d) reduced CRC risk in participants whose body mass was <70kg but not in those weighing ≥70kg. In addition, higher aspirin doses (≥ 325mg/d) reduced CRC risk in those weighing up to 80kg [48]. These findings highlight evidence gaps in respect of effective (and safe) doses of aspirin for CRC prevention in obese individuals.

6. Conclusions

There is now convincing evidence that excess adiposity is associated with increased bowel cancer risk and that the increased risk is apparent even among those with Lynch Syndrome who are genetically predisposed to the disease. It seems likely that genomic damage resulting from obesity-related inflammation is a key driver of colorectal tumorigenesis but mechanisms may also include altered insulin/ IGF1 signalling which may give tumour cells a competitive advantage. The importance of inflammation as a mediator of the effects of obesity is supported by the fall in CRC risk which follows sustained exposure to NSAIDs such as aspirin and the abrogation of the excess CRC risk in obese patients with Lynch Syndrome who were randomised to aspirin. Since the available evidence about the effects of weight loss in the obese on CRC risk is limited, this should be a high priority for future research.

In the meantime, to lower the global CRC burden, it would be prudent to institute effective public measures to reduce the development of obesity and to enable those who are obese to lose weight. Such interventions are likely to benefit all sections of the population including those at higher risk due to familial conditions such as Lynch Syndrome.

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References

- [1] WHO (2018) <http://www.who.int/cancer/prevention/en/>; [accessed 5 March 2018].
- [2] Cancer Research UK (2018) <http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer>; [accessed 5 March 2018].
- [3] Esteller M1, Levine R, Baylin SB, Ellenson LH, Herman JG. MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. *Oncogene*. 1998 Nov 5;17(18):2413-7.
- [4] Ning Y1, Wang L, Giovannucci EL. A quantitative analysis of body mass index and colorectal cancer: findings from 56 observational studies. *Obes Rev*. 2010 Jan;11(1):19-30. doi: 10.1111/j.1467-789X.2009.00613.x.
- [5] Omata F1, Deshpande GA, Ohde S, Mine T, Fukui T. The association between obesity and colorectal adenoma: systematic review and meta-analysis. *Scand J Gastroenterol*. 2013 Feb;48(2):136-46. doi: 10.3109/00365521.2012.737364.
- [6] Ma Y, Yang Y, Wang F, Zhang P, Shi C, Zou Y, Qin H. Obesity and risk of colorectal cancer: a systematic review of prospective studies. *PLoS One*. 2013;8(1):e53916. doi: 10.1371/journal.pone.0053916. Epub 2013 Jan 17. Review. PMID: 23349764.
- [7] Keum N, Lee DH, Kim R, Greenwood DC, Giovannucci EL. Visceral adiposity and colorectal adenomas: dose-response meta-analysis of observational studies. *Ann Oncol*. 2015 Jun;26(6):1101-9. doi: 10.1093/annonc/mdu563. Epub 2014 Dec 5. Review. PMID: 25480876 7.

- [8] Kantor ED, Udumyan R, Signorello LB, Giovannucci EL, Montgomery S, Fall K. Adolescent body mass index and erythrocyte sedimentation rate in relation to colorectal cancer risk. *Gut*. 2016 Aug;65(8):1289-95. doi: 10.1136/gutjnl-2014-309007. Epub 2015 May 18. PMID: 2598694
- [9] Freisling H, Arnold M, Soerjomataram I, O'Doherty MG, Ordóñez-Mena JM, Bamia C et al. Comparison of general obesity and measures of body fat distribution in older adults in relation to cancer risk: meta-analysis of individual participant data of seven prospective cohorts in Europe. *Br J Cancer*. 2017 May 23;116(11):1486-1497. doi: 10.1038/bjc.2017.106.
- [10] Keum N, Greenwood DC, Lee DH, Kim R, Aune D, Ju W et al. Adult weight gain and adiposity-related cancers: a dose-response meta-analysis of prospective observational studies. *J Natl Cancer Inst*. 2015 Jan 24;107(3). pii: dju428. PMID: 25618901
- [11] Maskarinec G, Harmon BE, Little MA, Ollberding NJ, Kolonel LN, Henderson BE et al. Excess body weight and colorectal cancer survival: the multiethnic cohort. *Cancer Causes Control*. 2015 Dec;26(12):1709-18. doi: 10.1007/s10552-015-0664-7.
- [12] Laake I, Larsen IK, Selmer R, Thune I, Veierød MB. Pre-diagnostic body mass index and weight change in relation to colorectal cancer survival among incident cases from a population-based cohort study. *BMC Cancer*. 2016 Jul 7;16:402. doi: 10.1186/s12885-016-2445-4.
- [13] Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F1, Straif K; International Agency for Research on Cancer Handbook Working Group. Body Fatness and Cancer--Viewpoint of the IARC Working Group. *N Engl J Med*. 2016 Aug 25;375(8):794-8. doi: 10.1056/NEJMSr1606602.

- [14] Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, Inflammation, and Cancer. *Annu Rev Pathol*. 2016 May 23;11:421-49. doi: 10.1146/annurev-pathol-012615-044359.
- [15] Johnson CM, Wei C, Ensor JE, Smolenski DJ, Amos CI, Levin B et al. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control*. 2013 Jun;24(6):1207-22. doi: 10.1007/s10552-013-0201-5.
- [16] Kiraly O, Gong G, Olipitz W, Muthupalani S, Engelward BP. Inflammation-induced cell proliferation potentiates DNA damage-induced mutations in vivo. *PLoS Genet*. 2015 Feb 3;11(2):e1004901. doi: 10.1371/journal.pgen.1004901.
- [17] Toubal A, Treuter E, Clément K, Venteclef N. Genomic and epigenomic regulation of adipose tissue inflammation in obesity. *Trends Endocrinol Metab*. 2013 Dec;24(12):625-34. doi: 10.1016/j.tem.2013.09.006.
- [18] Bardhan K & Liu K. Epigenetics and colorectal cancer pathogenesis. *Cancers (Basel)*. 2013 Jun 5;5(2):676-713. doi: 10.3390/cancers5020676.
- [19] Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer*. 2013 Nov;13(11):759-71. doi: 10.1038/nrc3611.
- [20] Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G et al. The gut microbiota and host health: a new clinical frontier. *Gut*. 2016 Feb;65(2):330-9. doi: 10.1136/gutjnl-2015-309990.
- [21] O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K et al. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun*. 2015 Apr 28;6:6342. doi: 10.1038/ncomms7342.

- [22] Choi J, Joseph L, Pilote L. Obesity and C-reactive protein in various populations: a systematic review and meta-analysis. *Obes Rev.* 2013 Mar;14(3):232-44. doi: 10.1111/obr.12003.
- [23] Flossmann E, Rothwell PM; British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. *Lancet.* 2007 May 12;369(9573):1603-13.
- [24] Burn J, Bishop DT, Mecklin JP, Macrae F, Möslin G, Olschwang S et al; CAPP2 Investigators. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. *N Engl J Med.* 2008 Dec 11;359(24):2567-78. doi: 10.1056/NEJMoa0801297. Erratum in: *N Engl J Med.* 2009 Apr 2;360(14):1470.
- [25] Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S et al; CAPP2 Investigators. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. *Lancet.* 2011 Dec 17;378(9809):2081-7. doi: 10.1016/S0140-6736(11)61049-0.
- [26] Rüschoff J, Wallinger S, Dietmaier W, Bocker T, Brockhoff G, Hofstädter F et al. Aspirin suppresses the mutator phenotype associated with hereditary nonpolyposis colorectal cancer by genetic selection. *Proc Natl Acad Sci U S A.* 1998 Sep 15;95(19):11301-6.
- [27] Movahedi M, Bishop DT, Macrae F, Mecklin JP, Moeslein G, Olschwang S et al. Obesity, Aspirin, and Risk of Colorectal Cancer in Carriers of Hereditary Colorectal Cancer: A Prospective Investigation in the CAPP2 Study. *J Clin Oncol.* 2015 Nov 1;33(31):3591-7. doi: 10.1200/JCO.2014.58.9952.

- [28] Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*. 2009 Mar 25;9:88. doi: 10.1186/1471-2458-9-88. Review.
- [29] Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ*. 2017 Nov 14;359:j4849. doi: 10.1136/bmj.j4849.
- [30] Schauer DP, Feigelson HS, Koebnick C, Caan B, Weinmann S, Leonard AC et al. Association Between Weight Loss and the Risk of Cancer after Bariatric Surgery. *Obesity (Silver Spring)*. 2017 Nov;25 Suppl 2:S52-S57. doi: 10.1002/oby.22002.
- [31] Schauer DP, Feigelson HS, Koebnick C, Caan B, Weinmann S, Leonard AC et al. Bariatric Surgery and the Risk of Cancer in a Large Multisite Cohort. *Ann Surg*. 2017 Sep 21. doi: 10.1097/SLA.0000000000002525. [Epub ahead of print]
- [32] Derogar M, Hull MA, Kant P, Östlund M, Lu Y, Lagergren J. Increased risk of colorectal cancer after obesity surgery. *Ann Surg*. 2013 Dec;258(6):983-8. doi: 10.1097/SLA.0b013e318288463a.
- [33] Afshar S, Kelly SB, Seymour K, Lara J, Woodcock S, Mathers JC. The effects of bariatric surgery on colorectal cancer risk: systematic review and meta-analysis. *Obes Surg*. 2014 Oct;24(10):1793-9.
- [34] Aravani A, Downing A, Thomas JD, Lagergren J, Morris EJA, Hull MA. Obesity surgery and risk of colorectal and other obesity-related cancers: An English population-based cohort study. *Cancer Epidemiol*. 2018 Apr;53:99-104. doi: 10.1016/j.canep.2018.01.002. Epub 2018 Feb 3.

- [35] Steinbach G, Heymsfield S, Olansen NE, Tighe A, Holt PR. Effect of caloric restriction on colonic proliferation in obese persons: implications for colon cancer prevention. *Cancer Res.* 1994 Mar 1;54(5):1194-7.
- [36] Terpstra OT, van Blankenstein M, Dees J, Eilers GA. Abnormal pattern of cell proliferation in the entire colonic mucosa of patients with colon adenoma or cancer. *Gastroenterology.* 1987 Mar;92(3):704-8.
- [37] Ponz de Leon M, Roncucci L, Di Donato P, Tassi L, Smerieri O, Amorico MG et al. Pattern of epithelial cell proliferation in colorectal mucosa of normal subjects and of patients with adenomatous polyps or cancer of the large bowel. *Cancer Res.* 1988 Jul 15;48(14):4121-6.
- [38] Mills SJ, Mathers JC, Chapman PD, Burn J, Gunn A. Colonic crypt cell proliferation state assessed by whole crypt microdissection in sporadic neoplasia and familial adenomatous polyposis. *Gut.* 2001 Jan;48(1):41-6.
- [39] Pendyala S, Neff LM, Suárez-Fariñas M, Holt PR. Diet-induced weight loss reduces colorectal inflammation: implications for colorectal carcinogenesis. *Am J Clin Nutr.* 2011 Feb;93(2):234-42. doi: 10.3945/ajcn.110.002683.
- [40] Sainsbury A, Goodlad RA, Perry SL, Pollard SG, Robins GG, Hull MA. Increased colorectal epithelial cell proliferation and crypt fission associated with obesity and roux-en-Y gastric bypass. *Epub* 2010 Dec 8. *Cancer Epidemiol Biomarkers Prev.* 2008 Jun;17(6):1401-10. doi: 10.1158/1055-9965.EPI-07-2874.
- [41] Kant P, Sainsbury A, Reed KR, Pollard SG, Scott N, Clarke AR et al. Rectal epithelial cell mitosis and expression of macrophage migration inhibitory factor are increased 3 years after Roux-en-Y gastric bypass (RYGB) for morbid obesity: implications for long-term neoplastic risk following RYGB. *Gut.* 2011 Jul;60(7):893-901. doi: 10.1136/gut.2010.230755.

- [42] Kant P, Perry SL, Dexter SP, Race AD, Loadman PM, Hull MA. Mucosal biomarkers of colorectal cancer risk do not increase at 6 months following sleeve gastrectomy, unlike gastric bypass. *Obesity (Silver Spring)*. 2014 Jan;22(1):202-10. doi: 10.1002/oby.20493.
- [43] Afshar S, Malcomson F, Kelly SB, Seymour K, Woodcock S, Mathers JC. Biomarkers of Colorectal Cancer Risk Decrease 6 months After Roux-en-Y Gastric Bypass Surgery. *Obes Surg*. 2018 Apr;28(4):945-954. doi: 10.1007/s11695-017-2953-6. PMID: 28990150
- [44] Ma C, Avenell A, Bolland M, Hudson J, Stewart F, Robertson C et al. Effects of weight loss interventions for adults who are obese on mortality, cardiovascular disease, and cancer: systematic review and meta-analysis. *BMJ*. 2017 Nov 14;359:j4849. doi: 10.1136/bmj.j4849.
- [45] Lean MEJ, Astrup A, Roberts SB. Making progress on the global crisis of obesity and weight management. *BMJ*. 2018 Jun 13;361:k2538. doi: 10.1136/bmj.k2538.
- [46] Fardet A, Druesne-Pecollo N, Touvier M, Latino-Martel P. Do alcoholic beverages, obesity and other nutritional factors modify the risk of familial colorectal cancer? A systematic review. *Crit Rev Oncol Hematol*. 2017 Nov;119:94-112. doi: 10.1016/j.critrevonc.2017.09.001.
- [47] Anderson AS, Dunlop J, Gallant S, Macleod M, Miedzybrodzka Z, Mutrie N et al. Feasibility study to assess the impact of a lifestyle intervention ('LivingWELL') in people having an assessment of their family history of colorectal or breast cancer. *BMJ Open*. 2018 Feb 1;8(2):e019410. doi: 10.1136/bmjopen-2017-019410. PMID: 29391383

[48] Rothwell PM, Cook NR, Gaziano JM, Price JF, Belch JFF, Roncaglioni MC et al. Effects of aspirin on risks of vascular events and cancer according to bodyweight and dose: analysis of individual patient data from randomised trials. Lancet. 2018 Jul 12. pii: S0140-6736(18)31133-4. doi: 10.1016/S0140-6736(18)31133-4. [Epub ahead of print]