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Adiposity, Mediating Biomarkers and Risk of Colon Cancer in the European Prospective Investigation into Cancer and Nutrition Study

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Novelty and impact statement:

Body fatness is now a well-established risk factor for colon cancer; however the potential mechanisms behind this relation are largely unknown. In a nested case-control study among 662 incident colon cancer cases matched within risk-sets to 662 controls, the metabolic biomarkers, high-density lipoprotein cholesterol, non-high-molecular-weight adiponectin and soluble leptin receptor showed to be main mediators of adiposity-colon cancer association. If confirmed by future research, these findings may be implicated in the targeted prevention of colon cancer.

Abstract

Adiposity is a risk factor for colon cancer, but underlying mechanisms are not well understood. We evaluated the extent to which eleven biomarkers with inflammatory and metabolic actions mediate the association of adiposity measures, waist circumference (WC) and body mass index (BMI), with colon cancer in men and women. We analysed data from a prospective nested case-control study among 662 incident colon cancer cases matched within risk-sets to 662 controls. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression. The percent effect change and corresponding CI-s were estimated after adjusting for biomarkers shown to be associated with colon cancer risk. After multivariable adjustment, WC was associated with colon cancer risk in men (top vs bottom tertile RR 1.68, 95% CI 1.06 - 2.65; P_{trend} = 0.02) and in women (RR 1.67, 95% CI 1.09 - 2.56; P_{trend} = 0.03). BMI was associated with risk only in men. The association of WC with colon cancer was accounted mostly for by three biomarkers, high-density lipoprotein cholesterol, non-high-molecular-weight adiponectin and soluble leptin receptor, which in combination explained 46% (95% CI 37 to 57%) of the association in men and 50% (95% CI 40 to 65%) of the association in women. Similar results were observed for the associations with BMI in men. These data suggests that alterations in levels of these metabolic biomarkers may represent a primary mechanism of action in the relation of adiposity with colon cancer. Further studies are warranted to determine whether altering their concentrations may reduce colon cancer risk.

Introduction

Adiposity is an established risk factor for colon cancer [1]; however the underlying mechanisms are poorly understood. Various data, including results from large prospective studies such as the European Prospective Investigation into Cancer and Nutrition (EPIC), suggest that visceral adiposity and related metabolic abnormalities may play an important role in colon carcinogenesis [2]. Thus, waist circumference (WC) - as a marker of abdominal fat accumulation - was equally strongly related to colon cancer risk in both sexes, whereas body mass index (BMI) - as a marker of general obesity - was related to risk of colon cancer among men but not among women, suggesting that WC may more accurately reflect the metabolic risk associated with cancer [3]. Several circulating biomarkers of metabolic processes were shown to be associated with colon cancer risk, including C-peptide as a marker of hyperinsulinemia [4], glycated hemoglobin (HbA$_{1c}$) as a marker of hyperglycemia [5], high-density lipoprotein cholesterol (HDL-C) as a marker of dyslipidemia [6], high sensitivity C-reactive protein (CRP-hs) as a marker of chronic low grade inflammation [7], and reactive oxygen metabolites (ROM) reflecting oxidative stress [8]. Insulin may also be involved in colorectal carcinogenesis through insulin-like growth factor 1 (IGF-1) acting as potent mitogen; although evidence on the association of circulating IGF-1 levels with colon cancer risk has not been convincing [9]. Visceral adipose tissue is metabolically more active compared to subcutaneous fat, secreting a variety of cytokines and hormones (adipokines), thereby highlighting the role of adipose tissue in regulating energy balance and potentially also in affecting angiogenesis and tumor growth [10, 11]. Thus, recent EPIC findings suggested an important role of soluble leptin receptor (sOB-R) [12] and non-high-molecular-weight (HMW) adiponectin in colon cancer risk [13]. However, it remains to be answered to what extent these metabolic biomarkers may explain the association of adiposity with colon cancer. It is also unclear to what extent the role of these potential mediating biomarkers differs between men and women given the known differences in body shape and fat accumulation between sexes.

164 Therefore, we aimed to investigate to what extent the association between adiposity, as

165 measured by WC and BMI, with colon cancer risk in the EPIC cohort may be mediated by

166 metabolic biomarkers shown to be related with colon cancer risk.

167 **Material and methods**

168 **Study population**

169 EPIC is a large prospective study with approximately 520 000 participants, aged 25 to

170 70 years at enrollment during the period from 1992 through 2000 and recruited from 23

171 centers in 10 European countries [Denmark, France, Germany, Greece, Italy, the Netherlands,

172 Norway, Spain, Sweden, and the United Kingdom][14]. Participants gave written informed

173 consent, underwent anthropometric measurements, and completed questionnaires on socio-

174 demographic and lifestyle characteristics, including detailed dietary assessment[14]. Blood

175 samples were collected from about 65% of the women and 93% of the men using standardized

176 protocols[14]. The participants' body weight and WC were mostly measured with the exception

177 of EPIC-Oxford center where weight and WC were derived from prediction equations based

178 on self-reports of all participants and measurements in a subsample of the Oxford cohort [15].

179 **Cohort follow-up and case ascertainment**

180 Incident cancer cases were identified through record linkage with regional cancer

181 registries or using a combination of methods, including health insurance records, cancer and

182 pathology registries, and active follow-up through study subjects and their next-of-kin.

183 Closure dates for the present study were defined as the latest date of complete follow-up for

184 both cancer incidence and vital status, and ranged from December 1999 to June 2003 for

185 centers using registry data, and from June 2000 to December 2002 for centers using active

186 follow-up procedures. For the present study, colon cancers were defined as tumors in the

187 cecum, appendix, ascending colon, hepatic flexure, transverse colon, splenic flexure, and

188 descending and sigmoid colon (C18.0-C18.7, according to the 10th Revision of the

189 International Statistical Classification of Diseases, Injury and Causes of Death), as well as

190 tumors that were overlapping or unspecified (C18.8 and C18.9).

191 **Nested case-control study**

192 A total of 662 incident cases of colon cancer (292 men, 370 women) were included in

193 the present analyses. An incidence density sampling protocol was used such that for each case

194 one control subject was chosen at random among appropriate risk sets consisting of all cohort

195 members alive and free of cancer (except non-melanoma skin cancer) at the time of diagnosis

196 of the index case. Matching characteristics were study center, sex, age, time of blood

197 collection, and fasting status. Women were also matched on menopausal status.

198 Premenopausal women were matched on phase of the menstrual cycle at blood collection, and

199 postmenopausal women were matched on current use of hormone replacement therapy.

200 **Exclusion criteria and management of missing data**

201 Excluded from the analysis were the subjects from Norway and the Malmö center in

202 Sweden which did not provide blood samples for the current study, as well as subjects with

203 missing information on anthropometric measurements (n=132). Data for the measurements of

204 several biomarkers was missing in cases and controls as follows: IGF1 (67/217); ROM (4/3);

205 TG (9/5); HDL-C (3/1); C-peptide (94/231), Hba1c (118/254); CRP-hs (31/40). Overall,

206 participants with missing information on biomarkers tended to be slightly more educated

207 (Supplementary Table 1). No substantial differences were seen in the other characteristics,

208 except for that male cases with missing values were less likely to be physically inactive, but

209 consumed more alcohol and red meat compared with all participants. However, these were

210 only 37 cases and in sensitivity analysis excluding these participants did not substantially

211 change risk estimates (data not shown). Under the assumption that values are missing at

212 random and after proving that the pattern of missing data was arbitrary, we applied multiple

213 imputation technique [16]. All variables in the regression model were included in the procedure.

214   Ten duplicate datasets were sampled from their predictive distribution based on the observed

215   data with the missing values replaced by imputed values.

216   **Laboratory Procedures**

217   Blood samples were processed, aliquotted into heat-sealed straws, and stored in liquid

218   nitrogen freezers (-196°C) [14]. Storage protocols differed in Denmark and Sweden, where tubes

219   were stored in the vapour phase of liquid nitrogen (-150 °C) or in -80°C freezers, respectively.

220   The blood collection and processing protocols are described in detail elsewhere [17]. Approval

221   was obtained from the ethics review board of the International Agency for Research on

222   Cancer, Lyon, France and the local review boards. The measurement of all biomarkers

223   included in the analysis, C-peptide [4], HbA_{1c} [5], TG, HDL-C [6], CRP-hs [7], IGF-1 [9], ROM [8],

224   adiponectin, non-HMW-adiponectin [13], and sOB-R [12] has been described elsewhere.

225   **Statistical methods**

226   Spearman partial correlation coefficients, adjusted for age at study recruitment and case-

227   control status were estimated to assess the correlations between baseline WC or BMI and

228   biomarkers; as well as among the biomarkers. Conditional logistic regression was used to

229   assess the association of WC and BMI with colon cancer. Relative risks (RRs), estimated

230   from odds ratios as derived from the risk set sampling design [18] and 95% confidence intervals

231   (CI-s) were computed. The multivariable model included possible confounders other than

232   those controlled for by matching: smoking status, education, alcohol, and physical activity.

233   Further adjustment for dietary factors (fish and shellfish, red and processed meat, fibre, fruits

234   and vegetables) did not change the risk estimates, therefore we have not included these

235   variables in the final multivariable model. Data was analysed according to tertiles based on

236   the distribution of WC or BMI among controls and *P*-values for linear trends across tertiles

237   were calculated using the median levels of WC or BMI as a metric variable. For the mediation

238   analysis, we selected biomarkers according to the criteria of Baron and Kenny, 1986 [19, 20]; in

239   short, these were biomarkers related to adiposity measures and shown in EPIC to be

240   independently associated with colon cancer risk. To explore potential mediation effect, we

241   first added each individual biomarker to the multivariable-adjusted model and examined the

242   relative change in the risk estimates across tertiles as well as changes in the linear trends.

243   Second, we explored the associations continuously per unit increase in WC or BMI and

244   estimated the percent of effect change in regression coefficients (β-s) after the multivariable

245   model was additionally adjusted for each of the individual biomarkers using the difference of

246   coefficients method. This method was originally proposed by Freedman and Schatzkin, 1992

247   [21, 22] and further adapted for Cox regression models by Lin, Fleming and De Gruttola, 1997 [23].

248   The corresponding 95% confidence intervals (CI) were calculated based on Fieller's theorem

249   [24]. Finally, to test the significance of mediation effect, we applied the Sobel's test [25, 26]. In

250   sensitivity analyses, we repeated the main multivariable adjusted analysis after excluding

251   cases diagnosed in the first 2 years of follow up (n=173); we also repeated the analysis for

252   markers that may be influenced by fasting status, such as C-peptide, after excluding non-

253   fasting participants (n = 642).

254   Two-sided *P*-values of less than 0.05 were considered to indicate statistical

255   significance. All statistical analyses were performed using SAS Enterprise Guide, Version 4.3

256   (SAS Institute, Inc., Cary, North Carolina, USA).

257   **Results**

258   The baseline characteristics of incident colon cancer cases and their corresponding controls in

259   men and women are presented in Table 1. Overall, cases of colon cancer had higher

260   concentrations of C-peptide, CRP-hs and ROM, and lower concentrations of non-HMW

261   adiponectin and sOB-R compared to controls. WC and BMI were highly correlated with each

262   other in both men (r = 0.84) and women (r = 0.83).

263   All biomarkers were correlated with both WC and BMI with the exception of IGF-1

264 (Table 2). The associations among the different biomarkers were in the expected direction and

265 ranged from weak to moderate strength (Table 3). Of note, since IGF-1 was not correlated to

266 obesity measures, it did not fulfill the requirements of being a potential mediating variable;

267 therefore we did not include it in subsequent mediation analyses.

268   In multivariable adjusted logistic regression, WC was associated with risk of colon

269 cancer in both men and women (top vs bottom tertile, RR 1.68, 95% CI 1.06 - 2.65, ; P_{trend} =

270 0.02 in men; RR 1.67, 95% CI 1.09 - 2.56, P_{trend} = 0.03 in women; Table 4). In subsequent

271 analyses, we assessed the association of colon cancer with WC continuously per 10 cm

272 increase and estimated the percent change in regression coefficients after adjustment for each

273 biomarker (Table 5). sOB-R, non-HMW-adiponectin and HDL-C were the three biomarkers

274 that explained to the greatest extent the association individually and in combination explained

275 46% (95% CI 37 to 57%) of the association in men and 50% (95% CI 40 to 65%) of the

276 association in women (Table 5). Factors that differentially explained the association by sex

277 were ROM, which accounted for 21% (95% CI 16 to 26%) of the association in men and 8%

278 (95% CI 9 to 12%) of the association in women; as well as C-peptide, accounting for 30%

279 (95% CI 22 to 40%) of the association in women and -9% (95% CI -14 to -4%) of the

280 association in men. When we included ROM (as a biomarker more strongly explaining the

281 association in men compared to women) in combination with the three most important

282 biomarkers (sOB-R, non-HMW adiponectin and HDL-C), the percent change in risk was 71%

283 (95% CI 58 to 86%) in men and 58% (95% CI 47 to 75%) in women; when instead we

284 included C-peptide (as a biomarker more strongly explaining the association in women

285 compared to men) in the same model, the respective values were 33% (95% CI 24 to 44%) in

286 men and 53% (95% CI 40 to 68%) in women (Table 5). The results from the Sobel's test

287 further confirmed that the association between WC and colon cancer was statistically

288 significantly mediated by sOB-R, non-HMW adiponectin and HDL-C (Supplementary Table

289 2).

290   BMI was statistically significantly associated with risk of colon cancer only in men

291 (top vs bottom tertile, RR 1.33, 95% CI 0.85 - 2.07, P_{trend} = 0 04), but not in women (RR 1.21,

292 95% CI 0.76 - 1.78, P_{trend} = 0.34)). In men, the highest percent change in β coefficients was

293 observed after the multivariable model for BMI (continuously per 5 units increase) was

294 further adjusted for a sOB-R, HDL-C, non-HMW adiponectin and ROM, which in

295 combination explained 90% (95% CI 85 to 132%) of the association (Supplementary Table 3).

296 No substantial differences in risk estimates were observed after excluding people diagnosed

297 with cancer during the first two years of follow-up, or non-fasting participants (for C-peptide)

298 (data not shown).

299   **Discussion**

300   In this nested case-control study, the three biomarkers that explained the largest

301 proportion of the association of adiposity with colon cancer risk were HDL-C, non-HMW

302 adiponectin and sOB-R. Factors that differentially explained the association in men and

303 women were ROM and C-peptide. These findings support the hypothesis that pathways

304 reflected by these biomarkers maybe responsible for mediating the effects of adiposity on

305 colon cancer risk.

306   As shown previously in EPIC [3], in our analysis, WC was associated with colon cancer

307 risk in men and in women; whereas BMI showed an association only among men. Since WC

308 is more strongly related to abdominal fat accumulation than BMI, these findings suggest that

309 it is particularly visceral fat that may increase colon cancer risk. Thus, more precise

310 characterization of the risk-defining obesity phenotype may be achieved in a simple way by

311 measuring WC in addition to BMI, but also by using metabolic biomarkers.

312   In our data, non-HMW adiponectin and sOB-R, accounted for the largest proportion of

313 the adiposity-colon cancer association. Adiponectin, the most abundant protein secreted by

314   adipose tissue, exerts insulin-sensitizing, anti-inflammatory, anti-atherogenic, pro-apoptotic,

315   and anti-proliferative properties [27]. In contrast to other adipokines, adiponectin is inversely

316   related to obesity and metabolic alterations [28]. It circulates in plasma as a trimer, a hexamer

317   and a high-molecular weight (HMW) form, and experimental evidence suggests that HMW

318   and non-HMW adiponectin fractions may have different biologic activities, such that the

319   HMW form may be more closely related to insulin sensitivity, whereas complexes with lower

320   molecular weight (i.e. non-HMW adiponectin) were suggested to have an anti-inflammatory

321   potential [29]. Recently, EPIC data suggested an important role of the non-HMW adiponectin

322   fraction in colon cancer risk [13]. In this study, circulating pre-diagnostic concentrations of total

323   adiponectin and non-HMW-adiponectin were inversely associated with risk of CRC,

324   independent of dietary and lifestyle factors. In contrast, HMW-adiponectin concentrations

325   were not statistically significantly related to CRC [13]. In humans, serum sOB-R was suggested

326   as a marker reflecting the impairment of leptin action in type 2 diabetes [30] and was inversely

327   associated with obesity [31] and insulin resistance [32, 33]. EPIC data further suggest that sOB-R

328   may play an important role in the development of CRC [12]. We observed that this protein was

329   strongly inversely associated with colon cancer risk, independent of adiposity measures,

330   baseline leptin concentrations and metabolic biomarkers [12]. Leptin concentrations were not

331   associated with colon cancer risk in this study population [12]. These data collectively implies

332   that adipokines, merely considered as markers of body fatness, may represent quite more

333   complex and strong pathogenic actions in colon carcinogenesis.

334   Our results further suggest that HDL-C may also potentially mediate the association

335   between adiposity and colon cancer. The question whether HDL-C may act as a secondary

336   epiphenomenon, i.e. reflecting impaired metabolic health, or is causally related to cancer is,

337   yet to be answered. In the EPIC data, HDL-C was associated with risk of colon cancer even

338   after accounting for metabolic syndrome, arguing against its role as simple epiphenomenon[6].

339   Although HDL-C is implicated in overall cancer risk in large cohort studies[34] and randomized

340   control trials[35], biological mechanisms that might account for a HDL-C–cancer relationship

341   are not well understood. On one side, low HDL-C may indirectly influence carcinogenesis, by

342   reflecting chronic conditions that increase inflammation and insulin resistance [36]. On the other

343   side, HDL-C may exert direct cancer-protective effects by regulation of apoptosis [37] and

344   cellular proliferation required for cancer growth and metastasis[38]. For example, HDL

345   mimetics were demonstrated to inhibit tumor growth and development in mouse models of

346   colon cancer[38]. In addition, HDL-cholesterol exerts direct anti-oxidant, immune-protective

347   and anti-inflammatory properties that may be relevant for colon cancer[36,39,40,41]. In the largest

348   to date prospective study on blood lipids and CRC risk within EPIC, HDL-C was

349   independently associated with CRC even after controlling for biomarkers of systemic

350   inflammation, insulin resistance and oxidative stress [6]. Interestingly, apart from established

351   pathways, recent evidence suggest that HDL-C may also decrease white adipose tissue mass,

352   increase energy expenditure, and promote the production of adipose-derived cytokines, such

353   as adiponectin[42]. Nevertheless, use of lipid lowering drugs, statins, in persons without cancer,

354   does not influence cancer incidence and cancer-related mortality[43]. However, evidence is

355   based on trials with relatively short trial period of approximately 5 years to find a protective

356   effect on invasive cancers. Further, most trials have been focused mostly on effects related to

357   reducing LDL cholesterol, and not on elevating HDL-cholesterol, that may be more important

358   for cancer risk. Further work on the various mechanisms of HDL-C action is warranted for

359   revealing new prophylactic strategies with potential relevance to cancer prevention.

360   Our findings further suggest that the extent to which some biomarkers may mediate

361   the association between WC and colon cancer differ in men and in women. Thus, higher

362   proportion of the association between WC and colon cancer was explained by ROM in men,

363   while by C-peptide in women. It is not clear why ROM may mediate the association between

364   abdominal adiposity and colon cancer in men but not in women. On one side, it may be

365   possible that this is due to the stronger correlation between ROM and WC in men than in

366   women in our data. On the other side, determinants of oxidative stress may be differentially

367   distributed in men and in women and thus influence the results. C-peptide, a marker for

368   insulin secretion that has a longer half-life than insulin, was consistently associated with risk

369   of colon cancer in a number of epidemiological studies [44, 45] including EPIC [4], though no

370   statistical heterogeneity of the results by gender was reported. It maybe speculated that WC in

371   women may be more closely related to insulin resistance state compared to men, which may

372   explain why C-peptide may mediate the association of WC with colon cancer in women but

373   not in men.

374   Our data do not support the hypothesis that circulating IGF-1 levels may mediate the

375   association of adiposity with colon cancer risk. Adiposity measures were inversely though

376   non-significantly associated with IGF-1 concentrations, thus making IGF-1 to fall beyond the

377   statistical requirement for a potential mediating variable. Similar inverse associations, that

378   were also statistically significant, have been reported in the National Health and Nutrition

379   Examination Survey (NHANES) III [46]. Furthermore, in EPIC IGF-1 was not related to

380   colorectal cancer and a recent meta-analysis of 9 prospective cohort studies suggested only a

381   modest role for elevated circulating IGF-I levels in the development of colorectal cancer [9].

382   Strengths of our study include the prospective design, the measured rather than self-

383   reported WC and BMI (from majority of EPIC centers), exploration of a wide range of

384   biomarkers, including novel adipokines, and the detailed information on a number of dietary

385   and lifestyle factors, that were used to control the analyses. Some limitations of the current

386   study should also be considered. A single assessment of biomarker concentrations at baseline

387   may be susceptible to short-term variation, which could lead to "bias toward the null".

388   However, previously, most of the biomarkers indicated high reliability of single

389   measurements over time[47]. We used C-peptide and HbA$_{1c}$ as long-term markers for insulin

390   and glucose concentrations [48]. Nevertheless, the adjustment for a single measurement or the

391   use of surrogate markers in our analysis may not have sufficiently represented the effects of

392   hyperinsulinemia and hyperglycemia. In our study data was missing on a number of

393   biomarkers, however no differences were seen between participants with and without data on

394   key variables with respect to population characteristics. Furthermore, to account for missing

395   values we have applied multiple imputation method considered as the most efficient missing

396   data method in molecular epidemiology studies[49]. In the present study, only a subset of

397   subjects was fasting at the time of blood draw. The fasting status may be important in

398   differentiating effects of chronically elevated blood insulin levels (i.e. higher fasting serum C-

399   peptide levels) and postprandially elevated blood insulin levels (i.e. higher nonfasting serum

400   C-peptide levels). However, previously no statistically significant heterogeneity in the

401   association of C-peptide and colon cancer by fasting status was observed[4]. In our data we

402   have conducted the analysis for C-peptide after excluding non-fasting participants and results

403   were not considerably changed. Further, some biomarkers that were studied in the analysis as

404   potential mediators are interrelated with each other. Thus, by adjusting for one biomarker, we

405   may also partially account for the effect of one or more other related biomarkers. Finally, it

406   should be noted that the mediators of the association between adiposity and colon cancer may

407   be simply statistical intermediates and markers of various pathogenic processes, but not on the

408   causal pathway to colon cancer[50].

409   In summary, these data suggest HDL-C, non-HMW adiponectin and sOB-R to be the

410   three biomarkers mediating to the greatest extent the association between adiposity and colon

411   cancer. While alterations in the levels of these metabolic biomarkers may represent a primary

412   mechanism of action in the relation of adiposity with colon cancer, they may also point to

413   targets for prevention of obesity-induced colon cancer. Further studies are warranted to

414   determine whether altering the concentrations of these metabolic biomarkers reduces colon

415   cancer risk.

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Table 1 Baseline characteristics[a] of colon cancer cases and controls, the European Prospective Investigation into Cancer and Nutrition Cohort (1992-2003)

Variable	Men		Women	
	Cases (n=292)	Controls (n=292)	Cases (n=370)	Controls (n=370)
Age, years, mean ± SD	59.3±6.9	59.3±6.9	58.6±7.5	58.6±7.5
University degree, %	21.6	24.3	14.0	13.2
Physically inactive, %	17.8	14.7	12.7	9.7
Smokers, %	28.1	25.3	20.0	17.0
Alcohol intake, g/day	15.8 (6.4-36.6)	12.1 (5.6-32.2)	3.2 (0.4-12.4)	4.4 (0.5-12.1)
Dietary factors				
Fibre, g/day, median (IQR)	22.4 (17.4-28.3)	23.6 (19.2-29.7)	22.1(17.3-27.2)	22.1 (17.8-26.1)
Fruits and vegetables, g/day, median (IQR)	336.8 (217.7-492.1)	361.5 (229.3-526.4)	428.8 (284.5-574-7)	446.4 (295.3-583.5)
Red and processed meat, g/day, median (IQR)	99.2 (64.6-134.1)	93.7 (55.1-130.1)	69.9 (42.0-98.4)	70.3 (45.1-95.8)
Fish and shellfish, g/day, median (IQR)	29.4 (16.7 -52.5)	32.5 (18.2-56.9)	24.3 (11.1-40.9)	26.6 (12.8-49.8)
Anthropometric factors				
Body mass index, kg/m^2, mean ± SD	27.3±4.0	26.5±3.4	26.4±4.8	26.1±4.3
Waist circumference, cm, mean ± SD	97.8±10.6	94.8 ±9.5	84.3±12.1	82.6±11.2
Waist to hip ratio, mean ± SD	0.96±0.1	0.94±0.06	0.81±0.8	0.81±0.07
Biomarkers				
Adiponectin, µg/mL, median (IQR)	5.4 (3.9-7.0)	5.3 (4.1-7.2)	8.3 (6.2-10.8)	8.4 (6.3-10.8)
HMW-adiponectin, µg/mL, median (IQR)	2.5 (1.5-3.7)	2.5 (1.7-3.7)	4.6 (3.2-6.7)	4.5 (3.1-6.4)
Non-HMW adiponectin, µg/mL, median (IQR)	2.7 (2.2-3.5)	3.0 (2.5-3.6)	3.6 (2.8-4.5)	3.9 (3.0-4.7)
sOB-R, ng/mL, median (IQR)	21.0 (17.3-24.3)	22.0 (18.3-26.8)	19.8 (15.5-24.2)	21.0 (16.9-26.2)
CRP-hs, mg/L, median (IQR)	2.8 (1.2-5.0)	1.9 (0.9-3.9)	3.3 (1.2-5.7)	2.7 (1.2-5.3)
HbA$_{1c}$, %, median (IQR)	5.8 (5.5-6.1)	5.7 (5.4-6.1)	5.8 (5.5-6.1)	5.7 (5.4-6.1)
C-peptide, ng/mL, median (IQR)	4.4 (3.2-6.6)	4.3 (3.0-6.6)	3.7 (2.7-5.4)	3.5 (2.5-5.5)
HDL-cholesterol, mmol/l, median (IQR)	1.2 (1.0-1.5)	1.3 (1.1-1.6)	1.5 (1.3-1.8)	1.5 (1.3-1.9)
Triglycerides, mmol/l, median (IQR)	1.7 (1.1-2.4)	1.5 (1.0-2.2)	1.3 (1.0-2.0)	1.3 (0.9-2.0)
IGF-1, ng/mL, median (IQR)	217.8 (179.0-258.4)	214.2 (170.7-261.2)	200.1 (155.9-247.2)	196.9 (155.2-249.6)
ROM, U/ml, median (IQR)	376.5 (334.5-418.0)	352.0 (317.0-396.0)	423.0 (385.0-472.0)	409.0 (371.0-453.0)

[a]Sex, age at recruitment, menopausal status and hormonal replacement therapy use were among the matching criteria.

n = number; SD = standard deviation; IQR = interquartile range; HbA$_{1c}$=glycated hemoglobin; IGF-1= insulin growth factor 1; HDL-cholesterol = high-density-lipoprotein cholesterol; CRP = C-reactive protein; ROM = reactive oxygen metabolites; HMW-adiponectin = high-molecular-weight adiponectin; sOB-R = soluble leptin receptor.

Table 2 Spearman partial correlations[a] of waist circumference and body mass index[b] with biomarkers in controls (n=662), the European Prospective Investigation into Cancer and Nutrition Cohort (1992-2003)

	Waist circumference		Body mass index	
	Men	Women	Men	Women
Waist circumference	-	-	0.84	0.84
Adiponectin	-0.22	-0.35	-0.22	-0.22
HMW-adiponectin	-0.20	-0.33	-0.21	-0.21
Non-HMW adiponectin	-0.20	-0.29	-0.20	-0.20
sOB-R	-0.36	-0.42	-0.37	-0.37
CRP-hs	0.31	0.30	0.23	0.31
HbA_{1c}	0.22	0.23	0.21	0.19
C-peptide	0.31	0.33	0.30	0.28
HDL-cholesterol	-0.22	-0.29	-0.24	-0.24
Triglycerides	0.28	0.30	0.26	0.26
IGF-1	-0.02[c]	-0.07	-0.02[c]	-0.02[c]
ROM	0.16	0.10	0.14	0.14

[a]Adjusted for age at recruitment and case-control status.

[b] Weight (kg)/height $(m)^2$.

[c]P-value > 0.05.

Note: All *P* values are statistically significant (*P*<.005) except for the marked instances.

HbA_{1c} = glycated hemoglobin; IGF-1= insulin growth factor 1; HDL-cholesterol = high-density-lipoprotein cholesterol; CRP-hs = high-sensitivity C-reactive protein; ROM= reactive oxygen metabolites; HMW-adiponectin = high-molecular-weight adiponectin; sOB-R = soluble leptin receptor.

Table 3 Spearman partial correlations[a] among biomarkers in controls (n=662)[b], the European Prospective Investigation into Cancer and Nutrition Cohort (1992-2003)

	Adiponectin	HMW-adiponectin	Non-HMW-adiponectin	sOB-R	CRP-hs	HbA$_{1c}$	C-peptide	TG	HDL-C	IGF-1	ROM
Adiponectin	1.00	0.96 (<.0001)	0.85 (<.0001)	0.31 (<.0001)	-0.16 (<.0001)	-0.12 (<.0001)	-0.20 (<.0001)	-0.29 (<.0001)	0.42 (<.0001)	-0.12 (.01)	0.007 (.44)
HMW-adiponectin		1.00	0.68 (<.0001)	0.28 (<.0001)	-0.12 (<.0001)	-0.10 (.002)	-0.17 (<.0001)	-0.28 (<.0001)	0.40 (<.0001)	-0.11 (<.0001)	0.03 (.31)
Non-HMW adiponectin			1.00	0.28 (<.0001)	-0.15 (<.0001)	-0.09 (.01)	-0.19 (<.0001)	-0.25 (<.0001)	0.35 (<.0001)	-0.10 (.001)	-0.02 (.31)
sOB-R				1.00	-0.19 (<.0001)	-0.10 (<.0001)	-0.31 (<.0001)	-0.24 (<.0001)	0.26 (<.0001)	-0.11 (<.0001)	-0.02 (.49)
CRP-hs					1.00	0.16 (.0003)	0.18 (<.0001)	0.15 (<.0001)	-0.15 (<.0001)	-0.17 (<.0001)	0.42 (<.0001)
HbA$_{1c}$						1.00	0.16 (<.0001)	0.15 (.0001)	-0.10 (.01)	-0.01 (.28)	0.17 (<.0001)
C-peptide							1.00	0.34 (<.0001)	-0.20 (<.0001)	0.07 (.53)	0.10 (.001)
HDL-cholesterol								1.00	-0.39 (<.0001)	-0.05 (.43)	0.16 (<.0001)
Triglycerides									1.00	-0.02 (.44)	-0.01 (.57)
IGF-1										1.00	-0.13 (.0001)
ROM											1.00

[a]Adjusted for sex and age at study recruitment.

[b]P values in parentheses.

HbA$_{1c;}$= glycated hemoglobin; HDL-cholesterol =high-density-lipoprotein cholesterol; CRP-hs = high-sensitivity C-reactive protein; ROM = reactive oxygen metabolites; HMW-adiponectin = high-molecular-weight adiponectin; sOB-R = soluble leptin receptor.

Table 4 Relative risks (95% confidence intervals) of colon cancer by tertiles of waist circumference before and after adjustment for biomarkers in men and women, the European Prospective Investigation into Cancer and Nutrition Cohort (1992-2003)

Tertiles	Men				Women			
	T1	T2	T3	P_{trend}[a]	T1	T2	T3	P_{trend}[a]
Median WC (cm)	86.6	95.0	104.7		71.7	80.4	93.0	
n, cases /controls	83/102	82/100	127/90		103/122	121/119	146/129	
Crude model[b]	Referent	1.03 (0.66 - 1.61)	1.82 (1.19 - 2.78)	0.0001	Referent	1.24 (0.85 - 1.82)	1.43 (0.97 - 2.12)	0.08
Multivariable model[c]	Referent	0.92 (0.57 - 1.40)	1.68 (1.06 - 2.65)	0.02	Referent	1.36 (0.91 - 2.03)	1.67 (1.09 - 2.56)	0.03
Further adjustment for								
Adiponectin	Referent	0.83 (0.56 - 1.46)	1.49 (0.92 - 2.42)	0.05	Referent	1.33 (0.89 - 2.00)	1.61 (1.03 - 2.53)	0.05
HMW-adiponectin	Referent	0.86 (0.53 - 1.41)	1.56 (0.97 - 2.52)	0.03	Referent	1.42 (0.94 - 2.15)	1.81 (1.15 - 2.84)	0.02
non HMW-adiponectin	Referent	0.81 (0.49 - 1.32)	1.44 (0.89 - 2.33)	0.07	Referent	1.26 (0.83 - 1.89)	1.43 (0.91 - 2.23)	0.15
sOB -R	Referent	0.79 (0.48 - 1.29)	1.32 (0.81 - 2.15)	0.17	Referent	1.25 (0.83 - 1.89)	1.42 (0.91 - 2.25)	0.15
CRP-hs	Referent	0.91 (0.57 - 1.46)	1.63 (1.02 - 2.59)	0.03	Referent	1.36 (0.91 - 2.03)	1.65 (1.07 - 2.53)	0.03
HbA$_{1c}$	Referent	0.89 (0.55 - 1.43)	1.60 (1.00 - 2.56)	0.03	Referent	1.36 (0.91 - 2.04)	1.62 (1.04 - 2.53)	0.04
C-peptide	Referent	0.94 (0.58 - 1.51)	1.75 (1.08 - 2.80)	0.01	Referent	1.33 (0.89 - 2.00)	1.57 (0.98 - 2.49)	0.07
HDL-cholesterol	Referent	0.82 (0.50 - 1.33)	1.46 (0.91 - 2.35)	0.06	Referent	1.29 (0.86 - 1.94)	1.51 (0.97 - 2.37)	0.08
Triglycerides	Referent	0.91 (0.57 - 1.47)	1.64 (1.03 - 2.64)	0.02	Referent	1.35 (0.90 - 2.02)	1.68 (1.04 - 2.56)	0.04
ROM	Referent	0.92 (0.57 - 1.49)	1.49 (0.93 - 2.39)	0.07	Referent	1.39 (0.93 - 2.09)	1.62 (1.05 - 2.49)	0.04

[a]P-value for trend (two sided) across tertiles is based on the median biomarker concentrations within tertiles as a continuous variable. Wald $\chi 2$ test was employed to evaluate the significance of the linear trend.

[b]Crude model is taking into account matching factors: age, sex, study centre, follow-up time since blood collection, time of the day at blood collection and fasting status; Women were further matched by menopausal status, phase of menstrual cycle at blood collection and postmenopausal women were matched by HRT use.

[c]Multivariable model was based on the crude model with additional adjustment for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol intake (continuous), and physical activity (inactive, moderately inactive, moderately active, active, or missing).

WC = waist circumference; HbA$_{1c;}$ = glycated hemoglobin; HDL-cholesterol =high-density-lipoprotein cholesterol; CRP-hs = high-sensitivity C-reactive protein; ROM = reactive oxygen metabolites; HMW-adiponectin = high-molecular-weight adiponectin; sOB-R = soluble leptin receptor; T = tertile; P_{trend}=P for trend across tertiles; All *P* values are two-sided.

Table 5 Relative risks, 95 % confidence intervals and regression coefficients for risk of colon cancer associated with waist circumference (continuously per 10 cm increase) and percent change in regression coefficients with adjustment for each additional biomarker, the European Prospective Investigation into Cancer and Nutrition Cohort (1992-2003)

	Men				Women			
	RR (95%CI)	β^b	% change[c]	95% CI[d]	RR (95%CI)	β^b	% change[c]	95% CI[d]
n, cases /controls	292/292				370/370			
Multivariable model[a]	1.33 (1.26 - 1.41)	0.29			1.20 (1.14 - 1.25)	0.18		
Further adjustment for								
Adiponectin	1.29 (1.22 - 1.37)	0.26	11	7 to 16	1.18 (1.12 - 1.24)	0.17	6	1 to 11
HMW-adiponectin	1.31 (1.24 - 1.39)	0.27	6	2 to 9	1.22 (1.16 - 1.28)	0.20	-12	-19 to -6
Non-HMW-adiponectin	1.27 (1.20 - 1.35)	0.24	16	11 to 21	1.14 (1.08 - 1.19)	0.13	28	20 to 36
sOB-R	1.21 (1.14 - 1.29)	0.19	33	25 to 42	1.14 (1.09 - 1.19)	0.13	28	20 to 38
CRP-hs	1.31 (1.24 - 1.39)	0.27	6	2 to 9	1.19 (1.14 - 1.25)	0.18	2	-1 to 5
HbA$_{1c}$	1.31 (1.24 - 1.39)	0.27	6	2 to 9	1.18 (1.13 - 1.24)	0.17	4	-1 to 8
C-peptide	1.37 (1.29 - 1.45)	0.31	-9	-14 to -4	1.13 (1.12 - 1.23)	0.16	30	22 to 40
HDL-cholesterol	1.26 (1.19 - 1.34)	0.23	19	14 to 25	1.16 (1.11 - 1.22)	0.15	16	11 to 22
Triglycerides	1.34 (1.26 - 1.42)	0.29	0.05	-	1.19 (1.14 - 1.25)	0.17	4	-2 to 9
ROM	1.26 (1.19 - 1.33)	0.23	21	16 to 26	1.18 (1.13 - 1.24)	0.17	8	9 to 12
sOB-R+non-HMW adiponectin+HDL-cholesterol	1.17 (1.09 - 1.24)	0.16	46	37 to 57	1.09 (1.04 - 1.15)	0.09	50	40 to 65
sOB-R+non-HMW adiponectin+HDL-C+ROM	1.08 (1.01 - 1.16)	0.08	71	58 to 86	1.08 (1.03 - 1.13)	0.07	58	47 to 75
sOB-R+non-HMW adiponectin+HDL-C+C-peptide	1.21 (1.14 - 1.29)	0.19	33	24 to 44	1.09 (1.09 - 1.15)	0.09	53	40 to 68

[a]Multivariable model was taking into account matching factors: age, sex, study centre, follow-up time since blood collection, time of the day at blood collection and fasting status; Women were further matched by menopausal status, phase of menstrual cycle at blood collection and postmenopausal women were matched by HRT use; with additional adjustment for smoking status (never, past, current, or unknown), education (no school degree or primary school, technical or professional school, secondary school, university degree, or unknown), alcohol intake (continuous), and physical activity (inactive, moderately inactive, moderately active, active, or missing).

[b]The β-coefficient (regression coefficient) is the natural log of the RR estimate.

[c]The percent change in the regression coefficient with adjustment for each additional biomarker compared with the multivariable model.

[d]The corresponding 95% confidence interval (CI) was calculated based on Fieller's theorem [24].

RR= relative risk; CI = confidence interval; HbA$_{1c}$ = glycated hemoglobin; IGF-1= insulin growth factor 1; HDL-cholesterol = high-density-lipoprotein cholesterol; CRP-hs = high sensitivity C-reactive protein; ROM = reactive oxygen metabolites; HMW-adiponectin = high-molecular-weight adiponectin; sOB-R= soluble leptin receptor.