CC/2016/01

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET. Part 3

The potential association of IGF-I with Colorectal cancer risk and with lung cancer risk

Introduction and background

1. As part of a horizon scanning paper (CC/2008/17) considered in November 2008, the Committee discussed the possible carcinogenic hazard from dietary insulin-like growth factor-1 (IGF-I). The discussion was prompted by the contents of a book which suggested that consumption of IGF-I in dairy produce could cause an increase in the risk of developing certain cancers. Since the use of bovine somatotropin (BST) was said to increase the amount of IGF-I in milk, concerns were expressed that increased dietary exposure to IGF-I from this source might increase the risk of consumers developing cancer.

2. Following this discussion, the COC then considered paper CC/09/08 in July 2009, which summarised the claims about the health effects of IGF-I that were presented in the book "Your Life in Your Hands" (Plant, 2007)[1]. The Committee drew a number of conclusions, which are given below:

a) *The book presented evidence on the role of IGF-I in cell proliferation and cancer in support of a claim that risks of certain cancers, particularly breast and prostate cancers, are increased by consumption of dairy products and that the increased risk is a result of the presence of IGF-I in milk. The evidence presented was incomplete, prone to bias and of inconsistent quality, so any conclusions drawn from the book must be regarded as provisional and would need to be confirmed following a fuller systematic search of the scientific literature before they could be acted upon.*

b) *The book identified that IGF-I has a role to play in the normal growth and development of tissues, and that locally high levels of IGF-I or increased sensitivity to IGF-I can also cause cancer cells to multiply.*

[1] Plant J, 2007 "Your Life in Your Hands", updated edition, Virgin Books Ltd, London. ISBN 978 0 7535 1204 3

c) *The book did not provide convincing evidence to justify its claim that the IGF-I in milk and dairy products (or in any other food) could cause consumers to have increased risks of developing certain cancers.*

d) *Information was provided on the amount of IGF-I in milk, but nothing was presented on the amounts of IGF-I in other foods*

e) *There is a potential for dietary IGF-I to come in contact with the cells lining the gastrointestinal tract. However, no information was presented on the concentrations of IGF-I that these cells could be in contact with.*

f) *No information was presented on the amount of breakdown of IGF-I that might occur in the gut lumen, although there was evidence from an in vitro study that casein and some other dietary proteins might give some protection from breakdown and there was evidence that partial breakdown to N-terminally truncated forms could increase the potency of IGF-I.*

g) *No information was presented on the amount of IGF-I that might be absorbed from the gut lumen into the bloodstream.*

h) *There was evidence that showed that IGF-I could cause increased mitosis and decreased apoptosis to occur in vitro in some cell lines, including several derived from cancer cells. It was also claimed that IGF-I caused differentiation of cells, but the references that were cited presented no evidence from experiments in support of the claim.*

i) *It was claimed in the book that increased blood concentrations of active IGF-I caused an increased risk of cancer. In support of this claim, the book made reference to several epidemiological studies that showed associations between blood levels of free IGF-I and risks of some cancers.*

Additional points made by the Committee were:

- It was considered highly unlikely that dietary IGF-I could elicit an effect in the gastrointestinal tract as it is unlikely that the cells of the intestinal epithelium would respond to luminal growth factors.
- IGF-I is unlikely to be absorbed from the gut to any great extent.

The Committee agreed that a systematic review of the risk of cancer from dietary IGF-I would be worthwhile. Members suggested that such a review could include:

a) Information from the EPIC cohort which showed a positive association between intake of dairy food and serum IGF-I levels.
b) Details of a study where boys received protein either in the form of meat or milk, which showed increased IGF-I levels only in those given milk.
c) Closer scrutiny of the conclusions of an epidemiology study by Ma *et al*., 2001.
d) Relevant information from the preclinical data package for human therapeutic use of IGF-I.

e) Information on whether dietary IGF-I contributes to circulating IGF-I levels.
f) Comparison of the mitogenic potency of IGF-I with a benchmark such as oestradiol.
g) Information on the amount of the high potency truncated form of IGF-I in milk.

3. Resources were not available to perform a systematic review. Instead the secretariat performed a narrative review that incorporated elements of a systematic review: recording details of literature searches and of the selection of articles used. The narrative review covers a wider selection of issues than would have been practical had a systematic review been performed.

4. As a result of the large database available, particularly of epidemiology studies, the review was split into several sections. In March 2012, the committee considered paper (CC/2012/06) which presented information on identity, physiological control of IGF-I, human physiological levels of IGF-I, IGF-I in food and tissues, the use of IGF-I as a human medicine, toxicology and other safety studies and information on the association between blood levels of IGF-I and breast cancer.

5. In November 2012 the committee then considered further data examining the association between IGF-I levels and the risk of prostate cancer.

6. The COC made a number of comments and drew a number of conclusions on breast and prostate cancer from this data. The minutes of the two meetings have been attached at Annex A. For the purposes of this paper it has been presumed that comments made on issues such as analytical techniques would apply to the studies on lung and colorectal cancer as well as those on breast and prostate cancer and this discussion has not been repeated.

7. Unfortunately due to a shortage of resource and to competing priorities, it was not then possible to make further progress on dietary IGF-I and cancer risk following the consideration of these papers. However it is now hoped that the IGF-I topic will be completed in a final paper in autumn/winter 2016 when the potential association between IGF-I and other cancers and mechanistic data will be considered. It is hoped that this topic can be completed in the next 12-18 months.

Colorectal cancer and lung cancer

8. The current paper considers data on the potential association between blood levels of IGF-I and colorectal cancer (Annex B) and lung cancer (Annex C).

9. Details of the original literature search, updated in 2012 are attached at Annex D. This has been subsequently updated on a topic by topic basis but will be completely updated once the work is approaching completion.

Questions for the Committee

10. Members are asked to consider the data in Annex B:

a) To make any general comments they may have on the data.

b) To consider whether there is an association between circulating IGF-I levels and the risk of colorectal cancer. If so, is it possible to draw any further conclusions on the level of IGF-I and the stage or severity of colorectal cancer.

11. Members are asked to consider the data in Annex C:

a) To make any general comments they may have on the data.

b) To consider whether there is an association between circulating IGF-I levels and the risk of lung cancer.

Secretariat
February 2016

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The potential association of IGF-I with Colorectal cancer risk and with lung cancer risk

Extracts from the minutes of previous COC meetings:

March 2012

Extract from CC/MIN/2012/2 -General aspects and breast cancer

ITEM 5: Possible Carcinogenic Hazard to Consumers from Insulin-like Growth Factor-I (IGF-I) in the Diet (CC/2012/06)

15. Dr N Wallis declared a personal specific interest as she is involved in research on IGF-I. Dr Wallis was excluded from participating in the discussion on this item. Professor A Boobis declared a lapsed personal specific interest having been member of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during the assessment of bovine somatotropin which, when used in cows, is said to increase the amount of IGF-I in milk. This was noted but not considered to preclude full participation in the discussion.

16. This topic had been considered previously as part of the November 2008 horizon scanning discussion (CC/2008/17) and further in paper CC/2009/08 which summarised the claims about the health effects of IGF-I presented in Dr Plant's book "Your Life in Your Hands"[2]. The paper prepared for the current meeting presented information on identity, physiological control of IGF-I, human physiological levels of IGF-I, IGF-I in food and tissues, use of IGF-I as a human medicine, toxicology and other safety studies and the association between blood levels of IGF-I and breast cancer.

17. Measurement of IGF-I was discussed and it was noted that differences were found between results from studies using plasma measurements compared to those using serum measurements. It was also highlighted that the use of different anti-coagulants for plasma sample collection affected results. There was little information on differences arising from the techniques

[2] Plant J, 2007 "Your Life in Your Hands", updated edition, Virgin Books Ltd, London. ISBN 978 0 7535 1204 3

used for sample measurement as both in house and commercial methods had been used, and the possibility of a lack of specificity in commercial approaches was noted. Finally, it was not clear from some studies whether total IGF-I (i.e. bound and unbound IGF-I) or free IGF-I was being measured in the samples.

18. With respect to physiological levels of IGF-I, there were some suggestions of discrepancy between the plasma levels in animals and those in humans, as far higher plasma levels were reported in a study in Wistar rats than in humans. However, it was not clear what the plasma level had been in the Sprague Dawley rats used for the two year bioassay, and therefore how this would compare with humans. For humans it was agreed that the IGF-I would reach a maximum during puberty and then decline with age.

19. In considering the levels found in food, it was queried whether there were numerous or only one truncated form of IGF-I found in milk. Though there was little information, it was considered that there was only one form. Members were informed that human and bovine IGF-I were indistinguishable. The effect of dietary exposure to IGF-I was discussed. It was apparent that dietary IGF-I increased serum concentrations of IGF-I. However it was not clear that this was necessarily a direct effect or may have been mediated by increased protein intake. Members noted that IGF-I would probably be broken down in the stomach and need to diffuse or be taken up from the gut against a concentration gradient and that it was possible that the effect of dietary IGF-I on serum IGF-I was due to protein load or an effect of intake of dairy foods.

20. The findings of the two-year carcinogenicity study were considered and it was noted that the dose at which mammary tumours occurred was higher than that associated with increased mortality and effects such as hypoglycaemia. It was also noted that IGF-I had been administered subcutaneously and there would have been 100% bioavailability, while bioavailability from dietary IGF-I would be much lower. It was suggested that, while route to route extrapolation would be difficult, a worst case screen could be used assuming 100% oral bioavailability and using a margin-of-exposure approach between rat and human plasma levels.

21. It was queried whether there had been any follow up to the clinical studies or from medicinal use. While nothing had been identified at the last literature search, given that IGF-I had been used as a medicine in the US since 1995 it was considered likely that there would be data available.

22. The physiological importance of early life exposure to high dietary IGF-I was highlighted and the need to bear this in mind when interpreting studies where neonates receive high doses.

23. In considering Annex 2 to this paper, the Committee agreed with the conclusions drawn in paragraphs 104 to 109, with some minor changes suggested for paragraph 109 to clarify that high parenteral doses cause a slight increase in malignant mammary tumours in mice, the effect of

maturation of the gut in neonatal animals and the assumptions regarding absorption from the gut.

24. The epidemiological database on the association between IGF-I and breast cancer included studies of different designs and with conflicting findings. A number of aspects were discussed. Differences were noted between results for pre- and post-menopausal women and it was highlighted that only one study had excluded peri-menopausal women. A difficulty in assessing and comparing the studies was that it was not always clear what adjustments had been made for confounding variables; it was agreed that this information should be included in the summary tables produced by the Secretariat where possible. Other points to take account of were which studies had measured IGF-I before diagnosis of cancer and how long the time period between measurement and diagnosis of cancer had been, and what associations other risks or confounders had with breast cancer in the studies.

25. It was noted that the three meta-analyses differed in their aims and findings. Only one had looked at the dose-response relationship and, while this meant there was less power, it was considered indicative of the quality of the individual studies as this meta-analysis excluded papers where there was not enough information to determine a dose-response relationship between serum IGF-I and breast cancer.

26. In conclusion, the epidemiology papers indicated a tendency for association between dairy protein and circulating IGF-I levels and also between IGF-I and breast cancer in premenopausal women. It was considered that it should be possible to assess the association between dietary protein and breast cancer through IGF-I in appropriate studies. Overall, the database was deemed insufficient to link dietary IGF-I directly with breast cancer

27. A further paper on other cancers would be prepared for a future meeting and a statement prepared in due course.

October 2012

Extract from CC/MIN/2012/2 - Prostate cancer

ITEM 7: Possible Carcinogenic Hazard to Consumers from Insulin-like Growth Factor-1 (IGF-1) in the Diet: IGF-1 and Prostate Cancer (CC/2012/16)

36. This topic had been considered previously in 2008 and 2009 and, most recently, at the April 2012 meeting. At that meeting the Committee was presented with a review of the identity and physiological control of IGF-1, human physiological levels of IGF-1, IGF-1 in food and tissues, the use of IGF-1 as a human medicine, toxicology and other safety studies, and information on the association between blood levels of IGF-1 and breast cancer. The paper presented at this meeting provided information on the

potential association between blood levels of IGF-1 and the risk of prostate cancer.

37. Overall the studies on IGF-1 and prostate cancer were considered inconsistent in terms of design, conduct and the results obtained. It was noted that many studies had been designed with a view to improving screening. There was also difficulty because some studies considered the stage of prostate cancer, but in others this was not reported. It was also noted that elevated IGF-1 may be a disease marker rather than a risk factor.

38. It was considered that while there was variability in the results of individual studies, the meta-analyses were more representative, and indicated an association between IGF-1 levels and the risk of prostate cancer. It was queried how the heterogeneity in the studies had been accounted for in the meta-analyses, for example, had meta-regression been used. In addition, information on how the meta analyses varied, e.g. in exclusion of papers, would be helpful in interpreting the differences. It was considered that the later meta-analyses were of better quality than the older ones.

39. While all of the studies reported were on endogenous levels of IGF-1, it was noted that there was no information on the possible effects of exogenous/dietary IGF-1 nor on the influence of exogenous factors on circulating IGF-1 levels. Hence, it is very difficult to interpret the implications of the findings for environmental IGF-1.

40. With respect to the conclusions of the review, it was noted that there were genome-wide association studies for prostate cancer, indicating the involvement of BRCA-1 and BRCA-2. A member proposed that SNPs were a more important element. It was also highlighted that the results were not necessarily inconsistent where they show the same trend, even if not all are statistically significant.

41. A final paper on lung, colorectal, ovarian and endometrial cancers would be prepared for a future meeting and this would also consider mechanistic elements. The outcomes from all three discussions would then be drawn together to form a final view on the carcinogenic hazard from IGF-1 in the diet.

Secretariat,
February 2016.

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IGF-I AND COLORECTAL CANCER

Introduction

1. Colorectal (bowel) cancer is the fourth most common cancer in the UK, with 41,900 cases being diagnosed in 2012, accounting for 12% of new cancer cases (Cancer Research UK, 2015). Rates have increased by more than 10% since the 1970s. Over four in ten cases occur in the over 75s. Other risk factors include family, history, diet, smoking, obesity, alcohol and exposure to ionising radiation; 54% of cases are thought to be preventable. Ethnicity may also be important; for example African-Americans have a higher incidence and mortality from colorectal cancer compared to Caucasians (Ochs-Balcom *et al*., 2014).

The IGF-I axis in colorectal cancer

2. The mechanism of IGF-I action and regulation was considered in detail in CC/2009/08 and CC/2012/06. In brief, IGF-I (also known as somatomedin C) is a peptide growth factor with a structure similar to insulin. It is largely produced in the liver in response to growth hormone. In circulation, IGF-I is largely bound to six binding proteins, most usually (>90%) IGFBP-3. The IGFBP-3 is cleaved by proteases which increase the availability of IGF-I. IGF-I acts via the IGF-I receptor (IGFIR). Levels of IGF-I are controlled via a feedback loop with free IGF-I inhibiting the secretion of growth hormone, which in turn reduces the levels of IGF-I production. Free IGF-I binds to IGFBP-2 and IGFBP-5 with a greater affinity than it binds to the IGF-I receptor so increases in the levels of these binding proteins will reduce IGF-I activity.

3. Possible mechanisms by which IGF-I could influence colorectal cancer were discussed by Cao *et al*. (2013). Serum IGF-I levels stimulated growth and metastasis in colon cancer through increased angiogenesis by the expression of vascular epithelial growth factor (VEGF) and inhibited cytokine-induced apoptosis of colon cancer cells. These effects could have important implications as circulating IGF-I levels contribute to higher invasive and metastatic potential of colon cancer cells. However, it is not known how IGF-I and IGFBPs act on early stages of colorectal carcinogenesis. IGFBP-3 in contrast, may be anti-mitogenic and pro-apoptotic (Suzuki *et al*., 2009). Expression of both IGF-I and IGF-IR is increased in most cancer cells (Olivo-Marston *et al*., 2009).

4. In addition, dietary IGF-I could come into direct contact with colorectal tissue if it was present in the gut in an intact or partially truncated form. This possibility is

considered in paragraphs 65-71 of Annex 2 to CC/2012/06. In brief, as a peptide IGF-I would be expected to be subject to proteolytic digestion in the gut. However data from neonatal or suckling animals suggests that biologically active IGF-I is found in the gut; there are fewer data on whether this could occur in adults. It has also been suggested that some dietary components such as albumin could protect IGF-I from digestion. The effects of dietary IGF-I will be considered in a future paper, but the amount of IGF-I that could be derived from food is very low. For example, the Veterinary Products Committee (1999) stated that milk consumption would add less than 0.1% to circulating IGF-I levels even if fully absorbed.

Clinical observations

Acromegaly

5. Patients with acromegaly have an increased risk of developing tumours of the gastrointestinal tract (particularly adenomas and adenocarcinomas of the colon) compared with normal subjects (Ron, *et al*., 1991; Cats, *et al*., 1996; Jenkins, *et al*., 1997; Colao, *et al.,* 1997; Bolfi *et al*., 2013).

6. It has been reported (Juul, *et al*., 1994) that acromegaly patients have high blood levels of growth hormone (GH) and of active IGF-I. Cats, *et al*. (1996) found that serum levels of GH and of IGF-I correlated with the rate of epithelial cell proliferation in the sigmoid colons of acromegaly patients, but others (Colao, *et al.,* 1997) found no correlation between numbers of colonic polyps and circulating levels of IGF-I or of growth hormone. Jenkins, *et al*. (2000) found significantly ($p<0.005$) higher mean serum IGF-I concentrations in acromegalics with newly detected adenomas at follow-up colonoscopy (390 ng/ml) than in acromegalics without new adenomas (231 ng/ml).

7. The colon cancer mortality rate was higher than expected (standard mortality ratio = 2.47) in acromegaly patients, with colon cancer mortality being highest in those with high post-treatment levels of growth hormone in their serum (Orme, *et al*., 1998).

Genetic polymorphism

8. Genetic polymorphisms in IGF pathway related genes including insulin and insulin signalling genes have been shown to influence circulating hormone levels, and in some studies the risk of colorectal neoplasia (LeRoy *et al*., 2011). A number of single studies are considered below and a meta-analysis has also been conducted. These have been arranged chronologically by biomarker.

IGF-I

9. In a study conducted in Taipei, Lin *et al*. (2010- abstract only) reported that older colorectal cancer patients had a higher frequency of the AA genotype of IGF-I compared to younger ones (12.7% vs 4.2%). However other clinicopathological

factors such as tumour location, differentiation, lymphovascular invasion and TNM[3] stage were not associated with the genotype. Mucinous differentiation (but not the other clinicopathological factors) was significantly associated with the CA/AA genotype of IGF-I.

10. Keku *et al*. (2012) compared genetic variations between colon cancer cases and matched controls in a White (297 cases, 530 controls) and an African-American population (231 cases, 306 controls). One of the variations investigated was a microsatellite $(CA)_n$ repeat polymorphism in the promoter region of *IGF-I* which has been shown to influence IGF-I production. The results were analysed by unconditional logistic regression. When typed as part of a case control study it was found that the IGF-I (CA)19 repeat was higher in White compared to African-American controls (50 and 31% respectively). Whites that were homozygous for this repeat had a nearly 2 fold increase in the risk of colon cancer (OR= 1.7; 95%CI 1.15-2.73) but not African-Americans (0.73; 0.50-1.51). The presence of the variation did not significantly affect the level of IGF-I In the blood.

11. In a case control study in an Iranian population, there was no association between four SNPs in IGF-I (rs6214), IGFBP-3 (rs3110697), INSR (rs1052371) and IRS2 (rs2289046) and the risk of CRC (Karimi *et al*., 2013). This was not affected by adjusting for confounders such as age, sex, BMI and smoking status) in a logistic regression model.

12. A case control study was conducted in a Dutch population of 1457 individuals with gastrointestinal cancer, of whom 544 had colorectal cancer, and 1457 matched controls (Ong *et al*., 2014). The study investigated the prevalence of SNPs rs6214 in the *IGF type I (IGF-I)* gene and rs6898743 in the growth hormone receptor (*GHR*) gene in patients with GI cancer and the controls. A number of associations were found between particular SNPs and oesophageal adenocarcinoma and oesophageal squamous cell carcinoma and head and neck cancer but not for colorectal cancer.

IGFIR

13. A classification tree was built to detect interactions in IGF and insulin-pathway related genes and metachronous (multiple separate occurrences) colorectal neoplasia among 1,439 subjects from two chemoprevention trials (LeRoy *et al*., 2011) The probability of colorectal neoplasia was greatest (71.8%) among carriers of any A allele for rs7166348 (*IGF1R*) and AA genotype for rs 1823023 (*P1K3R1*) (phosphoinositide-3-kinase). In contrast, carriers of any A at rs7166348 (*IGF1R*), any G for the (*P1K3R1*) variant, and AA for rs10426094 (*INSR*) (insulin receptor) had the lowest probability of colorectal neoplasia (14.3%). Logistic regression modelling showed that any A at rs7166348 (*IGF1R*) with the AA genotype for rs1823023 (*P1K3R1*) conferred the highest odds of colorectal neoplasia (OR 3.7: 95%CI 2.2-6.5) compared with carriage of GG at rs7166348 (*IGF1R*), any G allele at rs1823023 (*P1K3R1*), and the AA genotype at rs10426094 (*INSR*) which conferred the lowest odds (OR 0.22: 95%CI 0.07-0.66). Stratifying the analysis by parent study and intervention arm showed highly consistent trends in direction and magnitude of associations, with preliminary evidence of genotype effects on measured IGF-I levels

[3] TNM is the classification of malignant tumours

in a sub-group of subjects. The results were considered to support a role for genetic interactions in the insulin/IGF pathway genes in colorectal neoplasia risk.

IGFBP-3

14. Carrying *IGFBP-3* variant alleles (rs2854744) was associated with a lower level of IGFBP-3 protein, most notably in the white population (Keku *et al*., 2012); IGF-I levels were not measured.

Multiple markers

15. A single nucleotide (T to A) polymorphism in the human growth hormone gene, (human T1663A GH1 gene polymorphism), has been associated with reduced risk of colorectal cancer. Le Marchand, *et al.* (2002) found that A/A individuals had a statistically significantly ($p<0.05$) reduced risk of colorectal cancer (and colorectal adenomas) and relatively lower mean plasma levels of growth hormone and IGF-I and a lower IGF-I/IGFBP-3 ratio than T/T individuals. Mean plasma IGFBP-1 levels were significantly ($p<0.05$) greater in A/A individuals than in T/T individuals. Unconditional logistic regression was used to analyse the data. Adjusted odds ratios for colorectal cancer associated with T/T, T/A, and A/A genotypes were 1, 0.75 (95% CI = 0.43 - 0.90) and 0.62 (95% CI = 0.31 - 1.22), respectively (P_{trend} = 0.006). The authors concluded that the human T1663A gene polymorphism may confer lower levels of growth hormone and IGF-I and appears to be associated with a decreased risk of colorectal cancer.

16. African-Americans have a higher incidence and mortality from colorectal cancers compared to Caucasians, with the declining mortality rate seen in Caucasians not being observed in African-Americans (Ochs-Balcom *et al*., 2014). In the study by Ochs-Balcom *et al*., (2014) IGF-I, IGFBP1 and IGFBP3, and SNPs in the IGF-I receptor (*IGF1R*), IGF-II receptor (*IGF2R*) and insulin receptor genes were analysed in 1572 individuals who were scheduled for routine colonoscopy (as part of the Transdisciplinary Research on Energetics and Cancer (TREC) study). Of these, 432 were incident adenoma cases and 1139 were adenoma free controls. Logistic regression models were used to analyse the data. One SNP (rs 496601) in *IGFIR* was associated with adenomas in Caucasians only; the per allele adjusted OR; 95%CI is 0.73; 0.57-0.93. Similarly one *IGF2R* SNP (rs 3777404) was statistically significant in Caucasians; adjusted per allele OR is 1.53; 1.10-2.14. The authors suggested that the results indicated inherited genetic variance in the risk of adenomas that warranted further study.

Meta-analysis

17. A meta-analysis of 96 studies (including over 110,000 participants) was conducted by Chen *et al*. (2009). The studies used in the meta-analysis included those with case-control, cohort or cross-sectional design which looked at associations between circulating concentrations and genotypes (SNP) of IGF-I and IGFBP-3 and cancer risk. The Meta-analysis produced pooled odds ratio (OR) and weighted mean difference (WMD) values. The Q test, Egger's test and Begg's funnel plot were used to evaluate the heterogeneity and publication bias between the studies; there were no statistically significant differences in these parameters. The

number of studies used in different analyses varied; 65 studies were available for phenotype analysis (including 15, 212 cases and 27,913 controls) and 27 studies for potential functional polymorphisms. Nine studies were available for genotype-phenotype correlation analysis. Higher IGF-I circulating levels significantly increased cancer risk (OR; 95%CI = 1.15; 1.013-1.29, *p* = 0.014) including among colorectal cancer patients (1.28; 1.02-1.6, *p* = 0.031) using data from 9 studies, 1909 cases and 3783 controls. A higher IGF-I/IGFBP-3 molar ratio could increase the colorectal cancer risk with borderline significance (1.70; 0.98-2.96) (p value not given). Differences in the functional polymorphisms assessed (*IGF1* $(CA)_n$, *IGFBP3* A-202C and *IGFBP3* Gly32Ala) did not affect the overall cancer risk or the circulating IGF-I levels, but circulating IGFBP-3 levels could be influenced by *IGFBP3* A-202C.

Experimental studies

In vivo

18. Although high IGF-I levels have been reported to be associated with an increased risk of colon cancer in epidemiological studies and IGF-I has been reported to inhibit apoptosis and stimulate cell proliferation *in vitro*, it has not been possible to explore this in some animal models (Olivio-Marston *et al*., 2009). This is because IGF-I knockout mice have severe developmental abnormalities and most do not survive. Olivio-Marston *et al*. (2009) used a mouse model in which *IGF-I* was specifically deleted in the liver using a Cre/*loxP* system. These mice display a circulating IGF-I level which is reduced by 50-75% compared to wild type mice. In a pilot study, it was found that following treatment with azoxymethane (one injection per week for six weeks) to induce colon tumours, the IGF-I deficient mice had a significant inhibition of colon tumour multiplicity in the proximal area of the colon compared to wild type mice. A decrease in proliferation and an increase in apoptosis was observed in the IGF-I deficient mice. IGF-I deficiency also affected the location of the tumours, with more tumours occurring in the distal colon in the deficient mice compared to the wild type. The significance of this was unknown, although it was noted that proximal tumours were more aggressive because they had more DNA damage and fewer apoptotic cells. A significant reduction in the levels of seven out of 10 cytokines was also observed, suggesting that IGF-I could have an effect on inflammation, which could contribute to the tumorigenesis, since inflammation had been linked to colon cancer.

In vitro

19. In studies using colon carcinoma cells, levels of 10-20 ng/ml IGF-I (levels attainable in serum) induced transient growth of the cells, then growth arrest (Ewton *et al*., 2002). When IGF-I functioned as a mitogen, it blocked differentiation. Intestinal cell differentiation occurred once the cells had undergone the IGF-I initiated growth arrest and IGF-I and butyrate acted synergistically to induce maturation markers. The mitogenic effect of IGF-I was short lived in the colon carcinoma calls compared to similarly treated NIH-3T3 cells which continued to proliferate. Intestinal cells *in vivo* undergo a limited number of divisions, then growth arrest and completion of their maturation. IGFs found in intestinal tissue may control the timing of this process. Colon cancers may have developed strategies to overcome the IGF-I

mediated growth arrest. It has been reported that IGFBP-3 levels were higher in resected colon cancers compared with adjacent normal tissue. In the study, growth inhibition by IGF-I and IGF-II was blocked by concurrent addition of IGFBP-3 implying that colon cancers with elevated IGFBP-3 levels would be selected for *in vivo* because they could bind and inactivate high serum IGF-I levels and continue to proliferate.

20. IGF-I receptor mRNA expression was increased in human colorectal cancer cell line cultures (Guo, *et al*., 1992) and in human colorectal cancer tissues obtained at operation (Freier, *et al*., 1999). Conversely, blockade of the IGF-I receptor with a neutralising monoclonal antibody (αIR3) inhibited survival and growth of human colorectal cells *in vitro* (Lahm, *et al.,* 1992 & 1994). Recombinant human IGF-I induced the expression of vascular endothelial growth factor in colorectal cancer cells *in vitro* (Akagi, *et al*., 1998) , thus showing a potential for promoting colorectal tumour progression by inducing the development of blood vessels.

21. IGF-I and IGF-I receptor expression was higher in human CRC tissues associated with lymph node metastases and tumour TNM stage (Li *et al*., 2013). A higher lymphatic vessel density was found in CRC tissues compared to adjacent non-tumour tissue and was also correlated with lymphatic metastasis.

22. Li *et al*. (2013) also investigated the effect of IGF-I on CRC lymphangiogenesis. Transwell assays suggested that IGF-I increased the migration of CRC cells. *In vivo* nude mouse studies showed that treatment with 50 µg/kg IGF-I i.p for three weeks increased lymph vessel density in LoVo cell (a human CRC cell line) xenografts compared to vehicle controls.

Observational Studies

23. The available studies are tabulated in Table 1 below and described in the text following the table. The studies are separated into retrospective studies, prospective studies and meta-analyses.

Table 1: Summary of results of epidemiology studies of colorectal cancer risk associated with IGF-I and related substances

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
Retrospective studies						
Greek adults	41 cases; 50 controls	Immuno-radiometric assay consistent with methods used to extract free IGF-I	Gender, age, educational level.	Positive	Highest two tertiles of IGF-I and IGF-II associated with increased risk compared to lowest (OR 5.2, 95%CI 1.0-26.8)	Manousos *et al*., 1999
English men and women aged 55-64 y	60 men and 40 women	Radio immunoassay	Age, sex, current use of hormone replacement therapy, smoking, BMI, aspirin use	Positive (for high-risk adenomas)	Higher IGF-I and lower IGFBP-3 in those with high-risk adenomas, compared with those with no cancer or low-risk adenomas.	Renehan, *et al*., 2001
Japanese men	157 cases 311 controls		Self Defence Force rank, hospital, smoking, IGFBP-3, glucose	Positive after additional adjustment	Modest positive association with IGF-I.	Teramukai *et al.*, 2004.
Adults aged	239 cases; 517 controls	ELISA		None	No difference between IGF-I, IGF-II or IGFBP3 levels.	Keku *et al.,* 2005

[4] In many studies, it is unclear whether the IGF-I measured was free or attached to binding proteins since the experimental details are not always provided. The majority of studies use commercially available ELISA kits, which may or may not involve an acid alcohol extraction step to remove the binding proteins.

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
US adults attending for colonoscopy	164 cases 614 controls	-	Alcohol intake, waist/ hip ratio	Negative	Plasma IGFBP3 not associated with adenoma risk. Tissues IGFBP-3 mRNA was higher in cases.	Keku *et al*., 2008
US adults Caucasian, Japanese and Native Hawaiian	554 cases; 786 controls	ELISA following acid alcohol extraction to give free IGF-I	Age, race, ethnicity, sex, recruitment site. Energy, smoking, oestrogen use alcohol intake, folate intake BMI, waist and hip circumference	None	IGF-I and IGFBP-3 not associated with adenoma risk OR;95%CI = 0.83; 0.54-1.27, p= 0.26, 0.78;0.51-1.19, p=0.37 respectively (lowest vs highest quartile)	Le Marchand *et al.* 2010
US adults (DHS cohort)	167 adults	ELISA	Age, race, gender	Negative	No effect of IGF-I or IGFBP3 on recurrent adenoma.	Kang *et al*., 2013
Chinese adults (17-83 y)	24 polyps 13 CRC 13 controls	ELISA	-	Positive for adenomatous polyps and colorectal cancer	IGF-I levels higher in carcinoma and adenomatous polyp and CRC cases compared to controls (200.96 ± 55.92, 218.77± 88.93 and 98.37 ± 24.99 respectively)	Zhang *et al*., 2014
Turkish adults	48 cases 30 controls	ELISA	Age, BMI, visceral fat, waist circumference homeostasis metabolic assessment method	Positive	IGF-I levels higher in carcinoma and adenoma cases compared to controls (184.6 ± 61.6, 177± 87.6 and 108.9 ± 45.3 respectively)	Erarslan *et al*., 2014
US males	126	ELISA	Age, smoking	Negative	No association between IGF-I, IGF binding proteins and number or types of polyp.	Comstock *et al.,* 2014

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
US adults	410 cases 1070 controls	ELISA	Age, sex, family history, smoking, NSAID, BMI.	Negative in Caucasians Positive in African-Americans	IGF-I and IGFBP3 higher in cases than controls in both groups.	Ochs-Balcom *et al*., 2014
Prospective studies						
American male physicians, aged 40-84 y	193 cases; 318 controls	ELISA	Age, smoking, BMI, alcohol	Positive	RR=2.51, comparing the highest and lowest quintiles. Negative association with IGFBP-3 levels (RR=0.28).	Ma, *et al*., 1999 & 2001
American female nurses, aged 35–55 y	107 cases; 107 controls.	ELISA. Results stated to be consistent with those following acid chromatography	Age, fasting status, month of sampling, alcohol intake, BMI, IGF-I and IGFBP-3 adjusted for each other.	None	No significant association between plasma IGF-I and colorectal cancer. Negative association with IGFBP-3.	Giovannucci, *et al*., 2000
American women aged 35-65 y	102 cases; 200 controls	Double antibody immuno radiometric assay after acid ethanol extraction to give free IGF-I	Menopausal status, age, date of recruitment, time of blood sampling	None	No significant association between plasma IGF-I or IGFBP-3 and colorectal cancer. Negative trend with IGFBP-1.	Kaaks, *et al*., 2000
Chinese men aged 45-65 y	125 cases; 661 controls	Commercial radio-immunoassay. Unclear if free	Residence, age, time of blood sampling, age, weight, smoking, alcohol	None	No significant association between plasma IGF-I and colorectal cancer. Positive associations with IGF-II and IGFBP-2.	Probst-Hensch, *et al.,* 2001

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
Swedish men and women aged 30-70 y	580 cases; 336 controls	Double antibody immuno radiometric Assay	Sex, age, sub-cohort, date of blood sampling, fasting time.	None	No significant trends, but IGF- & IGFBP-3 levels had a slight positive association with colon cancer and a slight negative association with rectal cancer.	Palmqvist, *et al.,* 2002
American female nurses aged 35-55 y	182 cases; 364 controls	ELISA	Age, date of blood sampling, fasting status, smoking	Positive	Positive association between IGF-I and colorectal cancer when adjusted for IGFBP-1. RR=2.17 comparing upper and lower quartiles.	Wei, *et al.,* 2005
Adults	202 cases; 256 controls	ELISA	Age, race, education, polyp history, aspirin use, NSAID use, smoking family history of CRC	Positive	IGF-I, IGF-I/IGFBP3 and insulin levels associated with adenoma, particularly severe adenoma.	Schoen *et al.,* 2005
UK adult males	147 cases 440 controls	ELISA	Age, smoking, alcohol, BMI	None	IGF-I, IGFBP-1 and IGFBP-3 were not associated with colorectal cancer	Morris *et al*., 2006
Japanese men and women aged 40-69 y	375 cases; 750 controls	Total IGF-I by immuno-radiometric assay.	Smoking, alcohol, BMI, exercise, family history of CRC	None	IGF-I, IGFBP-1 and IGFBP-3 were not associated with colorectal cancer	Otani, *et al*., 2007
American post-menopausal women aged 50-79 y	438 cases; 816 controls	Total and free IGF-I by ELISA	Age, smoking, race/ethnicity physical activity, waist circumference, NSAID use, alcohol use, family history of CRC	Positive	The trend associating free IGF-I with colorectal cancer was of borderline significance. Hazard ratio = 1.53.	Gunter, *et al*., 2008

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
Finnish male smokers, aged 50-69 y	134 cases; 400 controls	ELISA	Smoking history, BMI, fibre intake, hypertension, physical activity	None	No association with IGF-I, IGFBP-3 or IGF-I/IGFBP-3 ratio.	Max, *et al*., 2008
Adults from polyp prevention trial	375 cases; 375 controls	Radio-immunoassay	Age, gender, body mass index, intervention group, aspirin, smoking, ethnicity, and education	Negative	Risk of adenoma recurrence reduced at high IGF-I and IGFBP3 levels.	Flood *et al*., 2008
Males from Wheat Bran Fibre Trial	299 no controls		Smoking history, BMI, alcohol use, family history of CRC	Negative	IGF-I reduced the risk of adenoma recurrence.	Jacobs *et al*., 2008
Adults 40-69 from JACC cohort	101 cases 303 controls	Immuno-radiometric assay	Area, age, BMI, cholesterol, smoking, alcohol, energy intake, protein intake.	Negative	No effect on CRC mortality.	Suzuki *et al*., 2009

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
European	1121 cases; 1121 control	Free IGF-I ELISA following acid alcohol extraction	BMI, ratio of waist to hip circumference, height, smoking status, education, physical activity, alcohol intake, dietary intakes of red meat, processed meat, dairy products, fruit, vegetables and fibre	Positive for colon cancer. None for rectal cancer	Slight association of IGF-I with colon cancer (not rectal cancer) in young (<50y) participants or those with low milk intakes. RR for an increase in serum IGF-I of 100 ng/mL = 1.43. No association with IGFBP-3.	Rinaldi, *et al*., 2010
US adults (HPFS and NHS cohort)	499 cases; 993 controls	ELISA	Smoking, alcohol intake, dietary intakes of red meat, processed meat, methionine, folate, calcium, family history of CRC	Positive	Risk associated with high IGF-I/IGFBP3 reduced by higher 25(OH)D levels.	Wu *et al*., 2011
Japanese adults	1520		Age, screening period, fasting duration, smoking, alcohol, family history of CRC, NSAID use, height, energy intake.	Positive	Increased IGF-I associated with colorectal adenoma (1.63; 1.08-2.48)	Yamaji *et al*., 2012 (abstract only)

Subjects	Number of subjects	How was IGF-I measured and was it free[4]?	Variables study controlled, matched or analysed for	Association between IGF-I levels in blood and colorectal cancer	Main results	Reference
Adults aged > 49y from IRAS cohort	143 individuals; 24 with polyps	Radioimmuno assay	Age, centre, race/ethnicity, gender, BMI, IGF-I and IGFBP-3 adjusted for each other.	Positive	Increasing IGF-I and IGF-I/IGFBP3 over a decade associated with polyps.	Soubry *et al*., 2012
US adults (PLCO cohort)	764 cases; 775 controls	ELISA	Age, race, sex, year of blood draw, BMI, smoking and education	Positive	Higher IGF-I at baseline associated with increased risk of colorectal adenoma (OR:95%CI for highest vs lowest was 1.80;1.30-2.47). IGF-I/IGFBP3 also associated with increased risk (1.49;1.07-2.08 for highest vs lowest)	Gao *et al*., 2012
Meta-analysis	-			*Positive*	*IGF-I levels were positively associated with colorectal cancer, whereas IGFBP-3 and IGF-I/IGFBP-3 ratio were less clearly associated*	*Renehan, et al., 2004*
Meta-analysis	-			*Positive*	*Positive association between IGF-I levels and risk of colorectal cancer (1.25;1.08-1.45)*	*Rinaldi, et al., 2010*
Meta-analysis	-			*Positive*	*Moderately positive association between IGF-I levels and risk of colorectal cancer*	*Chi et al., 2013*
Meta-analysis	-			*Positive for advanced colorectal carcinoma only*	*Moderately positive association between IGF-I levels and risk of advanced colorectal adenoma only*	*Yoon et al., 2015*

Retrospective studies

24. In a case control study reported by Manousos *et al*. (1999), IGF-I and IGF-II levels were higher in 41 Greek patients with colorectal cancer compared to 50 healthy controls, though this was not statistically significant (mean ± SEM IGF-I levels were 80.25 ± 5.05 and 78.83 ± 4.76 ng/ml in cases and controls respectively). Similarly, IGFBP-3 levels were non-significantly inversely associated with risk of colorectal cancer (mean ± SEM IGFBP-3 levels were 2.95 ± 0.15 and 2.79 ± 0.11 µg/ml in cases and controls respectively). However, individuals with both IGF-I and IGF-II values in the top two tertiles of their distributions (26 cases and 29 controls) had elevated ORs compared to individuals in the lowest tertile (4 cases and 11 controls) (OR; 95%CI = 5.2; 1.0-26.8). The data were analysed by logistic regression. It was concluded that high levels of IGF-I and IGF-II might be associated with colorectal cancer.

25. Renehan, *et al*. (2001) performed a study to investigate the hypothesis that circulating levels of IGF-I and IGFBP-3 predict the presence of colorectal adenomas (surrogate markers of colorectal cancer risk). Serum samples were collected from 442 participants at one study centre (Christie Hospital NHS Trust, Manchester, UK) within the Flexi-Scope Trial of healthy volunteers, aged 55-64 years, before they underwent diagnostic endoscopy. Only those individuals (60 men and 40 women) who had a complete screening colonoscopy to the caecum were included in this study. Of the examinations, there were 42 high-risk adenomas identified, 11 low-risk adenomas and 47 normal. Estimates of the relative risk (RR) for the adenomatous stages were calculated by unconditional logistic regression, adjusting for known risk factors. The mean serum levels of IGF-I and IGFBP-3 were similar in individuals with normal colonoscopy findings and those with low-risk adenomas. In contrast, in those with high-risk adenomas, mean serum IGF-I was increased (190(53) *versus* 169(54) µg/L; p=0.06) and IGFBP-3 was decreased (3.22(0.60) *versus* 3.47(0.62) mg/L; p=0.05) as compared with the combined results for normal colonoscopy and low-risk adenoma groups. Levels of IGF-I and IGFBP-3 were unaffected by removal of the adenomas.

26. The association between IGF-I and IGFBP-3 and colorectal cancers was investigated in Japanese men (Teramukai *et al*., 2004). In the study population there were 157 cases of histologically diagnosed colorectal adenomas and 311 controls with normal colonoscopy or non-polyp benign lesions in a consecutive series of 803 men undergoing a pre-retirement health check at two hospitals of the Self Defence Forces (SDF). After adjustment for rank in the SDF, hospital, smoking and IGFBP-3, a modest but statistically non-significant increase in the prevalence odds of colorectal adenomas was observed for the highest vs lowest quartile level of IGF-I. The increase was slightly greater with further adjustment for 2-h glucose concentration (OR;95%CI, 1.0-4.5, P_{trend} = 0.06). Men with high levels of IGFBP-3 showed only a minimal decrease in risk after adjustment for IGF-I. The association with IGF-I was less evident for advanced adenomas. Fasting and 2-h glucose and BMI were more strongly positively associated with colorectal adenomas than IGF-I, especially with advanced adenomas, independently of IGF-I or IGFBP-3. The authors concluded that plasma IGF-I and IGFBP-3 may be involved in colorectal tumorigenesis regardless of the stage in growth of the adenoma, but not as a mediator for the effects of being overweight or of hyperglycaemia.

27. In a case-control study by Keku *et al*. (2005) 239 adults with one or more adenomas were compared with 517 adenoma-free controls. The participants were drawn from patients undergoing colonoscopy. A number of serum parameters were analysed and biopsy samples were assessed for apoptosis. Higher insulin levels were associated with an increased risk of adenoma (highest two vs lowest quartiles) and lower levels of apoptosis. There were no differences in the circulating levels of glucose, IGF-I, IGF-II or IGFBP-3 between and adenoma cases and controls (mean ± SEM 121.4 ± 4.8 and 130.7 ± 3.9 ng/ml for IGF-I and 3,177 ± 80 and 3,255 ± 51 ng/ml for IGF-I and IGFBP-3 in cases and controls respectively). However, when considered separately, IGF-I levels were lower in male case compared to controls (126.6 ± 5.7 and 145.8 ± 6.3 respectively). No association was found between these variables and apoptosis.

28. Keku *et al*. (2008) investigated the expression of tissue and plasma IGFBP-3 in 580 patients (64 cases, 416 controls) undergoing colonoscopy. Tissue IGFBP-3 mRNA was measured in colon biopsy samples. A modest correlation was observed between tissue and plasma IGFBP-3 and plasma IGFBP-3 was not associated with an increased risk of adenoma (adjusted or unadjusted model) or with apoptosis. Tissue IGFBP-3 mRNA was significantly lower in cases than controls (1.13 and 1.28 (units unclear) respectively, $p = 0.03$). Subjects in the lower three quartiles of tissue IGFBP-3 expression were more likely to have adenomas (OR;95%CI for lowest vs highest in unadjusted model 1.6 (0.9-2.7) *p* trend 0.07); this was not affected by adjustment for waist/hip ratio, alcohol intake, sex and total calcium and the ORs were comparable when comparing the highest quartiles to any of the others. Low levels of apoptosis were significantly associated with increased risk of adenoma, but local IGFBP-3 expression was inversely associated with apoptosis. It was suggested that local IGFBP-in the colon may directly influence adenoma risk but IGFBP-3 may act through a pathway other than apoptosis to influence adenoma risk.

29. Similarly, high plasma C-peptide and low IGFBP-1 levels were associated with an increased risk of colorectal adenoma in a case-control study of C-peptide, a marker of insulin secretion, and IGF hormones in participants with pathologically-confirmed first-time adenoma (Le Marchand *et al*., 2010). The study compared 554 cases with 786 controls with normal endoscopy in a population of Caucasian, Japanese and Native Hawaiians. Unconditional logistic regression was used to establish OR;95% CI The association of C-peptide and IGFBP-I was not affected by other risk factors. Levels of IGF-I and IGFBP-3, BMI, and waist and hip circumference were not independently associated with adenoma risk. The adenoma cases were identified in the baseline screening exam at the Hawaii site of the Prostate, Lung, Colorectal Ovarian cancer screening trial (PLCO) cohort. There were a number of differences in consumption of different types of food between the cases and controls, with processed meat consumption being higher in cases, and calcium, fibre from vegetables and folate consumption being higher in controls.

30. In a study of 125 individuals with colorectal cancer, levels of IGF-I were significantly higher in individuals with locally advanced cancer compared to less advanced cancers (Kukliǹski *et al*., 2011). Levels were also higher in patients who were male, aged over 60 or who had mucigenous colorectal cancers.

31. In a study of Chinese patients, Serum IGF-I and mucosal IGF-I mRNA levels were higher in patients with adenomatous polyps (n=24) or carcinoma (n=13) compared to healthy controls (Zhang *et al*., 2013). The levels were 200.96, 218.77 and 98.37 ng/ml respectively. There was also a significant correlation between serum IGF-I and mucosal IGF-I mRNA in patients with adenomatous polyps. Since IGF-I was increased in patients with polyps as well as those with cancer it was proposed that IGF-I might be important in the development of both benign and malignant lesions and could be involved in malignant transformation.

32. The association between insulin and related growth factors and recurrent adenomas was investigated by Kang *et al*. (2013). The analysis included 167 individuals from the Diet and Health Study cohort with one or more adenomas detected at a baseline colonoscopy who had a subsequent surveillance colonoscopy; blood samples were taken at baseline. No significant associations were found between insulin, glucose, IGF-I (OR; 95%CI= 0.7; 0.3-1.5), IGF-II (1.0; 0.5-2.3), IGFBP-3 (1.0; 0.5-2.1) or anthropometric measures and recurrent adenomas.

33. In a cross-sectional study by Erarslan *et al*. (2014) 48 Turkish patients with either colorectal carcinoma or adenoma were compared with 30 matched controls. Serum IGF-I levels were in higher in the patients with carcinoma or adenoma compared to the controls. IGF-I levels (ng/ml, mean ± SD) were higher in female carcinoma patients 198.25 ± 70.20 compared to adenoma patients 167.63 ± 108.94 but higher in male adenoma patients 176.15 ± 56.94 compared to carcinoma patients 183.43 ± 72.58. The levels in male and female controls were 102.21 ± 45.50 and 120.45 ± 44.59 in females and males respectively.

34. In a study by Comstock *et al*. (2014) into colon polyps and insulin-related serum factors, 126 asymptomatic men were recruited at colonoscopy and blood samples taken for the analysis of a number of serum factors including IGF-I and IGFBPs 1 to 7. No associations were found between polyp number (0, 1, 2, or ≥ 3) and type (no polyps, hyperplastic polyps or tubular adenoma) and IGF-I, IGFBP-3, IGFBP7 or IGF-I/IGFBP-3 ratio (it is noted that no other associations were found, but not all the data were presented).

35. In a study by Ochs-Balcom *et al*. (2014) (see also para 11) IGF-I, IGFBP-1 and IGFBP-3, and SNPs in the IGF-I receptor (*IGF1R*), IGF-II receptor (*IGF2R*) and insulin receptor genes were with measured in 1572 individuals who were scheduled for routine colonoscopy. Of these, 432 were incident adenoma cases and 1139 were adenoma free controls. The IGF-I levels were higher in Caucasians compared to African-Americans for both cases and controls, with similar differences being apparent for IGFBP3. Logistic regression models revealed a statistically significant association between IGF-I and cancer in African-Americans only with OR; 95% CIs of 1.68; 1.06-2.68 and 1.68; 1.05-2.71 respectively for the second and third tertile compared to the first. In Caucasians, cases had higher IGF-I levels than controls. No associations were observed between IGF-I/IGFBP ratio and colorectal adenoma. Covariates controlled for included age, sex, family history of colorectal cancer, smoking, NSAID use and BMI. No associations were found between the biomarkers and advanced adenoma, but it was noted that the sample size was small. The

authors suggested that the results indicated an ethnic difference in the associations of IGF pathway biomarkers and the risk of adenomas that warranted further study

Prospective studies

36. Ma, *et al*. (1999 & 2001) performed a nested case-control study within the Physicians' Health Study cohort (a total of 22,071 healthy men aged 40 to 84 years in 1982 with blood samples available from 14,916 of the men), using prospectively collected plasma from 193 men within the cohort who had developed colorectal cancer in the following 13 years and 318 age- and smoking-matched control men. The risk of colorectal cancer was compared with dietary factors and with initial plasma levels of IGF-I, IGF-II and IGFBP-3. IGF-II was not associated with risk of colorectal cancer. After controlling for IGFBP-3, age, smoking, body mass index (BMI) and alcohol intake, men in the highest quintile of plasma IGF-I levels had an increased risk of colorectal cancer compared with the lowest quintile (relative risk = 2.51) – see Table 2 below. After controlling for IGF-I and the other co-variants, men with higher plasma IGFBP-3 had a lower risk (relative risk = 0.28, comparing extreme quintiles). The risk was highest in those with high initial ratio of plasma IGF-I to plasma IGFBP-3 – see Tables 3 and 4. The authors concluded that men with a high IGF-I to IGFBP-3 ratio have an increased risk of colorectal cancer. The associations between colorectal cancer and dietary factors were described in the section entitled "Influence of diet on IGF-I levels and cancer risk; Studies in humans".

Table 2: Relative risks (RR) of colorectal cancer in subjects with different initial plasma levels of IGF-I and of IGFBP-3 (Ma, *et al*., 1999 & 2001)

	Quintile					**Quintile 5 95% CI**	**P-value for trend***
	1 (referent)	**2**	**3**	**4**	**5**		
IGF-I							
Case/control	33/63	50/64	36/64	40/64	34/63		
Mean (ng/mL)	115	156	183	212	273		
RR¥	1	1.68	1.30	1.51	1.36	0.72-2.55	0.51
IGFBP-3							
Case/control	44/63	49/64	41/64	41/64	18/63		
Mean (ng/mL)	2161	2660	2996	3395	3984		
RR¥	1	1.26	1.03	1.04	0.47	0.23-0.95	0.07
IGF-I and IGFBP-3							
RR for IGF-I §	1	1,89	1.66	2.14	2.51	1.15-5.46	0.02
RR for IGFBP-3 §	1	1.08	0.77	0.69	0.28	0.12-0.66	0.005

* All p-values are two-sided.
¥Adjusted for age, smoking, body mass index and alcohol intake.
§Adjusted for age, smoking, body mass index, alcohol intake and IGF-I or IGFBP-3.

Table 3: Relative risks (RR)¥ of colorectal cancer in relation to initial serum levels of both IGF-I and IGFBP-3 (Ma, *et al*., 1999 & 2001)

	IGFBP-3		
IGF-I	**Tertile 1**	**Tertile 2**	**Tertile 3**
Tertile 1			
RR	1	2.24	0.87
95% confidence interval	referent	1.10-4.56	0.21-3.57
No. case subjects/No control subjects	43/72	28/25	4/9
Tertile 2			
RR	1.54	1.30	0.50
95% confidence interval	0.78-3.07	0.67-2.53	0.21-1.19
No. case subjects/No control subjects	21/30	27/44	9/32
Tertile 3			
RR	4.15	1.61	0.94
95% confidence interval	1.13-15.19	0.81-3.17	0.49-1.80
No. case subjects/No control subjects	8/4	25/37	28/65

¥ Adjusted for age, smoking, body mass index and alcohol intake.

Table 4: Relative risks (RR)¥ of colorectal cancer in relation to molar ratio of plasma IGF-I to plasma IGFBP-3 at the beginning of the study (Ma, *et al*., 1999 & 2001)

	IGF-I/IGFBP-3 (ng/mL)			***P*trend**
	Tertile 1	**Tertile 2**	**Tertile 3**	
Median	0.18	0.22	0.27	
No case subjects /No control subjects	50/98	54/100	77/98	
RR	1	1.21	1.84	0.01
95% confidence interval	Referent	0.73-1.98	1.12-3.01	

¥ Adjusted for age, smoking, body mass index, alcohol intake, multivitamin use, aspirin use, exercise and intake of skim/low fat milk.

37. Giovannucci, *et al*. (2000) reported the results of a prospective case-control study nested within the Nurses' Health Study (NHS) cohort of United States female nurses. Between 1989 and 1990, blood samples were collected from 32,826 of the NHS participants, aged between 35 and 55 years. For eligibility as a case or control in this study, cohort members must have supplied a blood sample, must have been free of cancer (other than non-melanoma skin cancers) prior to supplying the blood sample and must have undergone sigmoidoscopy or colonoscopy after giving the blood sample but prior to 1st June 1994. Seventy-nine new cases of adenocarcinoma of the colon or rectum were identified and matched with 158 controls from the cohort. Ninety cases of intermediate/late stage adenoma and 107 cases of early-stage adenoma were matched with equal numbers of controls. Controls were matched to each case on the basis of age, fasting status when giving blood, and the month of blood sampling. Plasma levels of IGF-I and IGFBP-3 were measured in the prospectively collected samples. The relative risks of development of the different tumour types were estimated for each tertile of the distributions of levels of IGF-I and IGFBP-3. When the data were adjusted for alcohol intake and BMI and IGF-I and IGFBP-3 were adjusted for each other, there were statistically significant trends ($p \leq 0.005$) for decreased risks of colorectal cancer and intermediate/late-stage

adenoma with increasing plasma levels of IGFBP-3, but there were no significant (p>0.05) dose-related trends associated with IGF-I levels. Comparison of the highest and lowest tertiles showed a RR of 1.21 associating IGF-I with colorectal cancer risk (although there was a slightly decreased risk [RR = 0.91] in the middle tertile).

38. Kaaks, *et al*. (2000) performed a case-control study nested within a cohort of 14,275 women in New York City of ages 35-65 years. Blood samples had been obtained from participants between March 1985 and June 1991. Serum from 102 women who subsequently developed colorectal cancer and from 200 cancer-free controls from the cohort was analysed for C-peptide (a marker for insulin secretion), IGF-I and IGFBP-1, -2 and -3. The controls were matched with the cases on the basis of menopausal status, age, date of recruitment, and time of day of blood collection. Logistic analysis was used to relate cancer risk to the levels of the various peptides measured in serum. Comparison of the risks associated with different quintiles of serum levels, there were statistically significant (p<0.05) positive trends for increased risks of colorectal cancer associated with increasing levels of C-peptide and decreasing levels of IGFBP-1, with respective ORs of 2.92 and 0.48 when the lowest quintile was compared with the highest (see Table 5). Positive trends for increased risk with increasing levels of IGF-I and IGFBP-3 were not statistically significant (p>0.05).

Table 5: Odds ratios (ORs) for colorectal cancer for quintiles of serum levels of C-peptide, IGF-I and IGFBPs (Kaaks, *et al*., 2000)

	Quintile					*p* for trend
	1	2	3	4	5	
C-peptide	1.00	0.56	1.92	1.26	2.92	0.001
IGF-I	1.00	1.49	1.30	1.52	1.88	0.25
IGFBP-1	1.00	0.57	0.43	0.30	0.48	0.02
IGFBP-2	1.00	1.00	1.12	0.93	0.38	0.06
IGFBP-3	1.00	1.70	1.15	0.92	2.46	0.19

39. A study of the risk of colorectal cancer associated with serum levels of IGF-I, IGF-II and IGFBP-3 was performed on subjects from a cohort of 18,244 Chinese men aged 45-65 living in Shanghai (Probst-Hensch, *et al.,* 2001). Blood serum samples were taken from men in the cohort between 1986 and 1989. Concentrations of IGF-I, IGF-II and IGFBP-3 were measured in serum samples from 125 men from the cohort who developed colorectal cancer in the 12 years following the blood sampling and from 661 controls from the cohort, who were matched for neighbourhood of residence, age, and time of sample collection. Comparison of quintiles of serum levels showed that IGF-I had no statistically significant (p<0.05) effect on the risk of colorectal cancer (OR of the highest quintile as compared with the lowest = 1.52; 95% CI = 0.82-2.85). However, IGF-II and IGFBP-2 exhibited significant positive associations with colorectal cancer risk when cases were confined to those diagnosed within 8 years of enrolment (OR for highest *vs.* lowest quintile = 2.74 and 2.85, respectively).

40. The association of colon cancer risk with pre-diagnostic plasma levels of IGF-I and IGFBP-3 was investigated in a case-control study nested within the Northern Sweden Health and Disease Cohort (Palmqvist, *et al.,* 2002). The participants were aged 30 to 70 years on recruitment. Concentrations of IGF-I and IGFBP-3 were

measured in pre-diagnostic plasma from 168 men and women who had developed cancer of the colon (n=110) or rectum (n=580 and from 336 controls selected from the cohort, matched for sex, age, sub-cohort, date of blood sampling and fasting time. Conditional logistic regression analyses showed increases in colon cancer risk with increasing levels of IGF-I and of IGFBP-3 (see Table 6). There were also decreases in rectal cancer risks with increasing levels of IGF-I and of IGFBP-3 (also in Table 6). None of the trends were statistically significant ($p<0.05$).

Table 6: Odds ratios of colorectal cancers for quartiles of plasma IGF-I and IGFBP-3 (Palmqvist, *et al.,* 2002)

	Quartile				p_{trend}
	1	2	3	4	
IGF-I adjusted for IGFBP-3					
Colorectum					
OR	1.00	1.18	1.18	1.27	0.56
95% CI		0.65-2.14	0.60-2.29	0.62-2.63	
Colon					
OR	1.00	1.81	2.16	2.47	0.08
95% CI		0.83-3.98	0.91-5.14	0.93-6.53	
Rectum					
OR	1.00	0.50	0.38	0.43	0.23
95% CI		0.15-1.69	0.10-1.41	0.11-1.59	
IGFBP-3 adjusted for IGF-I					
Colorectum					
OR	1.00	0.82	1.31	1.32	0.20
95% CI		0.44-1.54	0.68-2.54	0.66-2.67	
Colon					
OR	1.00	0.86	1.70	1.75	0.07
95% CI		0.39-1.91	0.73-3.94	0.72-4.22	
Rectum					
OR	1.00	0.79	0.81	0.58	0.45
95% CI		0.27-2.30	0.25-2.66	0.16-2.16	

41. Wei, *et al.* (2005) conducted a prospective nested case-control study among the 32,826 American women, aged 35-55 years, of the Nurses' Health Study who had provided a blood sample in 1989 in 1990. After excluding diabetics, 182 incident colorectal cancer cases were identified over 10 years of follow-up. Each case was matched to two controls on the basis of year of birth, date of blood collection and fasting status. Plasma concentrations C-peptide, glycosylated haemoglobin (HbA1c), IGF-I, IGFBP-1 and IGFBP-3 were measured. C-peptide levels were weakly associated with colon cancer: top quartile *versus* bottom quartile multivariable relative risk (MVRR) = 1.76, 95% CI = 0.85-3.63. Plasma levels of HbA1c were not associated with cancers of the colon or colorectum. There was a statistically significant (p=0.03) trend for higher relative risk of colon cancer with higher plasma levels of IGF-1, but only when the data were adjusted for plasma IGFBP-1 concentrations (see Table 7). IGFBP-1 was inversely associated with risk of colon cancer: MVRR = 0.28, 85% CI = 0.11-0.75. IGFBP-3 was not associated with cancers of the colon or colorectum, but there was a statistically significant (p=0.01) trend for higher relative risk of colon cancer with higher plasma IGF-I/IGFBP-3 ratio (see Table 7).

Table 7: Relationship between plasma levels of IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio and risks of cancers of the colon (Wei, *et al*., 2005)

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
IGF-1					
Median (ng/mL)	95	133	170	230	
Cases/Controls	26/69	31/62	33/66	44/65	
RR* (95% CI)	1.00	1.14 (0.59-2.20)	1.38 (0.71-2.71)	1.95 (0.97-3.91)	0.09
RR† (95% CI)	1.00	1.19 (0.60-2.37)	1.46 (0.07-3.08)	2.17 (0.96-4.88)	0.03
IGFBP-3					
Median (ng/mL)	2920	3823	4602	5443	
Cases/Controls	30/64	36/71	37/62	34/65	
RR* (95% CI)	1.00	1.08 (0.56-2.12)	1.36 (0.71-2.61)	1.20 (0.62-2.30)	0.62
RR† (95% CI)	1.00	0.85 (0.41-1.78)	1.04 (0.51-2.16)	0.81 (0.38-1.75)	0.12
IGF-I/IGFBP-3					
Median	0.09	0.12	0.14	0.18	
Cases/Controls	24/67	31/67	34/62	48/66	
RR† (95% CI)	1.00	1.88 (0.90-3.94)	2.33 (1.11-4.87)	2.82 (1.35-5.88)	0.01

* Adjusted for BMI, physical activity, pack-years smoked, and alcohol as continuous variables, family history of colorectal cancer, aspirin use, history of screening, menopausal status, and use of hormone replacement therapy.

† Adjusted for BMI, physical activity, pack-years smoked, and alcohol as continuous variables, family history of colorectal cancer, aspirin use, history of screening, menopausal status, use of hormone replacement therapy and IGF- or IGFBP-3.

42. Schoen *et al*. (2005) conducted a study of 458 asymptomatic individuals drawn from the PLCO cohort; of these 202 participants had colorectal adenomas, of which 70 were advanced. Compared to controls, levels of IGF-I, the ratio of IGF-I/IGFBP-3 and insulin were associated with adenomas, particularly advanced ones. In an unadjusted logistic regression analysis using sex-specific cut points, subjects in the top quartile of IGF-I, IGF-I/IGFBP and insulin had an increased risk of adenoma compared to those in the lowest quartile. The OR; 95%CIs were 1.7; 1.0-2.9, p_{trend} = 0.05, 1.9; 1.1-3.3, p_{trend} = 0.01, and 2.1;1.2-3.6, p_{trend} = 0.04 for IGF-I, IGF-I/IGFBP3 and insulin respectively. These findings were not significantly affected by adjustment for age, race, education, history of polyp, aspirin use, NSAID use, smoking or family history of CRC. When the case group was limited to advanced adenomas, the effect was more pronounced with ORs; 95%CIs of 2.8; 1.3-6.2, p_{trend} = 0.006, 2.3; 1.0-5.2, p_{trend} = 0.04, and 2.3; 1.1-49, p_{trend} = 0.14 for IGF-I, IGF-I/IGFBP3 and insulin respectively. Comparison of the non-advanced adenoma group to the controls did not demonstrate an association with IGF-I. Additional adjustments for BMI, visceral fat, insulin or IGF-I did not change the relationship between insulin or IGF-I and adenomas, nor did exclusion of diabetics or mutual adjustment of IGF-I and IGFBP-3 or mutual adjustment of IGF-I and insulin. The authors suggested that that insulin and IGF-I might stimulate non-advanced adenomas to become advanced. Or, the adenomas may result in increased levels of IGF-I and insulin.

43. In a nested case-control study among the BUPA cohort, IGF-I, IGF-II and IGFBP3 levels were measured in serum samples from 1051 UK men with cancer and 3142 controls matched for age and duration of sample storage (Morris *et al*., 2006). In the analysis for colorectal cancer risk, 147 cases and 440 controls were included. The maximum follow up period was 15 years. The median IGF-I level was 124 ng/ml in controls and 122 ng/ml in cases; the median IGFBP-3 level was 3.2 µg/ml in both. Associations with 14 cancers were considered in the study, including colorectal cancer; no associations were found between IGF-I, IGF-2 or IGFBP-3 and colorectal cancer (see Table 8). A meta-analysis of this cancer site was also conducted (see paragraph 57). In this analysis, IGF-I was associated with an increased risk (1.37; 1.05-1.7) of colorectal cancer.

Table 8: Relationship between plasma levels of IGF-I, IGF-II, and IGFBP-3 and risks of colorectal cancer (Morris, *et al*., 2006)

	Quartile				P_{trend}
	1	**2**	**3**	**4 (95%CI)**	
IGF-I	1.0	1.20	1.39	1.10 (0.56-2.18)	0.65
IGF-2	1.0	1.70	1.84	1.59 (0.67-3.75)	0.40
IGFBP3	1.0	0.90	1.06	0.72 (0.37-1.37)	0.46

Adjusted for age, smoking, alcohol and BMI

44. Otani, *et al*., (2007) investigated the association between plasma levels of C-peptide, IGF-I, IGFBP-1 or IGFBP-3 and the risk of colorectal cancer in a prospective nested case-control study. During an 11½-year follow-up, 375 newly diagnosed colorectal cancer cases were identified within a cohort of 38,373 adults (aged 40-69 years on recruitment) within the Japan Public Health Centre-Based Prospective Study (JPHC Study) who had returned a filled-in baseline questionnaire and provided a blood sample. Two matched controls from the cohort were selected for each case. The odds ratio of colorectal cancer for plasma levels of each protein was estimated using the conditional logistic regression model adjusted for potential confounding factors. There was an association of increased levels of C-peptide with increased risk of cancers of the colorectum and of the colon (but not of the rectum) in men, however, there was no such association in women. The other peptides (IGF-I, IGFBP-1 and IGFBP-3) were not associated with colorectal cancer (see Table 9).

Table 9: Odds Ratios (and 95% confidence intervals) of colon and rectal cancers for plasma levels of C-peptide, IGF-I, IGFBP-1 and IGFBP-3 (Otani, *et al*., 2007)

MEN:

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
Colon					
C-peptide	1.0	2.1 (0.96-4.6)	2.6 (1.0-6.3)	3.5 (1.2-10)	0.025
IGF-I	1.0	0.69 (0.33-1.4)	0.73 (0.33-1.6)	0.82 (0.36-1.9)	0.89
IGFBP-1	1.0	1.5 (0.75-3.1)	1.3 (0.57-3.0)	1.6 (0.62-4.0)	0.47
IGFBP-3	1.0	1.6 (0.74-3.3)	1.4 (0.64-3.1)	1.6 (0.67-3.7)	0.41
Rectum					
C-peptide	1.0	1.8 (0.40-8.0)	3.8 (0.83-18)	2.2 (0.47-10)	0.24
IGF-I	1.0	0.74 (0.20-2.8)	1.1 (0.25-5.1)	0.96 (0.16-5.7)	0.92
IGFBP-1	1.0	0.86 (0.16-2.8)	0.21 (0.045-0.94)	0.30 (0.053-1.7)	0.056
IGFBP-3	1.0	1.0 (0.31-3.4)	0.98 (0.21-4.7)	0.89 (0.19-4.2)	0.82

WOMEN:

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
Colon					
C-peptide	1.0	0.65 (0.29-1.4)	0.92 (0.41-2.0)	0.72 (0.28-1.8)	0.54
IGF-I	1.0	0.99 (0.48-2.1)	1.1 (0.48-2.5)	0.64 (0.24-1.7)	0.43
IGFBP-1	1.0	1.2 (0.57-2.5)	0.73 (0.31-1.7)	1.3 (0.47-3.5)	0.95
IGFBP-3	1.0	1.3 (0.61-2.7)	0.88 (0.37-2.1)	1.4 (0.54-3.5)	0.52
Rectum					
C-peptide	1.0	0.88 (0.34-2.3)	0.46 (0.14-1.5)	0.76 (0.23-2.5)	0.82
IGF-I	1.0	1.1 (0.40-2.9)	1.4 (0.39-5.2)	1.4 (0.31-6.0)	0.75
IGFBP-1	1.0	0.95 (0.37-2.5)	0.28 (0.075-1.1)	0.79 (0.21-2.9)	0.45
IGFBP-3	1.0	0.83 (0.32-2.2)	0.67 (0.20-2.2)	0.77 (0.20-3.0)	0.73

45. Gunter, *et al*. (2008) conducted a nested case-cohort investigation of colorectal cancer among non-diabetic subjects enrolled in the Women's Health Initiative Observational Study, a prospective cohort of 93,676 post-menopausal women. Fasting baseline serum specimens from all incident colorectal cancer cases (n=438) and a random sub-cohort (n=816) of subjects from the large cohort were analysed for insulin, glucose, total IGF-I, free IGF-I, IGFBP-3 and oestradiol. Comparing extreme quartiles, insulin (hazard ratio (HR); 95% CI = 1.73; 1.16-2.57, p_{trend} = 0.005), waist circumference (1.82;1.22-2.70, p_{trend} = 0.001), and free IGF-1

(1.35; 0.92-1.98, p_{trend} = 0.05) were each associated with colorectal cancer incidence in multivariate models. However, these associations each became non-significant when adjusted for one-another. Oestradiol levels, in contrast, were positively associated with risk of colorectal cancer (1.53; 1.05-2.22) even after control for insulin, free IGF-I and waist circumference. The authors suggested the existence of at least two independent biological pathways that are related to colorectal cancer: one that involves endogenous oestradiol and a second broadly associated with obesity, hyperinsulinaemia and free IGF-I.

46. No associations between colorectal cancer risk and circulating levels of IGF-I, IGFBP-3 and IGF-I/IGFBP-2 ratio were found in a case-control study nested in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (Max, *et al*., 2008). In the main study, 29133 Finnish male cigarette smokers, aged 50-69, had been recruited between 1985 and 1988. Serum samples were taken at the time of recruitment. Incident cancer cases were identified through the Finnish Cancer Registry: 134 cases of colorectal cancer had been diagnosed by 31st December 1997 (range = 5-12 years; median= 9 years after serum sampling). A sub-cohort of 400 cancer-free control subjects was randomly selected from survivors of the first 5 years of follow up. The serum-based biomarkers (IGF-I, IGFBP-3 and IGF-I/IGFBP-2 ratio) were not significantly associated with incident colorectal cancer overall or by anatomic subsite (proximal and distal). BMI, total energy intake, alcohol intake and physical activity level did not modify the risk estimates ($P_{interaction}$ > 0.05 for each comparison).

47. The levels of IGF-I and IGFBP-3 were measured in 375 subjects with and 375 subjects without a recurrent adenoma during the course of a polyp prevention trial to determine their baseline concentrations (Flood *et al*., 2008). To estimate the relative risk of adenoma recurrence over the course of 4 years of follow up for each of these serum measured, ORs and 95% CIs were calculated using multivariable logistic regression modelling adjusting for age, gender, body mass index, intervention group, aspirin, smoking, ethnicity, and education. For both IGF-I and IGFBP-3, risk was decreased in the highest compared with the lowest quartile (for IGF-I: CI; 95%CI was 0.65; 0.41-1.01 (*p* trend = 0.02) and for IGFBP3 0.66; 0.42-1.05 (*p* trend = 0.14)). The associations were greater for advanced adenomas (for IGF-I: 0.51; 0.1-1.29 (*p* trend = 0.09) and for IGFBP3 0.32; 0.13-0.82(*p* trend = 0.04)). The results were considered unexpected since previous studies had generally reported an increased risk of disease with high levels of IGF-I. A number of possible explanations were considered, including that cachexia and under nourishment were common and thus might depress IGF-I levels, resulting in an inverse association even when IGF-I had acted as a promoter of disease at an earlier stage. Since the Polyp Prevention Trial was a recurrence study, advanced neoplasia could have existed at the qualifying stage which would have affected IGF-I at baseline. Patients with advanced cancer at the qualifying stage would also be more likely to have recurrent adenoma. It was noted, however, that this was only theoretical since none of the patients had advanced cancer. The possible effect of IGF-I on tumour recurrence being via insulin or the inflammatory pathway was also discussed. Adjusting the IGF-I and IGFBP-3 results for each other did not affect the individual associations with tumour recurrence.

48. Jacobs *et al*. 2008 reported that IGF-I levels were inversely associated with the recurrence of colorectal adenoma. In this study, plasma levels of IGF-I, IGFBP-1 and IGFBP3 were measured at baseline in 299 male participants of the Wheat Bran Fibre Trial who were then followed prospectively for recurrence of their adenomatous lesions. In cross-sectional analyses, plasma IGF-I was significantly positively associated with the presence of any adenomas with villous features [advanced] p= 0.04. In contrast, IGF-I levels were inversely associated with colorectal adenoma recurrence, with adjusted OR;95%CI of 0.55;0.29-1.01 and 0.49;0.26-0.91 for the second and third tertiles of IGF-I respectively compared with the first tertile (p_{trend} = 0.02). The inverse association was stronger for advanced adenoma recurrence than for non-advanced recurrence. It was concluded that once an adenoma was removed, high IGF-I levels reduced the odds of the formation of new lesions in the colorectum. No significant associations were observed for IGFBP-1, IGFBP-3 or IGF-I/IGFBP-3. In the discussion, it was suggested that IGF-I might have different properties in the presence of tumour-associated defects in signalling, noting the dual role of IGF-I proposed by Ewton *et al*. (2002). IGF-I levels were similar in the normal and overweight men, suggesting that IGF-I was not the mediator of the increased risk of colorectal cancer associated with increasing body size.

49. The association between colorectal cancer risk with serum IGF-I, total IGFBP-3 and intact IGFBP-3 was investigated in a large case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Rinaldi, *et al*., 2010). Between 1992 and 1998, blood samples were taken prospectively from participants from eight European countries. Those who developed cancer by December 2002 were identified from national cancer registries. Investigators compared 1,121 cases of colorectal cancer with 1,121 matched controls. Relative risks (RR) for colon and rectal cancers and 95% confidence intervals (CI) were calculated in relation to quintile categories serum IGF-I concentrations by conditional logistic regression. Possible confounders that were considered for to use for adjustment included body mass index, ratio of waist to hip circumference, height, smoking status, education, physical activity, alcohol intake and dietary intakes of red meat, processed meat, dairy products, fruit, vegetables and fibre. The results showed no associations with risk of colorectal cancer overall. Sub-group analyses showed some moderate positive associations of IGF-I levels with risk: in younger participants (less than 55 years-old) for colon cancer only (RR per quintile increase = 1.18; 95% CI = 1.00-1.39) and among participants whose milk intake was in the lowest tertile of the population distribution (RR for an increase in serum IGF-I of 100 ng/mL = 1.43; 95% CI = 1.13-1.93). There were no statistically significant ($p>0.05$) increases in colorectal cancer risk for an increase of 100 ng/ml of serum IGF-I associated with dietary intakes of dairy calcium, non-dairy calcium, dairy proteins, non-dairy proteins, red and processed meat, red and processed meat plus poultry and fish, fruit and vegetables, and fibre. Neither total IGFBP-3 nor intact IGFBP-3 were associated with risk of colorectal cancer with colon or rectal cancers separately.

50. In a nested case control study combining data from the Health Professionals Follow Up study (HPFS) and the Nurses Health study cohort, additive and multiplicative interactions were examined between baseline plasma 25 hydroxyvitamin D (25(OH)D) and IGF-I, IGFBP-3 as well as C peptide levels in 499 cases and 992 matched controls (Wu *et al*., 2011). The cases included were

diagnosed after the date of blood draw 1993-5 up to 2002 (HPFS) and 1989-1990 up to 2008 (NHS). The analytes were deemed high or low depending on whether they were above or below the median. Compared to participants with high 25(OH)D and low IGF-I/IGFBP3, participants were at elevated risk of colorectal cancer when 25(OH)D was low (OR; 95%CI, 2.05; 1.43-2.92) but not when 25(OH)D was high (1.20; 0.84-1.71). Similarly, compared to participants with high 25(OH)D and low IGF-I/IGFBP3 and low c-peptide levels (reference group). Having a high c-peptide and IGF-I/IGFBP3 ratio did not increase risk further. The authors concluded that improving vitamin D status may help lower the risk of colorectal cancer associated with higher IGF-I/IGFBP-3 ratios and C-peptide levels.

51. A nested case control study was conducted in the PLCO cohort of adults aged 55-74 at baseline to test whether IGF-I, IGF-II and IGFBP-3 levels were associated with an increased risk of colorectal adenoma as assessed at baseline (Gao *et al*., 2012). A total of 764 advanced, left-sided colorectal adenoma cases and 775 controls frequency matched for ethnicity and gender and without evidence of a left sided polyp on sigmoidoscopy were included in the study. Logistic regression was used to estimate the Odds Ratios (ORs) and 95%CI for the association adjusting for age, race, sex, year of blood draw, BMI, smoking and education. Higher IGF-I levels were associated with increased adenoma risk. IGF-II levels were also associated with increased risk for the first vs fourth quartile but this association was no longer significant after adjustment for IGF-I. IGFBP-3 levels were not associated with risk.

Table 10: Odds Ratios (and 95% confidence intervals) of colon and rectal adenomas for plasma levels of IGF-I, IGF-II, IGFBP-3, IGF-I/IGFBP-3 and IGF-II/IGFBP-3 ratio (Gao, *et al*., 2012)

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
IGF-I	1.0	1.58 (1.16-2.16)	1.42 (1.04-1.93)	1.80 (1.30-2.47)	0.002
IGF-II	1.0	1.16 (0.86-1.57)	1.12 (0.82-1.53)	1.49 (1.09-2.02)	0.02
IGFBP3	1.0	1.06 (0.78-1.43)	1.20 (0.88-1.62)	1.32 (0.98-1.79)	0.05
IGF-I/ IGFBP3 molar ratio	1.0	1.12 (0.82-1.53)	1.17 (0.84-1.62)	1.49 (1.07-2.08)	0.02
IGF-II/ IGFBP3 molar ratio	1.0	1.15 (0.85-1.55)	1.28 (0.95-1.74)	1.34 (0.99-1.82)	0.04
(IGF-I + IGF-II)/ IGFBP3 molar ratio	1.0	1.22 (0.90-1.66)	1.34 (0.98-1.82)	1.50 (1.11-2.03)	0.007

52. Plasma concentrations of c-peptide, IGF-I, and IGFBP-1 and IGFBP-3 were measured in 1520 healthy Japanese volunteers who underwent total colonoscopy and the association between these biomarkers and colorectal adenoma was cross-sectionally investigated by gender (Yamaji *et al*., 2012). Unconditional logistic regression was used to estimate ORs and 95%CIs after adjustment for confounding. A positive association of C-peptide and IGF-I with colorectal carcinoma was observed in men as was an inverse association with IGFBP-1. In contrast, no

measurable association was found in women. The gender difference was significant for C-peptide (p_{trend}= 0.03) and "marginally significant" (p_{trend} = 0.14 and 0.12) for IGF-I and IGFBP3. The authors concluded that the insulin and IGF-I axis acted differently by gender at least in the early stages of neoplasia.

Table 11: Odds Ratios (and 95% confidence intervals) of colorectal adenoma for plasma levels of C-peptide, IGF-I, IGFBP-1 and IGFBP-3 (Yamaji, *et al*., 2012)

MEN:

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
C-peptide	1.0	2.04 (1.31-3.17)	1.89 (1.23-2.92)	2.62 (1.71-4.01)	<0.001
IGF-I	1.0	1.38 (0.92-2.08)	1.40 (0.92-2.13	1.63 (1.09-2.46)	0.02
IGFBP-1	1.0	0.89 (0.60-1.31)	0.85 (0.57-1.26)	0.49 (0.32-0.75)	0.002
IGFBP-3	1.0	1.36 (0.90-2.04)	1.46 (0.97-2.20)	1.42 (0.94-2.14)	0.10

WOMEN:

	Quartile				P_{trend}
	1	**2**	**3**	**4**	
C-peptide	1.0	0.72 (0.40-1.28)	0.87 (0.49-1.53)	0.98 (0.56-1.71)	0.84
IGF-I	1.0	0.85 (0.49-1.49)	0.92 (0.53-1.59)	0.79 (0.44-1.43)	0.52
IGFBP-1	1.0	1.22 (0.70-2.11)	0.88 (0.49-1.57)	1.05 (0.60-1.86)	0.86
IGFBP-3	1.0	1.27 (0.72-2.23)	0.88 (0.49-1.57)	1.31 (0.76-2.29)	0.58

Adjusted for age, screening period, and the duration if fasting, cigarette smoking, alcohol consumption, family history of CRC, NSAID use, height and energy intake. The associations were weakened by further adjustment for BMI and physical activity.

53. Soubry *et al*. (2012) compared the changes in the level of IGF-I and the ratio of IGF-I/IGFBP-3 over a decade to the presence of colorectal adenoma in 143 individuals aged 40-69 (of whom 24 were diagnosed as having adenomatous polyps) in the Insulin Resistance and Atherosclerosis Study (IRAS) cohort. Circulating IGF-I and IGF-I/IGFBP3 were measured at three time points within a ten year follow up period (1992-4, 1997-9 and 2002-4). The random error of circulating IGF-I measurements was estimated to be 12-17.6% for lower and higher values respectively, therefore change patterns were "no increase" (±15% and/or <15% from baseline and "ever increase" (at least one increase > 15%). Although the mean levels IGF-I and IGF-I/IGFBP-3 declined with age as expected, approximately 30% of the participants showed an increase of at least 15% ("ever increase") in one or both variables compared to baseline. Using logistic regression, a possible association between "ever increase" in IGF-I and IGF-I/IGFBP-3 and the presence of colorectal adenoma was reported: ORs were 3.81 (95%CI 1.30-10.8) and 2.83 (1.00-8.22) respectively. No association was found when analysing the actual levels at any time point. It was suggested that the increase may represent a disturbed GH/IGF-I homeostasis which could favour the development of pre-cancerous lesions such as

colorectal adenoma. Variables considered in the analysis included age, gender, study centre, BMI and a previous report of polyps. The stability of the associations were supported by sensitivity analyses, looking at including individuals with only 2 measurements, excluding those with hyperplasia and increasing the cut off point for "ever increase". The authors considered that the presence of adenomas was unlikely to influence IGF-I levels because although transformed cells could produce IGF-I it was unlikely to be present in sufficient quantity to influence circulating levels. The small sample was noted to be a possible limitation.

Mortality

54. A prospective observational study was performed among 373 patients with non-metastatic colorectal cancer nested within the NHS and HPFS cohorts (Wolpin *et al*., 2009). In these patients high circulating C-peptide and IGFBP-1 was associated with decreased mortality. However there were no associations between IGF-I or IGFBP-3 and mortality. It was stated by the authors that these are the only two components of the GH/IGF-I axis not closely associated with lifestyle factors.

55. A nested case control study set among the Japan Collaborating Cohort Study for Evaluation of Cancer Risk (the JACC study) was carried out by Suzuki *et al*., 2009. This investigated the associations between serum IGF-I, IGF-II and IGFBP-3 in a Japanese population. In the JACC study, 127,500 individuals were recruited at baseline, 110,792 were followed and blood samples were taken from 39,242 individuals. A total of 101 risk sets for colorectal cancer fatalities (1 case and 3 controls) were assessed using conditional logistic regression modelling adjusting for BMI, smoking, alcohol consumption and family history of colorectal cancer. The ORs and 95%CIs for colorectal cancer mortality among the highest tertiles of IGF-I, IGF-II and IGFBP3 compared to the lowest tertiles were 1.01; 0.49-2.10, 1.02; 0.55-1.91 and 1.22; 0.63-2.38 respectively. No linear trends were observed. The lack of any association was not altered by additional adjustment for mutual markers.

Meta-analyses

56. Renehan, *et al*. (2004) performed a meta-analysis as part of a systematic review. Databases were searched for epidemiological studies published between January 1996 and December 2002 that considered the associations of circulating concentrations of IGF-I and of IGFBP-3 with the risks of cancers of the prostate, colorectum, pre-menopausal breast, post-menopausal breast and lung. On considering the association between IGF-I and the risk of colorectal cancer, the prospective studies of Ma, *et al*., 1999; Giovannucci, *et al*., 2000; Kaaks, *et al*., 2000; Probst-Hensch, *et al*., 2001 and Palmqvist, *et al*., 2001 were evaluated. All of the studies had odds ratios of >1, and taken as a whole there was an odds ratio of 1.58, with a 95% confidence interval of 1.11-2.27. On considering the association between IGFBP-3 and the risk of colorectal cancer, there was an odds ratio of 0.77 (95% CI = 0.36-1.66). The two studies of US health professionals (Ma, *et al.,* 1999 and Giovannucci, *et al*., 2000) showed a decreased risk with increased levels of IGFBP-3 (OR = 0.28; 95% CI = 0.15-0.54), but the other studies showed an increased risk (OR = 1.44; 95% CI = 0.93-2.23). The authors concluded that IGF-I levels were positively associated with colorectal cancer, whereas IGFBP-3 and IGF-I/IGFBP-3 molar ratio were less clearly associated.

57. Morris *et al*., (2006) performed a meta-analysis on the association between IGF-I, IGF-II and IGFBP and a number of cancer sites including colorectal cancer. The studies included were Giovannucci, *et al*., 2000; Palmqvist, *et al.,* 2002; Kaaks, *et al*., 2000; Hunt *et al.,* 2002; Probst-Hensch, *et al.,* 2001; Ma, *et al*., 1999; Nomura *et al*., 2002 as well as the data from their own study. The meta-analysis used a random effects model, including nested case-control studies and comparing the highest quartile and the lowest quartiles. Publication bias was assessed by examining the funnel plot for each site and testing whether the regression of the study estimate with the study precision was significant. Sources of heterogeneity (year of publication, type of sample, time from sample collection to diagnosis and study location) were assessed with sub-group analysis. There was no evidence of publication bias. There was a positive association between IGF-I and colorectal cancer (OR=1.37; 95%CI 1.05-1.78) (though this was not significant; *p*=0.68) and no evidence of heterogeneity between studies; a similar pattern was observed for IGF-II. For IGFBP-3 there was no association (0.98: 0.64-1.51) but there was evidence of heterogeneity which was not explained by any of the sub-group analyses.

58. Rinaldi, *et al*. (2010) performed a meta-analysis of ten prospective studies (Ma, *et al*., 1999; Manousos *et al*., 1999; Giovannucci, *et al*., 2000; Kaaks, *et al*., 2000; Probst-Hensch, *et al.,* 2001; Hunt *et al.,* 2001[5]; Nomura *et al*., 2002; Palmqvist, *et al.,* 2003; Wei, *et al.,* 2005; Otani, *et al*., 2007; Gunter, *et al*., 2008; Max, *et al*., 2008; Rinaldi, *et al*., 2010). All studies combined, there was a total of 2,862 case subjects and 4,966 control participants. When computing the meta-analysis following the Greenland and Longnecker method, there was a moderately positive association between IGF-I levels and risk of colorectal cancer, with a RR of 1.07 and a 95% CI of 1.01 – 1.14. Using the DerSimonian *et al*. method of meta-analysis, comparing highest *vs.* lowest reported categories of IGF-I levels, gave similar results, with a RR of 1.13 and a 95% CI of 0.97 – 1.32.

59. Chi *et al*. 2013 conducted a meta-analysis of 19 studies containing 5,155 cases and 9,420 controls. These comprised 16 prospective nested case control studies and 3 case control studies (Ma, *et al*., 1999; Giovannucci, *et al*., 2000; Kaaks, *et al*., 2000; Probst-Hensch, *et al.,* 2001; Palmqvist, *et al.,* 2002; Nomura *et al*., 2003; Wei, *et al.,* 2005; Morris *et al*., 2006; Jenab *et al*., 2007; Otani, *et al*., 2007; Tripkovic *et al*., 2007; Gunter, *et al*., 2008; Max, *et al*., 2008; Suzuki *et al*., 2009; Rinaldi, *et al*., 2010; Ollberding *et al*., 2012). The analysis used maximally adjusted OR comparing the highest with lowest categories as the principle effect measure. High level IGF-I was associated with an increased risk of CRC (OR 1.25; 95%CI 1.16-2.01). Sub group analysis showed that the increased cancer risk by IGF-I was more distinguished in colon cancer (1.35; 1.04-1.75) and colorectal cancer risk in Caucasians (1.32; 1.12-1.56).

60. In a recent meta-analysis (Yoon *et al*., 2015) a total of 12 studies were assessed (11 studies, 3038 cases for IGF-I, 12 studies and 3208 cases for IGFBP3

[5] Hunt et al. (2002) investigated whether IGF-2 was associated with colorectal cancer risk in women. As part of the study, IGF-I was also analysed. The study was a nested case control study including 102 cases and 200 matched controls from a cohort of 14,275 women in the New York University Women's Health Study. It has not been considered further in this review.

and 7 studies and 1867 cases for IGF-I/IGFBP-3). The studies used were: Comstock *et al*., 2014; Ochs-Balcom *et al*., 2014; Kang *et al*., 2013; Yamaji *et al*., 2012; Le Marchand *et al*., 2010; Flood *et al*., 2008; Jacobs *et al*., 2008; Keku *et al*., 2008; Keku *et al*., 2005; Schoen *et al*., 2005; Teramuki *et al*., 2002; Giovannucci *et al*., 2000. The summary ORs of occurrent colorectal adenoma for the highest vs lowest category of IGF-I, IGFBP3 and IGF-I/IGFBP3 ratio were 1.13; 0.95-1.34, 0.99; 0.84-1.16 and 1.05; 0.86-1.29 respectively indicating that there were no associations. Higher IGF-I levels and IGFBP3/IGF-I ratios were significantly associated with decreased risk of recurrent colorectal adenoma (0.60; 0.42-0.85 and 0.65; 0.44-0.96 respectively). A stratified analysis indicated that IGF was associated with advanced but not non-advanced colorectal adenoma (2.21; 1.08-4.52 and 0.89; 0.55-1.45). The finding that IGF-I levels and IGFBP3/IGF-I ratios were significantly associated with decreased risk of recurrent colorectal adenoma, in contrast with the generally accepted view of the stimulatory effect of IGF-I was considered. In addition to this being a chance finding, it was noted that IGF-I was mostly associated with advanced colorectal adenoma. Recurrence rates of adenoma were very high and the vast majority of the recurrent adenomas do not advance. Secondly, since the analysis of studies of recurrent adenoma is conditional on having occurrent adenoma, the comparison may implicitly be between the colorectal adenomas arising in causal pathways influenced by IGF-I and those arising from pathways not influenced by IGF-I (e.g. inflammation or insulin). In this case, high IGF-I may not be a direct causal protective factor, but may appear relatively protective if other pathways associated with the initial colorectal adenoma are associated with a higher rate of recurrence.

Summary and discussion: IGF-I and risk of colorectal cancer

61. Colorectal cancer is the fourth most common cancer in the UK. Risk factors include family, history, diet, smoking, obesity, alcohol and ionising radiation. Some examples of genetic polymorphism have been reported. It has been suggested that IGF-I may influence the occurrence of colorectal cancer through direct contact in the gut lumen (via ingestion) as well as by elevated blood levels. A number of mechanisms explaining the possible effect of IGF-I have been suggested. These include increased angiogenesis via VEGF or the inhibition of cytokine mediated apoptosis.

62. Patients with acromegaly and thus elevated GH and IGF-I levels are thought to have an increased risk of developing tumours of the gastrointestinal tract compared to normal subjects.

63. Studies comparing circulating serum or plasma IGF-I levels in patients with colorectal cancer and controls have reported both increased levels of IGF-I in the cancer patients and no difference between the two groups. Since cancers may produce their own growth factors, the results are difficult to interpret.

64. The results of the prospective studies investigating levels of IGF-I and colorectal cancer risk are also inconsistent. Some studies report an association between IGF-I and others report no association.

65. One explanation for this may be the different study designs and the range of potentially confounding factors that may influence the results. These may include issues such as age, diet and time from sample collection to diagnosis. Data on ethnicity are often absent which may be important if particular polymorphisms are relevant to IGF-I levels. The choice of assay may also be important since it is unclear to what extent active IGF-I is measured by the different procedures. IGF-I concentrations may be measured and reported as total or free IGF-I or this may not be specified. Some studies adjust the IGF-I results for IGFBP-3 and vice versa, and others present information on the IGF-I/IGFBP-3 molar ratio.

66. Several meta-analyses have also been performed. These also produced conflicting results, although were more generally positive.

67. Results for an association of colorectal cancer risk with IGFBP-3 are also inconsistent. It has been suggested that high IGFBP-3 is protective by taking free IGF-I out of circulation, but the results from the studies are inconsistent.

Secretariat
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REFERENCES

Akagi Y, Liu W, Zebrowski B, Xie K and Ellis LM, 1998, “Regulation of vascular endothelial growth factor expression in human colon cancer by insulin-like growth factor-I”, Cancer Res., 58: 4008-4014.

Bolfi, F., Miot, H.A., Resende, M., Mazeto, G.M., Romeiro, F.G., Yamashiro, F.D.S., Nunes, V.S. (2013). Frequency of various types of neoplasia in a group of acromegalic patients, Arg. Bras. Endcrinol. Metabol., 57, 612-617.

Cancer research UK (2015) http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/bowel-cancer#heading-Zero

Accessed November, 2015.

Cao, H., Wang, G., Meng, L., Shen, H., Feng, Z., Liu, G., Du J. (2012). Association between circulating levels of IGF-I and IGFBP-3 and lung cancer risk; A meta-analysis. PLOS one, 7, 1-8.

Cats A, Dullaart RP, Kleinbeuker J, Kuipers F, Sluiter W, Hardonk M, and de Vries EGE, 1996, “Increased epithelial cell proliferation in the colon of patients with acromegaly”, Cancer Res., 56: 523-526.

Chen W., Wang, S., Tian, T., Bai, J., Hu, Z., Xu, Y., Dong, J., Chen, F., Shen, H. (2009) Phenotypes and genotypes of Insulin-like Growth Factor 1, IGF-Binding Protein-3 and Cancer Risk, European Journal of Cancer Genetics, 17, 1668-1675.

Chi, F., Wu, R., Zeng, Y.C., Xing, R., Liu, Y. (2013). Circulation insulin-like growth factor peptides and colorectal cancer risk: an updated systematic review and meta-analysis. Mol. Biol. Rep, 40, 3583-3590.

Colao A, Balzano A, Ferone D, Panza N, Grande G, Marzullo P, Bove A, Iodice G, Merola B and Lombardi G, 1997, “Increased prevalence of colonic polyps and altered lymphocyte subset pattern in the colonic *lamina propria* in agromegaly”, Clin. Endocrinol. (Oxf.), 47: 23-28.

Comstock, S., Xu, D., Hortos, K., Kovan, B., McCaskey, S., Pathak, D., Fenton, J. (2014). Association of Insulin-Related Serum Factors with Colorectal Poly Number and Type in Adult Males. Cancer Epidemiology, Biomarkers and Prevention, 23, 1843-1851

Erarslan E., Coşkun, Türkay, C., Köktener, A., Aydoğan,T. (2014). IGF-I levels and visceral fat accumulation in colonic neoplasia. Clinics and Resrach in Hepatology and Gastroenterology, 38, 99-105.

Ewton D.Z., Kansra, S., Friedman E. (2002). Insulin-like Growth Factor-I has a biphasic effect on Colon Carcinoma cell through transient inactivation of forkhead1, initially mitogenic then mediating growth arrest and differentiation. Int J Cancer, 98, 665-673.

Flood, A., Mai V., Kahle, L., Rosen, C.J., Lanza, E., Schatzkin, A. (2008). Serum concentrations of insulin-like growth factor and insulin-like growth factor binding protein-3 and recurrent colorectal adenomas. Cancer Epidemiol Biomarkers Prev, 17, 1493-1498.

Freier S, Weiss O, Eran M, Flyvbjerg A, Dahan R, Nephesh I, et al., 1999, "Expression of the insulin-like growth factors and their receptors in adenocarcinma of the colon", Gut, 44: 704-708.

Gao Y, Katki H, Graubard B, Pollak M, Martin M, Tao Y, Schoen RE, Church T, Hayes RB, Greene MH, Berndt SI. Serum IGF1, IGF2 and IGFBP3 and risk of advanced colorectal adenoma. Int J Cancer. 2012 Jul 15;131(2): E105-113.

Giovannucci E, Pollak M, Platz EA, Willett WC, Stampfer MJ, Majeed N, Colditz GA, Speizer FE and Hankinson SE, 2000, "A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women", Cancer Epidemiol. Biomarkers Prev., 9: 345-349.

Gunter M.J., Hoover DR, Yu H, Wassertheil-Smoller S, Rohan T.E, Manson J.E, Howard, BV, Wylie-Rosett J, Anderson GI, Ho GY, Kaplan RC, Li J, et al., 2008, "Insulin, insulin-like growth factor-I, endogenous estradiol and risk of colorectal cancer in premenopausal women", Cancer Res., 68: 329-337.

Guo Y.S., Narayan S., Yallampalli C and Singh P, 1992, "Characterization of insulin-like growth factor-I receptors in human colon cancer", Gastroenterology, 102(4, part 1): 1101.

Hunt, K.J., Toniolo, P., Akhmedkhanov, A., Lukanova, A., Dechaus, H., Rinalsi, S., Zeleniuch-Jacquotte, A., Shore, R.E., Riboli, E., Kaaka, R. (2002). Insulin-like growth factor II and colorectal cancer risk in women. Cancer, Epidemiology and Biomarkers, 11, 901-905.

Jacobs, E.T., Martínez, M.E., Alberts, D.S., Ashbeck, E.L., Gapstur, S.M., Lance P., Thompson P.A., (2008) Plasma insulin-like growth factor I is inversely associated with colorectal adenoma recurrence: a novel hypothesis. Cancer Epidemiol Biomarkers Prev, 17, 300-305.

Jenab, M., Riboli, E., Cleveland RJ and multiple others (Epic). Serum C-Peptide, IGFBP-1 and IGFBP-2 and risk of Colon and Rectal Cancers in the European Prospective Investigation into Cancer and Nutrition. Int. J of Cancer, 121, 368-376.

Jenkins PJ, Fairclough PD, Richards T, Lowe DG, Monson J, Grossman A, Wass JAH and Besser M, 1997, "Acromegaly, colonic polyps and carcinoma", Clin. Endocrinol. (Oxf), 47: 17-22. .

Jenkins PJ, Frajese V, Jones AM, Camacho-Hubner C, Lowe DG, Fairclough PD, *et al*., 2000, "Insulin-like growthfactor-1 and the development of colorectal neoplasia in acromegaly", J. Clin. Endocrinol. Metab., 85(9): 3218-3221.

Juul A, Main K, Blum WF, Lindholm J, Ranke MB and Skakkabaek NE, 1994, “The ratio between serum levels of insulin-like growth factors (IGF)-I and the IGF binding proteins (IGFBP 1, -2 and -3) decreases with age in healthy adults and is increased in acromegalic patients”, Clin. Endrinol., 41: 85-93.

Kaaks R, Toniolo P, Akhemedkhanova A, Lukanova A, Biessy C, Dechaud H, Rinaldi S, Zeleiuch-Jacquotte A, Shore RE and Riboli E, 2000, “Serum C-peptide, insulin-like growth factor (IGF)-I, IGF-binding proteins and colorectal cancer risk in women”, J. Natl. Cancer Inst., 92(19): 1592-1600.

Kang, M., Peery, A., Locklear, C., Galanko, J., Sandler, R., Keku, O. (2013). Plasma, Insulin, Glucose, IGF-I, IGF-II, and IGFBP3 and risk of recurrent colorectal adenomas. J Gastroenterol. Hepatol. Res, 14, 531-535.

Kang, M., Peery, A., Locklear, C., Galanko, J., Sandler, R., Keku, O. (2013). Plasma, Insulin, Glucose, IGF-I, IGF-II, and IGFBP3 and risk of recurrent colorectal adenomas. J Gastroenterol. Hepatol. Res, 14, 531-535.

Karimi, K., Mahmoudi, T., Karimi, N., Dolatmoradi, H., Arkani, M., Farahani, H., Vahedi, M., Parsimehr, E., DAabiri, R., Nobakht, H., Asadi, A., Zali, M.R. (2013). Is there an association between variants in candidate insulin pathway genes IGF-I, IGFBP-3, INSR, IRS2 and risk of colorectal cancer in the Iranian population? Asian Pac. J. Cancer Prev. 14, 5011-5016.

Keku, T.O., Lund, P.K., Galanko, J., Simmons, J.G., Woosley, J.T., Sandler, R.S. (2005). Insulin Resistance, Apoptosis, and Colorectal Adenoma Risk, Cancer Epidemiology Biomarkers and Prevention, 14, 2076-

Keku, T.O., Sandler, R.S. Simmons, J.G., Galanko, J., Woosley, J.T., Proffitt, M., Omofoye, O., McDoom, M., Lund, P. (2008). Local IGFBP3 m RNA Expression, apoptosis and the Risk of Colorectal Cancer. BMC Cancer, 8, 143-152.

Kukliński, A., Kamocki, Z., Cepowicz, D., Gryko, M., Czyżewska, J., Pawlak K., Kędra, B., (2011). Relationships between insulin-like growth factor I and selected clinic-morphological parameters in colorectal cancer patients. Pol Przegl. Chir, 83, 250-7.

LeRoy, E.C., Moore, J.H., Hu, C., Martínez, M.E., Lance, P., Duggan, D., Thompson, P.A. (2011). Genes in the Insulin and Insulin-like Growth Factor Pathway and Odds of Metachronus Colorectal Neoplasia. Hum. Genet, 129, 503-512.

Lahm H, Suardet L, Laurent PL, Fischer JR, Cevhan A, Givel, JC, and Odartchenko N, 1992, “Growth regulation and co-stimulation of human colorectal cancer cell lines by insulin-like growth factor I, II and transforming growth factor alpha”, Brit. J. Cancer, 65: 341-346.

Lahm H, Amstad P, Wyniger J, Yilmaz A, Fischer JR, Ceyan A, Givel JC, et al., 1994, “Blockade of the insulin-like growth factor-I receptor inhibits growth of human colorectal cancer cells: evidence of a functional IGF-II-mediated atocrine loop”, Int. J. Cancer, 58: 452-459.

Le Marchand L, Donlon T, Seifried A, Kaaks R, Rinaldi S and Wilkens LR, 2002, “Association of a common polymorphism in the human GH1 gene with colorectal neoplasia”, J. Natl. Cancer Inst., 94(6): 454-460.

Li, Z-J., Ying, X-J., Chen, H-L., Ye, P-J., Chen, Z-L., Li, G., Jiang, H-F., Liu, J., Zhou, S-Z. (2013). Insulin-like growth factor-I induces lymphangiogenesis and facilities lymphatic metastasis in colorectal cancer. World Journal of Gastroenterology, 19, 7788-7794.

Lin J.S., Shen, M.Y., Lin, T.C., Wang, H.S., Yang, S.H., Li, A.F., Chang, S.C. (2010). Distribution of a single nucleotide polymorphism of insulin-like growth factor-1 in colorectal cancer patients and its association with mucinous adenocarcinoma. Int. J.Biol.Markers. 25, 195-9.

Ma J, Pollak M, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ, 1999, “Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3”, J. Natl. Cancer Inst., 96(7): 620-625.

Ma J., Giovannucci E, Pollak M, Chan JM, Gaziano JM, Willett W and Stampfer MJ, 2001, “Milk intake, circulating levels of IGF-1 and risk of colorectal cancer in men”, J. Natl. Cancer Inst., 93(17): 1330-1336.

Manousos, O., Souglakos, J., Bosetti C., Chatzidakis, V., Trichopoulos D., Adami H.O., Mantzaros, C. (1999) IGF-I and IGF-II in relation to colorectal cancer. Int J Cancer, 83, 15-17.

Max JB, Limburg PJ, Ogunseitan A, Stolzenberg-Solomon RZ, Vierkant RA, Pollak MJ, Sellers TA, Virtamo J, Cerhan JR and Albanes D, 2008, “IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio: no association with incident colorectal cancer in the alpha-tocopherol beta-carotene”, Cancer Epidemiol. Biomarkers Prev., 17: 1832-1834.

Nomura, A.M.Y., Stemmermann, G.N., Lee, J., Pollak, M.N. (2003). Serum insulin-like growth factor I and subsequent risk of colorectal cancer among Japanese-American men. American Journal of Epidemiology, 158, 424-431

Ochs-Balcom, H., Vaughn, C., Nie, J., Chen, Z., Thompson, C., Parekh, N., Tracy, R., Li, L. (2014). Racial Differences in the Association of Insulin-like Growth Factor Pathway and Colorectal Adenoma risk. Cancer Causes Control, 25: 161-170.

Olivio-Marston, S.E., Hursting, S.D., Lavigne, J., Perkins, S.N., Maarouf, R.S., Yakar, S., Harris, C.C. (2009). Genetic reduction of circulating Insulin–like Growth Factor-1 inhibits Azoxymethane-induced colon tumorigenesis in mice. Mol Carcino, 48, 1071-1076.

Ong, J., Saloman, J., te Morsche, R.H.M., Roelofs, H.M., Witteman, B.J.M., Dura, P., Lacko, M., Peters, W.H.M.. (2014). Polymophisms in the insulin-like growth factor axis are associated with gastrointestinal cancer. PLOS One, 9 (3) 890916.

Orme SM, McNally RJ, Cartwright RA and Belchetz PE, 1998, “Mortality and cancer incidence in acromegaly: a retrospective cohort study. United Kingdom Acromegaly Study Group”, J. Clin. Endocrinol. Metab., 83(8): 2730-2734.

Otani T, Iwasaki M, Sasazuki S, Inoue M and Tsugane S, 2007, “C-peptide, insulin-like growth factor-I, insulin-like growth factor binding proteins and risk of colorectal cancer in a nested case-control study: The Japan public health center-based prospective study”, Int. J. Cancer, 120: 2007-2012.

Palmqvist R, Hallmans G, Rinaldi S, Biessy C, Stenling R, Riboli E and Kaaks R, 2002, “Plasma insulin-like growth factor-I and insulin-like growth factor binding protein-3, and risk of colorectal cancer: a prospective study in northern Sweden”, Gut, 50: 642-646.

Probst-Hensch N, Yuan J, Stanczyk F, Gao Y-T, Ross RK and Yu MC, 2001, “IGF-1, IGF-2 and IGFBP-3 in prediagnostic serum: association with colorectal cancer in a cohort of Chinese men in Shanghai”, Brit. J. Cancer, 85(11): 1695-1699.

Renehan AG, Painter JE, Atkin WS, Potten CS, Shalet SM and O’Dwyer ST, 2001, “High-risk colorectal adenomas and serum insulin-like growth factors”, Br. J. Surg., 88: 107-113.

Renehan AG, Zwahlen M, Minder C, O’Dwyer ST, Shalet SM and Egger M, 2004, “Insulin-like growth factor (IGF)-I, IGF binding protein-3 and cancer risk: systematic review and meta-regression analysis”, Lancet, 363: 1346-1353.

Rinaldi S, Cleveland R, Norat T, Biessy C, Rohrmann S, Linseisen J, Boeing H, Pischon T, Panico S, Agnoli C, Palli D, Tumino R, Vineis P, Peeters PH, van Gils CH, Bueno-de-Mesquita BH, Vrieling A, Allen NE, Roddam A, Bingham S, Khaw KT, Manjer J, Borgquist S, Dumeaux V, Torhild Gram I, Lund E, Trichopoulou A, Makrygiannis G, Benetou V, Molina E, Donate Suárez I, Barricarte Gurrea A, Gonzalez CA, Tormo MJ, Altzibar JM, Olsen A, Tjonneland A, Grønbaek H, Overvad K, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Slimani N, Boffetta P, Jenab M, Riboli E and Kaaks R., 2010, “Serum levels of IGF-I, IGFBP-3 and colorectal cancer risk: results from the EPIC cohort, plus a meta-analysis of prospective studies”, Int. J. Cancer, 126(7): 1702-15.

Ron E, Gridley G, Hrubec Z, Page W, Arora S and Fraumeni JF Jr, 1991, “Acromegaly and gastrointestinal cancer”, Cancer, 68: 1673-1677.
Suzuki, S., Kojima, M., Tokudome, S., Suzuki, K., Ozasa, K., Ito, Y., Tajima, K., Nakachi, K., Watanabe, Y., Tamakoshi, A. (2009). Insulin-like Growth Factor (IGF)-I, IGF-II, IGF Binding Protein-3, and Risk of Colorectal Cancer: a Nested Case-control Study in the JACC Study. Asian Pacific j of Cancer Prev. 10, JACC Serum Component Supplement, 45-49.

Schoen R.E., Weissfeld, J.L., Kuller, L.H., Thaete, F.L., Evans, R.W., Hayes R.B., Rosen C.J. (2005). Insulin-like growth factor-I and insulin are associated with the presence and advancement of adenomatous polyps. Gastroenterology, 129, 464-475

Soubry, A., Il'yasova, D., Sedjo, R., Wang, F., Byers, T., Rosen, C., Yashin, A., Ukraintseva, S., Haffner, S., D'Ajostina R. (2012). Increase in circulating levels of IGF-I and IGF-I/IGFBP3 molar ratio over a decade is associated with colorectal adenomatous polyps. Int J Cancer., 131:512-517.

Suzuki, S., Kojima, M., Tokudome, S., Suzuki, K., Ozasa, K., Ito, Y., Tajima, K., Nakachi, K., Watanabe, Y., Tamakoshi, A. (2009). Insulin-like Growth Factor (IGF)-I, IGF-II, IGF Binding Protein-3, and Risk of Colorectal Cancer: a Nested Case-control Study in the JACC Study. Asian Pacific j of Cancer Prev. 10, JACC Serum Component Supplement, 45-49.

Teramukai, S., Lee, R., Eguchi, H., Odat, T., Kono, S. (2002). Insulin-like Growth Factor (IGF)-I, IGF-Binding Protein-3 and Colorectal Adenomas in Japanese Men. Jpn. J. Cancer Res, 93; 1187-1194.

VPC, 1999, "Report of the Working Group on the Safety of Recombinant Bovine Somatotropin (rBST)", Veterinary Products Committee, Veterinary Medicines Directorate, Woodham Lane, New Haw, Surrey, England.

Wolpin, B.M., Meyerhardt, J.A., Chan, A.T., Ng, K., Chan, J.A., Wu, K., Pollak, M.N., Gionannucci, E.L., Fuchs. C.S. (2009). Insulin, the insulin like growth factor axis, and mortality in patients

Wei EK, Ma J, Pollak MN, Rifai N, Fuchs CS, Hankinson SE and Govannucchi E, 2005, "A prospective study of C-peptide, of insulin-like growth factor-I, insulin-like growth factor binding protein-1 and the risk of colorectal cancer in women", Cancer Biomarkers Prev., 14: 850-855.

Wu, K., Feskanich, D., Fuchs, C.S., Chan, A.T., Willett, W.C., Hollis, B.W, Pollak, M.N., Giovannucci, E. (2011). Interactions between plasma levels of 25-hydroxyvitamin D, insulin-like growth factor (IGF)-I and C-peptide with risk of colorectal cancer. PLoS One 2011; 6(12);e28520.

Yamaji, T., Iwasaki, M., Sasazuki, S., Tsugane S., T. (2012). Gender difference in the association of Insulin and the Insulin-like growth factor axis with colorectal neoplasia. Int J Obesity, 36, 440-447.

Yoon, Y.S., Keum, N.N., Zhang, X., Cho, E. (2015). Circulating levels of IGF-I, IGFBP-3, and IGF-I/IGFBP-3 molar ratio and colorectal adenomas. X=Cancer Epidemiology, [in press]

Zhang, M., Hu, Z., Huang, J., Shu, Y., Dai, J., Jin, G., Tang, R., Dong, J., Chen, Y., Xu, L., Huang, X,, Shen H. (2010) 3' untranslated region polymorphism in IGF-I predicts survival of non-small cell lung cancer in a Chinese population. Clin. Cancer Res. 16, 1236-1244.

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET – Part 3

IGF-I and Lung cancer.

Introduction

1. Lung cancer is the third most common cancer in the UK. In 2013, 45,500 new cases were diagnosed accounting for 13% of new cancer cases. Survival rates are low (around 5%) with35, 371 deaths occurring in the UK in 2012. Lung cancer is considered to be 89% preventable (Cancer Research UK, 2016) with risk factors being lifestyle factors notably smoking, certain environmental exposures and exposure to ionising radiation.

2. Lung Cancer can be divided into two types; Non-Small Cell lung cancer and small cell lung cancer.

3. The majority of lung cancers are Non-small cell lung cancers (NSCLC); these can be broadly sub-divided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma sub-types, although in poorly differentiated tumours, the histological distinctions are less clear (Scagliotti and Novello, 2012).

4. SCLC (oat cell cancer) about 12% of lung cancers. It is largely associated with smoking since it rarely occurs in in non-smokers (Cancer Research UK, 2016).

The IGF-I axis in lung cancer

5. As previously, the mechanism of IGF-I action and regulation was considered in detail in CC/2009/08 and CC/2012/06. In brief, IGF-I (somatomedin C) is a peptide growth factor with a structure similar to insulin. It is largely produced in the liver in response to growth hormone. In circulation, IGF-I is largely bound to six binding proteins, most usually (>90%) IGFBP-3. IGFBP-3 is cleaved by proteases which increase the availability of IGF-I. IGF-I acts via the IGF-I receptor. Levels of IGF-I are controlled via a feedback loop with free IGF-I inhibiting the secretion of growth hormone, in turn reducing the levels of IGF-I production. Free IGF-I binds to IGFBP-2 and IGFBP-5 with a greater affinity than it binds to the IGF-I receptor so increases in the levels of these binding proteins will reduce IGF-I activity.

6. Human lung cancer cells have higher levels of IGF-II than normal tissue and over expression occurs in both NSCLC and SCLC (discussed Velcheti and Govindan, 2006). The over expression of IGF-II results from aberrant regulation of

the genomic imprinting mechanism of IGF-II and mesoderm specific transcript genes. IGF-II has not been considered in detail in this review.

Expression of IGF-I pathway factors

7. Disruption of the balance of IGF-I and IGFBP-3 has been implicated in the aetiology and progression of lung and other cancers (Zhang *et al*., 2010). Expression of IGF-I receptors has been demonstrated in the bronchial epithelial cells of normal lung and in primary lung cancer (22/24 cases) being most prominent in squamous cell carcinoma (Kaiser *et al*., 1993).

8. Positive IGF-IR expression was found in 80.9% of 47 biopsy samples from NSCLC patients, with 19.1% of samples being negative (Dilli *et al*., 2013). There was no association between IGF-IR expression and histological sub-type, local invasion, lymph node and metastasis status or survival

9. The levels of IGF-I and IGF-II were higher in bronchial tissue samples with high grade dysplasia than in those containing normal epithelium, hyperplasia and squamous metaplasia (Kim *et al*. 2009). Derivation of human bronchial cell lines with activation mutation in *KRAS*(V12) or loss of p53 overexpressed IGF-I and IGF-II. The transformed characteristics of these cells were significantly supressed by inactivation of IGF-IR or inhibition of IGF-I or IGF-II expression but enhanced by overexpression of IGF-IR or exposure to the tobacco carcinogens 4-(methylnitrosamino)-I- (3-pyridyl)-I-butanone and benzo(*a*)pyrene. The authors concluded that airway epithelial cells produced IGFs in an autocrine or paracrine manner and could act jointly with tobacco carcinogens to enhance lung carcinogenesis.

10. In further work by the same group (Kim *et al*., 2011), IGF-I but not IGF-II protein levels in NSCLC were significantly higher than those in normal and hyperplastic bronchial epithelium. IGF-I and IGFBP-3 levels in NSCLC tissue specimens were significantly correlated with phosphorylated IGF-IR expression. The impact of IGFBP-3 expression on the activity of tissue driven IGF-I in lung cancer development was investigated using transgenic mice with a lung specific human transgene (tg), a germline-null mutation of IGFBP-3, or both. The authors reported that IGF-I was overexpressed in NSCLC, leading to activation of IGF-IR, and that IGFBP-3, depending on its expression level, either inhibits or potentiates IGF-I actions in lung carcinogenesis.

11. The expression patterns of IGF-I, IGF-II, IGF-1R and phosphorylated IGF-1R (p IGF-1R) were assessed immunohistochemically using tissue microarrays in tumour samples taken from 352 patients (Kim *et al*., 2014). Detailed clinical and pathological information was available for the majority of the patients and this included demographic data, smoking history, pathologic tumour node metastasis staging, overall survival time and recurrence free survival time. IGF-I and IGF-II were largely expressed in the cytoplasm of the cell and was not obviously different between tumours of different stages. The expression of IGF-I was higher in patients with adenocarcinoma than in those with squamous cell carcinoma whereas for IGF-2, expression was higher in patients with squamous cell carcinoma. IGF-I

expression was also higher in patients with mutated epidermal growth factor receptors (mt EGFR) than in those with wild type EGFR, while IGF-II expression was higher in patients with wild type EGFR. Patients with low levels of IGF-I expression had longer overall survival rates than those with high levels of expression, sub group analysis revealed a significant difference in overall survival in adenocarcinoma patients only. It was noted that the study was a follow up to earlier work which indicated that IGF-IR and insulin receptor expression was associated with poor survival in NSCLC patients.

12. A study by Chang *et al*., (2002b) investigated IGFBP-3 expression in tumour samples from 74 patients with primary pathological stage 1 non-small cell lung cancer (NSCLC). Reduced IGF-I expression was found in 42/74 samples (56.8%) and this was more frequent in large cell carcinoma than squamous cell carcinoma and adenocarcinoma, although this difference was not statistically significant. The reduction was not associated with other factors such as age, sex, histological grade, and smoking history. Significant statistical correlation was found between IGFBP-3 expression and disease specific survival was noted (p= 0.019 by log rank test). Although not statistically significant, patients with decreased IGFBP-3 expression had shorter overall, disease free and event free survival rates that did patients normal IGFBP-3 expression. In multi-variate analysis, the level of IGFBP-3 expression remained an independent factor for predicting a shorter diseases specific survival probability. The authors suggested that IGFBP-3 was acting as a tumour suppressor and plays an important part in determining biological aggressiveness in early NSCLC.

Potential mechanisms

13. The epithelial-mesenchymal transition is a process in which cells undergo a switch from an epithelial phenotype (characterised by lateral, apical, and basal membranes, polarized distribution of cellular components, cell-cell interactions with tight junctions and lack of mobility) to a mesenchymal phenotype (with loose cell-cell interactions, no polarization, and potential for motility) is potentially an important part of the mechanism for NSCLC progression (Scagliotti and Novello, 2012). IGF-II and IGF-IR are highly expressed in epithelial differentiated NSCLC tumours while IGF-II and IGF-2R are highly expressed in transitional tumours. NSCLC tissue levels of IGF-IR and circulating IGF-I are correlated with expression of epithelial and mesenchymal markers respectively.

14. Pavelić *et al*. 2005 reported that increased expression (at mRNA and protein level) of IGF-I and IGF-IR and decreased apoptosis were found in large-cell carcinomas and adenocarcinomas obtained from a tissue bank. Cell treatment with IGF-I increased telomerase activity, whereas treatment with αIR3, which inhibits the activity of IGF-IR, decreased it. The authors noted that increased telomerase activity could be involved in cells becoming immortal as part of the carcinogenic process.

15. Cellular functions of IGF-regulated signalling are influenced by the expression of a variety of receptor docking proteins, including four different insulin receptor substrate proteins (Dziadziuszko *et al.,* 2008). Downstream signalling is primarily through thephosphatidylinositol-3 kinase Akt pathway and the mitogen activated protein kinase pathway, resulting in increased cell proliferation and apoptosis

inhibition. Ligand-driven activation is influenced by upstream endocrine factors (particularly for IGF-I), imprinting (for IGF-2) by multiple circulating and tissue based IGF binding proteins/proteases and by the expression of the IGF-II clearance receptor. Deregulation of IGF signalling has been described in both small cell and NSC lung cancer.

16. Free IGF-I was correlated with tumour vimentin expression and inversely, with-cadherin suggesting a possible role of IGF-I in tumour de-differentiation (Gualberto *et al*., 2011).

17. Mazzaccoli *et al*. (2012) reported that the Growth Hormone/IGF-I axis was severely disrupted in lung cancer patients compared with healthy controls, resulting in a loss of circadian rhythmicity of hormone secretion. It was suggested that this could have a role in the progression of neoplastic disease.

18. Peng *et al*. (2013) conducted global profiling for miRNAs in a cohort (n=81) of stage 1 non-small cell lung cancers and determined that miR-486 was the most down regulated miRNA in tumours compared with adjacent uninvolved lung tissues, suggesting that miR-486 loss may be important in lung cancer development. It was found that miR-486 directly targeted components of insulin growth factor signalling including IGF-I, IGF-I receptor and phosphoinositide-3-kinase, regulatory subunit 1(alpha) (PIK3R1 or p85a) and functions as a tumour suppressor of lung cancer both *in vitro* and *in vivo*. To assess the role of miR-486 *in vivo*, both A549 and H1299 cells (with or without overexpressing miR-486) and were injected s.c into nude mice. Within 24 –days of injection, over expression on miR-486 in both H1299 cells caused a substantial reduction in tumour volume. The A 549 cells overexpressing miR-486 did not form any detectable xenograft tumours whereas the control cells did form tumours. Overall, the data were taken to show that miR-486 functioned as a tumour suppressor.

19. Tang *et al*. (2013) reported that oestrogen and IGF-I acted synergistically to promote lung tumours in mice with urethane induced tumours. It was suggested that this could be related to the MAPK and AKT signalling pathways. This followed earlier work (Tang *et al*., 2012) in which it was reported that oestrogen up regulated IGF-I signalling pathways in lung cancer via the β oestrogen receptor.

Clinical observations

Genetic variation

20. Han *et al*. (2008) investigated the association between single nucleotide polymorphisms (SNPs) of the IGFBP-3 gene and susceptibility to lung cancer and the effect of the SNPs on the serum levels of IGFBP-3, total IGF-I and free IGF-I in a Korean population. Initial screening was conducted in the promoter region of the IGFBP-3 gene in 41 randomly selected lung cancer patients, two polymorphisms (-1590 C>A;-202 A>C) were identified in 415 Korean lung cancer patients and 415 matched controls. The genotype and allele frequency distribution of each SNP did not differ significantly between cases and controls but two polymorphisms were associated with increased risk for lung cancer in females and never smokers.

Functional analysis in the H1703 cell lines using DNA fragments containing a-C haplotype of the promoter region showed significantly decreased transcriptional activity. There was a negative correlation between the number of polymorphic alleles in -1590/-202 loci and serum levels of IGFBP-3 but this was not statistically significant in the test for linear trend. The authors suggested that IGFBP-3 polymorphisms might be a genetic risk factor for lung cancer by means of decreased IGFB-3 expression among females or never smoking Koreans.

21. Zhang *et al*., (2010) selected and genotyped tagging potentially functional SNPs in IGF-I and IGFBP-3 in a case cohort of 568 patients with diagnosed NSCLC in a Chinese population. It was found that rs5742714C/G in the 3'untranslated region of IGF-I was associated significantly with NSCLC survival after adjustment for demographic and clinicopathologic factors (age, sex, smoking, histology, stage, surgical operation and chemotherapy or radiotherapy status., showing an improved median survival time in patients carrying the CG/GG variant genotypes. The median survival time was 28.5 months for CG/GG individuals compared with 23.0 months in CC individuals, adjusted HR of),77; 0.60-0.99, p for heterogeneity test = 0.045. It was noted that some of the SNPs had been linked to the prognosis of other cancers or altered circulating levels of IGFBP-3 in other studies and this had not been replicated in the Zhang study. However, it was suggested that this could be due to differences in genetic background, different tumour sites or therapeutic issues.

Acromegaly

20. Although acromegaly is associated with an increased risk of certain cancers, notably colorectal cancer and thyroid cancer due to the increased levels of serum IGF-I in these patients and the subsequent mitogenic effects, lung cancer is thought to be less common in non-smoking acromegalic individuals compared to other cancers; however, one case of a female patient with a pulmonary epidermoid carcinoma was reported by El Aziz *et al*. (2012).

Methylation

21. Eighty three patients with pathological type 1 NSCLC were investigated for promoter hyper-methylation of IGFBP-3, by methylation-specific PCR (Chang *et al* 2000). Hypermethylation of the IGFBP-3 promoter was found in 51 (61.5%) of the tumours. Methylation status was not associated with other factors such as age, sex, histological type, histological grade, and smoking history. However patients with a hypermethylated IGFBP-3 promoter had a significantly lower 5 year disease specific disease free and overall survival rate than those without. In multi-variate analysis, hypermethylation of the IGFBP-3 promoter remained an independent factor for predicting a shorter diseases specific survival probability. The frequency of hypermethylation did not vary with histology. The authors suggested that the hypermethylation of the promoter provided the cancer cells with a growth advantage in a manner akin to deletions or mutation and that the finding was consistent with the role of IGFBP-3 in the invasion and metastasis of lung cancer.

Experimental studies

In vitro

22. NSCLC cell lines of several different histologies show increased proliferation in response to IGF-I as well as producing IGF-I in culture (Favoni *et al*., 1994 discussed Scagliotti and Novello, 2012). In mouse models engineered to overexpress human IGF-IA in pulmonary epithelial cells, the frequency of premalignant adenomatous hyperplastic lesions was significantly increased with controls. Over expression of IGF-I in lung epithelium also induced pulmonary adenocarcinomas in a majority of mice aged >18 months with evidence of downstream activation of the Erk1/Erk2 or p38 mitogen- activated protein kinase pathways. In addition, IGFBP-3 has dose-dependent anti-tumour activity in murine Lewis lung cancer models (Scagliotti and Novello, 2012).

23. Using microarrays, Rajski *et al*. (2010) investigated the expression patterns of genes induced by IGF-I in primary lung fibroblasts. The initial work to derive the expression signature was conducted in breast cancer cells (MCF-7) and stromal cells (CCl-171 fibroblasts), before assessing the signature against microarray data sets for breast and lung tumours. The GO Termfinder tool was used to analyse the signature; from this it was determined that the fibroblast derived IGF-I was significantly enriched for genes involved in biological processes such as M phase, mitotic cell cycle, mitosis and cell cycle, cell division as well as angiogenesis, the p53 pathway and integrin and Wnt signalling. The expression of the lung fibroblast signature was described as coherent and provided a basis for dividing the tumours into two groups. Patients with a high expression of the signature had a significantly shorted disease free survival (p = 0.001; 45% vs 82% after 5 years, HR; 95% CI 2.1; 1.2-3.8) than patients with lower expression if the signature. Since the expression signature was derived from breast and lung fibroblasts and could be applied to both as a prognostic tool, it was suggested that the response of stromal fibroblasts to IGF-I might be a universal feature of cancer.

Observational studies

24. The available studies are tabulated in Table 1 below and described in the text following the table. They are divided into retrospective studies, prospective studies and meta-analyses.

Table 1: Summary of results of epidemiology studies of lung cancer risk associated with IGF-I and related substances

Subjects	Number of subjects	How was IGF-I measured and was it free[6]?	Variables study controlled, matched or analysed for	Association between IGF-I levels and lung cancer	Main results	Reference
Retrospective studies						
Males	37 cases 25 controls	Radioimmuno assay	-	Positive	IGF-I higher in patients	Bhatavdekar *et al.,* 1994 -abstract only available
Korean lung cancer patients	41 cases; 20 controls	IGF by Radioimmuno assay, IGFBPs by Western blotting		Negative	Levels of IGF-I and IGFBP-3 lower in lung cancer patients	Lee *et al*., 1999
Americans (white, black & hispanic), aged 60.6 to 63.4 y	204 cases; 218 controls	Free-Immunoassay following acid ethanol extraction.	Age, sex, ethnicity, smoking status, BMI, family history of cancer.	Positive	OR=2.13 comparing concentrations of IGF-I in cases and controls. Negative association with IGFBP-3.	Yu, *et al*., 1999
Americans (white, black & hispanic), aged 60.6 to 63.4 y	183 cases; 227 controls	ELISA	Age, sex, ethnicity, smoking status, BMI, family history of cancer.	Positive	OR=2.06, comparing risks in upper and lower quartiles of IGF-I. Negative association with IGFBP-3.	Wu *et al*., 2000
Korean patients	77 cases	ELISA	Age, sex, smoking status, histology.	Negative	IGF-I associated with improved prognosis and survival	Han *et al*., 2006
Polish adults	38 cases (25 NSCLC) 10 No controls	ELISA		Positive	IGF-I higher and IGFBP-3 lower in patients compared with healthy controls	Izycki *et al*., 2006

[6] In many studies, it is unclear whether the IGF-I measured was free or attached to binding proteins since the experimental details are not always provided. The majority of studies use commercially available ELISA kits, which may or may not involve an acid alcohol extraction step to remove the binding proteins.

Subjects	Number of subjects	How was IGF-I measured and was it free[6]?	Variables study controlled, matched or analysed for	Association between IGF-I levels and lung cancer	Main results	Reference
Chinese lung cancer patients	35 cases 14 controls	Radio immunoassay		Positive	IGF-I higher and IGFBP-3 lower in patients compared with healthy controls	Wang *et al*., 2004 (abstract only)
Lung cancer patients	24 cases; 12 controls	Free IGF-I measured by two site immuno-radiometric assay.	Tumour diameter, histological type, localisation of tumour.	None (in serum)	IGF-I and IGFBP-3 lower in the epithelial lining fluid of patients.	Ünsal *et al*., 2005
German adults	34 patients 13 controls	ELISA. It was noted that the quotient of each sample was calculated.		None	No differences in IGF-I or IGFBP-3 between patients and healthy controls	Matuschek *et al*., 2011
US adults	100 NSCLC patients	Immunobeads	Sex, age, race, smoking, tumour histology, fasting state	None	No association between IGF-I and lGFBP-3 and prognosis.	Shersher *et al*., 2011
Greek adults	77 NSCLC patients	Total by radio-immunoassay	Age, smoking, weight loss, metastasis, histologic sub type.	Positive	Associated with overall survival	Vlachostergios *et al*., 2011
Chinese adults	80 NSCLC patients 45 BPL controls	ELISA	No	Positive	Pre-operative IGF-I associated with tumour size and poor prognosis	Fu *et al*., 2013
Prospective studies						
American women aged 32 to 70 y	93 cases; 186 controls		Age, date of blood sampling, menopausal status, menstrual cycle, smoking.	None	No association between lung cancer and levels of IGF-I and IGFBP-1, -2 & -3.	Lukanova, *et al*., 2001

Subjects	Number of subjects	How was IGF-I measured and was it free[6]?	Variables study controlled, matched or analysed for	Association between IGF-I levels and lung cancer	Main results	Reference
Chinese men aged 45 to 64 y	230 cases 659 controls	Radioimmuno assay	Neighbourhood, age, time of sample smoking.	Negative	Reduced risk associated with high IGF-I and IGFBP-3. ORs 0.70 & 0.52, respectively, comparing upper & lower quartiles.=	London, *et al*., 2002
Heavy smokers (aged 50 to 69 y) or asbestos workers (aged 45 to 69 y) in USA,	159 cases; 297 controls	ELISA	Age, sex, year of sampling, year of blood draw, smoking	None	No significant association between IGF-I and lung cancer, but positive association for IGFBP-3: OR=2.35 comparing upper and lower quartiles.	Spitz, *et al*., 2002
Individuals in the JACC study	194 cases 9351 controls	Free IGF-I measured by immune-radiometric assay	Area, gender, age, smoking, BMI, IGFBP-3	Positive	Increased IGF-I associated with increased risk of lung cancer (1.74: 1.08-2.81)	Wakai *et al*., 2002
Male smokers (Finland) from ATBC cohort.	200 cases; 400 controls	ELISA	Age, intervention arm, BMI, smoking	None	No significant association between IGF-I or IGFBP-3 and lung cancer	Ahn *et al*., 2006
UK male professionals	167 cases; 498 controls	ELISA	BMI, alcohol, smoking	None	No significant association between IGF-I, IGF-2 or IGFBP-3 and lung cancer	Morris *et al*., 2006
Meta-analysis	-			*None*	*No association between IGF-I and lung cancer when results from all 4 studies are considered. OR=1.01*	*Renehan, et al., 2004*
Meta-analysis	-	-	-	*None*	*No significant association between IGF-I or IGFBP-3 and lung cancer*	*Morris et al., 2006*

Subjects	Number of subjects	How was IGF-I measured and was it free[6]?	Variables study controlled, matched or analysed for	Association between IGF-I levels and lung cancer	Main results	Reference
Meta-analysis	-			*None*	*No association between IGF-I and lung cancer. Inverse association between IGFBP-3 and lung cancer risk*	*Chen, et al., 2009*
Meta-analysis	-			*None*	*No association between IGF-I and lung cancer. Inverse association between IGFBP-3 and lung cancer risk*	*Cao, et al., 2012*

Retrospective studies

25. Levels of IGF-I were significantly higher in 37 male patients with lung cancer compared with 25 age matched controls (Bhatavdekar *et al*., 1994).

26. Yu, *et al*. (1999) looked at blood levels of IGF-I, IGF-II and IGFBP-3 in 204 newly-diagnosed lung cancer patients and in 218 control subjects, matched for sex, age and ethnicity. The case subjects were consecutive patients (aged 60.6 to 63.4 years) with lung cancer registered at the University of Texas M.D. Anderson Cancer Centre. There was an elevated mean IGF-I and a reduced mean IGFBP-3 in lung cancer patients compared with controls, aged 61.8 to 64.2 years. The risk of lung cancer in subjects having upper quartile levels of IGF-I had an odds ratio of 2.06 (95% CI = 1.19–3.56) as compared with those in the lower quartile. The OR for high quartile plasma levels of IGFBP-3 was 0.48 (95% CI = 0.25-0.92) when adjusted for IGF-I level. There was no association between IGF-II levels and lung cancer.

27. Wu, *et al*. (2000) reported that cases in Yu, *et al*. (1999) study had an increased sensitivity to mutagens, as assessed by cytogenetic analysis of lymphocytes from cases and controls after *in vitro* exposure to bleomycin and benzo[a]pyrene diol epoxide (BPDE). The study by Wu *et al*., 2000 used the same study population, but the analysis was done in 183 case patients and 227 controls. The patients were matched by sex, age, ethnicity and smoking status. Levels of IGF-I, IGF-II and IGFBP-3 were also analysed in the study. The levels of IGF-I were significantly higher in case patients compared to controls (OR; 95% CI = 2.13; 1.20-3.78). There were no significant differences in IGF-II. High IGFBP-3 was associated with a reduced risk of lung cancer, 0.59; 0.33-1.05 in cases compared to controls. Mean levels of IGF-I and IGF-I/IGFBP-3 ratio were non-significantly higher in patients with advanced or poorly differentiated disease than in patients with early or well differentiated disease. Variation in IGF-I was not associated with any histological type or tumour stage. When mutagen sensitivity was added to the model, the OR increased to 8.88; 3.67-21.50 for high IGF-I and bleomycin sensitivity combined and 13.53; 4.48-40.89 for benzo[*a*]pyrene diol epoxide (BPDE) combined. When all three risk factors were combined, the OR was 17.09; 41.16-70.27). It was concluded that individuals with genetic instability and higher proliferation potential were at enhanced risk of lung cancer.

28. Serum IGF-I and IGFBP levels were compared in 41 patients with lung cancer (SCLC =9, NSCLC = 32) and healthy controls (Lee *et al*., 1999). The serum IGF-I level was significantly lower in lung cancer patients than controls (207.9 ± 62.6 vs 281.3 ± 53.9 ng/ml, $p < 0.01$). Patients with SCLC had lower IGF-I levels than those with NSCLC (194 ± 62.9 vs 258.4 ± 27.8 $p < 0.01$). Patients with squamous cell carcinoma tended to have lower IGF-I levels than those with adenocarcinoma, but this was not significant. The concentration of IGFBP-3 in lung cancer was 48% of that in the controls (7.43 ± 5.42 vs 15.43 ± 5.42, $p = 0.05$), whereas IGFBP-2 was markedly elevated. There were no differences in IGFBP levels between individuals with SCLC and those with NSCLC.

29. IGF-I and IGFBP-3 were measured in the plasma of 78 patients with lung cancer, 35 with benign pulmonary disease and 14 healthy controls. Serum IGF-I and IGF-I/IGFBP-3 were significantly higher in the lung patients than in the other 2 groups (570.67 ± 185.80, 466.53 ± 142.42 and 427.66 ± 141.19 for IGF-I

respectively). There were no significant differences in IGFBP-3 levels between the three groups. The serum level of IGF-I was higher in patients with lymphoid node metastasis and the levels of IGFBP-3 were lower than those without metastasis. IGFBP-3 was lowest in patients with bone metastasis. IGF-I was lower post chemotherapy.

30. Ünsal *et al*. 2005 measured IGF-I and IGFBP-3 in the bronchiolar lavage fluid (BAL) and the serum of 24 lung cancer patients and 12 healthy controls. Patients with other conditions which could affect serum IGF-I levels were specifically excluded as were women using hormone replacement therapy. The BAL was standardised for albumin to account for dilution and the data expressed as epithelial lining fluid (ELF). Serum IGF-I and IGFBP-3 were lower in lung cancer patients (126.9 ± 63.4 and 167.6 ± 56.5 ng/ml for IGF-I and 2277.6 ± 614.0 and 2874.7 ± 861.9 ng/ml for IGFBP-3 respectively), but the difference was not statistically significant. Similarly, IGF-I and IGFBP-3 were non-significantly lower in ELF (3157.6 ± 3119.0 and 4456.8 ± 4850.8 ng/ml for IGF-I and 1272.0 ± 680.1 and 1048.5 ± 867.5 ng/ml for IGFBP-3 respectively). However, the IGF-I/IGFBP-3 ratio was significantly lower in ELF. Mean IGF-I levels in ELF were lower in patients with distant metastasis, while the IGF-I/IGFBP-3 ratio was lower in patients with distal and nodal metastasis. Tumour stage was negatively correlated with IGF-I levels in ELF and serum IGF-I/IGFBP-3 ratio. There were no significant differences in sex, BMI or smoking between the two groups, but the controls were significantly younger than the patients.

31. Plasma levels of IGF-I, IGF-II and IGFBP-3 were analysed pre-treatment in 77 Korean patients with advanced NSCLC (Han *et al*., 2006). IGF-2 and IGFBP-3 levels were elevated in female patients, non-squamous cell carcinoma and never smokers. In a univariate Cox proportional hazards model, higher levels of IGF-I, IGF-II and IGFBP-3 were predictive of longer progression-free (p = 0.001, 0.006 and 0.007 respectively) and overall survival (p = 0.025, <0.0001 and 0.001 respectively). Multivariate analysis indicated that IGF-I and IGFBP-3 were independent factors for progression-free survival (p = < 0.0001 and 0.001 respectively). In addition, IGF-I, IGF-II and IGFBP-3 were independently predictive for overall survival (p= 0.004, 0.001 and 0.043 respectively).

32. Serum levels of IGF-I in NSCLC and SCLC lung cancer patients compared with healthy controls (Izycki *et al*., 2006) and were unaffected by four cycles of chemotherapy. IGFBP-3 levels were lower in NSCLC patients compared with controls both before and after treatment. In SCLC patients, IGFBP-3 levels were significantly lower than the controls (10 healthy volunteers) before treatment, and while still lower after treatment, the difference was no longer significant. Neither the histological type of NSCLC nor clinical staging had any association with serum levels of IGF-I and IGFBP-3.

33. Serum IGF-I, IGFBP-3 and IGF-I:IGFBP-3 ratio were not associated with survival in 56 patients with inoperable NSCLC (Meek *et al*., 2009).

34. Serum IGF-I levels were not different in 34 patients with lung cancer compared to 13 healthy controls (Matuschek *et al*., 2011). Limited data are provided on IGF-I and IGFBP-3 and none on the concentrations measured. The levels of IGF-I

did not change following radiotherapy. Similarly, IGFBP-3 did not differ between groups and was also unaffected by treatment.

35. Pre-treatment serum levels of IGF-I, IGFBPs 1 to 7 and C-peptide were measured in 100 NSCLC patients prior to treatment (Shersher *et al*., 2011). Of the 100 patients, 59 had no metastatic progression whereas 41 had positive lymph nodes. No association was found between IGF-I levels and recurrence free survival. Low levels of IGFBP-5 correlated with poor recurrence free survival and a positive nodal status, whereas IGFBP-7 correlated with a positive nodal status but did not have an association with recurrence free survival. No significant correlation was found between IFGFBPs-5 and 7 and sex, age, race, smoking history, tumour histology or fasting state.

36. Vlachostergios *et al*. 2011 measured serum IGF-I levels in 77 NSCLC patients to establish whether they correlated with inflammatory and weight loss status and with clinical outcome. IGF-I correlated with age, histologic subtype, albumin and C-reactive protein. In univariate analysis, IGF-I was related to time to progression and overall survival. It was concluded that IGF-I correlated with systemic inflammation and appeared to play an independent predictive role in metastatic non-small cell lung cancer. Although significant, the HRs calculated for IGF-I are very small (1.00; 0.99-1.01 for overall survival, p= 0.0042 and 1.01; 1.00-1.01 for time to progression) suggesting no real association.

37. Fu *et al*. (2013) reported that pre-operative serum IGF-I concentrations were elevated and correlated with clinicopathological parameters and overall survival in 80 patients with NSCLC compared to 45 cases with benign liver lesions who served as controls. Serum IGF-I concentration were significantly higher in patients with NSCLC (21.59 ± 9.04 ng/ml) compared to those with benign pulmonary lesions (12.37 ± 4.51 ng/ml, p = 0.0003). Concentrations were also higher in patients with larger tumours, more advanced tumours and were also significantly correlated with poor prognosis and local lymph node metastasis.

Prospective studies

38. Lukanova, *et al*. (2001) reported the results of a case-control study nested with the New York Women's Health Study Cohort of 14,275 women, aged 32-70 years, who attended a mammography screening clinic between March 1985 and June 1991 and had not been pregnant or used any hormonal medications in the preceding 6 months. Blood samples were collected at the time of recruitment. Serum IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, insulin and cotinine were measured in blood samples from 93 women from the cohort who were diagnosed with lung cancer at least 6 months after recruitment and 186 matched control women from the cohort. Two controls were matched to each case on the basis of age, date of blood sampling, menopausal status, day of menstrual cycle and smoking status at the time of blood collection. Mean serum levels of IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3 were not significantly different between cases and controls. Univariate logistic regression analyses showed no association of lung cancer risk with serum levels of IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3.Similar results were obtained with multivariate analyses, including adjustments for cotinine, time since last meal, BMI, IGF-I or IGFBP-3. Exclusion of cases diagnosed within 3 years of recruitment or restriction of analyses to adenocarcinomas only did not alter these results. The

authors concluded that the results of the study gave no evidence of any association between pre-diagnostic serum levels of IGF-I, IGFBP-1, IGFBP-2 or IGFBP-3 and lung cancer risk in women.

39. London, *et al*. (2002) reported the results of a prospective study of men in Shanghai, China, which examined the association between serum levels of IGF-I or IGFBP-3 and subsequent risk of lung cancer. Between January 1986 and September 1989, all men aged 45-64 years living in a defined area of the city were invited to participate in a study of diet and cancer. A total of 18,244 men were enrolled in the study. At recruitment each subject was interviewed and a blood sample was taken. Follow-up was by annual re-contact with participants and by reviewing cancer reports from the Shanghai Cancer Registry and by death certificates. By March 1997, 259 cases of lung cancer had been identified. Three control participants were allocated to each case, matched on the basis of neighbourhood of residence, age at interview and time of sample collection. Increased serum levels of IGF-I were not associated with increased risk of lung cancer. However, high serum levels of IGFBP-3 were associated with a reduced risk of lung cancer (see Table 2).

Table 2: Odd ratios (ORs) for lung cancer by quartiles of IGF-I and IGFBP-3 in men in Shanghai (London, *et al*., 2002)

	Quartiles				P_{trend}
	1	**2**	**3**	**4**	
IGF-I					
Mean serum conc (ng/mL)	80	111	135	181	
Cases/controls	73/167	54/162	52/171	51/159	
Unadjusted OR (95% CI)	1.0	0.76 (0.50-1.15)	0.68 (0.45-1.04)	0.70 (0.45-1.10)	0.36
IGFBP-3					
Mean serum conc (ng/mL)	1279	1676	1984	2506	
Cases/controls	67/160	62/173	60/166	41/160	
Unadjusted OR (95% CI)	1.0	0.85 (0.56-1.28)	0.81 (0.53-1.26)	0.52 (0.31-0.88)	0.04

40. Spitz, *et al*. (2002) reported the results of a lung cancer case-control study nested in the placebo arm of the β-Carotene and Retinol Efficacy Trial (CARET) in heavy smokers (men and women) and in asbestos workers (all men). 159 cases of lung cancer were identified amongst cohort members from whom serum had been collected at least 3 years prior to diagnosis. Each case was matched with 2 controls from the cohort on the basis of age, sex, ethnicity, year of enrolment, year of blood sample, and exposure category (heavy smoker or asbestos worker). The cases were significantly (p<0.001) heavier smokers than the controls, with mean pack-years of 58.7 and 45.9, respectively. Levels of both IGF-I and IGFBP-3 were higher in cases (158 & 30700 ng/mL) than in controls (153 & 29400 ng/mL), but the differences were not significant (p = 0.52 and p = 0.17). There was no significant association between IGF-I levels and lung cancer risk, there was a significant dose-related association between IGFBP-3 and lung cancer with an odds ratio (OR) of 2.35 between the lower and upper quartiles of IGFBP-3 levels (see Table 3). There was no significant

association between IGF-I/IGFBP-3 ratio and ORs for the different quartiles. The authors noted that risks associated with elevated IGFBP-3 tended to be higher in current smokers and recent quitters. They further noted that current smoking or recent cessation may exert a suppressive effect on IGF-I levels that might obscure a relatively modest association between IGF-I levels and lung cancer risk.

Table 3: Odds ratios (ORs) associating serum levels of IGF-I and IGFBP-3 with lung cancer risk in smokers and asbestos workers (Spitz, *et al*., 2002)

	Quartiles				P_{trend}
	1	**2**	**3**	**4**	
IGF-I					
Cases/controls	38/73	36/80	42/73	43/71	
OR (95% CI)	1.00	0.79 (0.42-1.49)	0.83 (0.43-1.61)	0.64 (0.31-1.33)	0.29
IGFBP-3					
Cases/controls	30/72	39/75	37/76	53/74	
OR (95% CI)	1.00	1.36 (0.69-2.65)	1.40 (0.68-2.89)	2.35 (1.13-4.92)	0.03

Data analysed in a conditional logistic regression model with adjustment for BMI, smoking status, pack-years of smoking, exposure population and IGF-I or IGFBP-3.

41. A nested case control study was conducted among individuals in the Japan collaborative cohort (Wakai *et al*., 2002). IGF-I, IGF-II and IGFBP-3 were measured in 194 case subjects who subsequently died of lung cancer in an eight year follow up period and from 9351 controls. The odds ratios were adjusted for smoking and other covariates (see Table 4 below). Overall, the risk of lung cancer death was decreased at higher levels of IGF-II and IGFBP-3. After controlling for IGFBP-3, higher levels of IGF-I were associated with an increased risk of death. To exclude the possibility of latent lung cancer, the analysis was limited to those followed for at least 3 years (158 cases, 9311 controls). This strengthened the negative associations of IGF-2 and IGFBP-3 and lung cancer death, but the association with IGF-I decreased, suggesting that latent cancer could partly explain the association

Table 4: Odds ratios (ORs) associating serum levels of IGF-I and IGFBP-3 with lung cancer risk in Japanese adults (Wakai *et al*., 2002)

All participants

	Quartiles				
		2	**3**	**4**	
IGF-I ng/ml	79	110	140	180	
Cases/controls	44/2122	49/2192	38/2443	63/2594	
OR (95% CI)	1.00	1.28 (0.83-1.97)	1.01 (0.62-1.63)	1.74 (1.08-2.81)	0.043
IGF-II ng/ml	440	540	610	710	
Cases/controls	81/2198	32/2230	34/2373194	47/2550	
OR (95% CI)	1.00	0.41 (0.27-0.63)	0.47 (0.31-0.71)	0.67 (0.46-0.98)	0.018
IGFBP-3 µg/ml	2.13	2.68	3.13	3.81	
Cases/controls	77/2298	40/2342	36/2339	41/2372	
OR (95% CI)	1.00	0.55 (0.37-0.81)	0.54 (0.36-0.82)	0.67 (0.45-1.01)	0.037

Subjects followed for > 3 years

	Quartiles				
		2	**3**	**4**	
IGF-I ng/ml	80	110	140	180	
Cases/controls	43/2128	40/2167	29/2366	46/2650	
OR (95% CI)	1.00	1.08 (0.68-1.70)	0.82 (0.49-1.37)	1.32 (0.78-2.21)	0.41
IGF-II ng/ml	440	540	610	710	
Cases/controls	81/2198	32/2230	34/2373194	47/2550	
OR (95% CI)	1.00	0.42 (0.27-0.65)	0.41 (0.26-0.66)	0.54 (0.35-0.84)	0.001
IGFBP-3 µg/ml	2.13	2.68	3.13	3.81	
Cases/controls	68/2297	33/2321	30/2329	27/2364	
OR (95% CI)	1.00	0.51 (0.33-0.78)	0.51 (0.33-0.80)	0.50 (0.31-0.80)	0.002

42. A nested case control study was carried out within the Alpha-Tocopherol, Beta-Carotene Cancer prevention Study (ATBC) cohort of male smokers from Finland (Ahn *et al*., 2006). A random sample of 200 lung cancer cases were drawn from the case group and matched with 400 eligible controls. Generalized linear models, adjusted for age as a continuous variable were sued to estimate means and SDs by case–control status. Unconditional logistic regression was used to calculate Odds ratios and 95% CIs. The final multivariate models included factors which changed the estimated effect by ≥ 10%. These were age, intervention arm, BMI and years of smoking. Both IGF-I and IGFBP-3 levels were slightly higher in controls than cases. IGF-I and IGFBP-3 were inversely associated with lung cancer risk but once adjusted for BMI and years of smoking, the association was no longer statistically significant. The fully adjusted OR; 95%CI (highest vs lowest quartile) was 0.76; 0.39-1.49) for IGF-I and 0.71; 0.35-1.47) for IGFBP-3.

43. IGF-I, IGF-II and IGFBP-3 were measured in 1051 men with cancer and 3142 controls as part of a nested case control study conducted in the BUPA study cohort of 21,250 professional men (Morris *et al*., 2006). Associations with 14 different cancers were assessed with 167 cases of lung cancer being assessed against 498 controls, maximum follow up was 15 years. BMI, alcohol consumption and smoking were assessed as possible confounders. No significant associations were found between lung cancer and any of the serum markers (see Table 5 below).

Table 5: Odds ratios (ORs) associating serum levels of IGF-I and IGFBP-3 with lung cancer risk (Morris *et al*., 2006)

	Quartiles				P_{trend}
	1	**2**	**3**	**4**	
IGF-I					
OR (95% CI)	1.00	1.00	1.23	1.21 (0.62-2.35)	0.45
IGF-2					
OR (95% CI)	1.00	1.21	1.22	0.82 (0.39-1.73)	0.61
IBFBP-3					
OR (95% CI)	1.00	0.90	1.39	1.70 (0.87-3.30)	0.06

Meta analyses

44. Renehan, *et al*. (2004) performed a meta-analysis as part of a systematic review of the associations of circulating concentrations of IGF-I and of IGFBP-3 with the risks of cancers of the prostate, colorectum, pre-menopausal breast, post-menopausal breast and lung. Databases were searched for epidemiological studies published between January 1996 and December 2002. On considering the association of IGF-I with lung cancer risk, four studies (Yu, *et al*., 1999; Lukanova, *et al*., 2001; London, *et al*., 2002; Spitz, *et al*., 2002) were looked at. When all four studies were considered together comparing the lowest and highest IGF-1 categories, there was an odds ratio of 1.01 (95% CI = 0.49-2.11). Three of the studies had odds ratios of less than one, with an odds ratio for the three studies combined of 0.74 (0.47-1.14). The other study (Yu, *et al.,* 1999) had an odds ratio of 2.75 (0.47-1.14). For IGFBP-3, three of the studies had odds ratios of less than one, with the other study (Spitz, *et al*., 2002), which investigated only heavy smokers and asbestos workers, giving an odds ratio of 2.35 (95% CI = 1.13-4.92) when comparing the highest and lowest quartiles of IGFBP-3 levels. Reduced IGFBP-3 was associated with increased lung cancer risk in this study but not in the other studies. The odds ratio for all studies was 0.83 (95% CI = 0.38-1.84), or 0.53 (95% CI = 0.34-0.83) if the Spitz study was omitted. The authors noted that heavy smoking can cause a reduction in circulating IGFBP-3 in a dose-related manner (citing Kaklamani, *et al*., 1999), and they claimed this showed that that attenuation of IGFBP-3 associations is not unexpected.

45. Morris *et al*. (2006) (see paragraph 39) conducted a meta-analysis. This included five studies; London *et al*., 2002; Wakai *et al*., 2002; Spitz, *et al*., 2002; Lukanova *et al*., 2001 and the data produced by Morris *et al*., 2006 resulting in a total of 843 cases and 11,072 controls. The meta-analysis used a random effects model, including nested case-control studies and comparing the highest quartile and the lowest quartiles. Publication bias was assessed by examining the funnel plot for each site and testing whether the regression of the study estimate with the study precision was significant. Sources of heterogeneity (year of publication, type of sample, time from sample collection to diagnosis and study location) were assessed with sub-group analysis. The odds ratio; 95%CI between the highest and lowest

quartiles of IGF-I was 1.02; 0.80-1.31. There was also no statistically significant association between IGFBP-3 and lung cancer (0.98; 0.61-1.58). There was significant heterogeneity in the IGFBP-3 analysis; this could be reduced by the exclusion of the study by Spitz *et al*. (2002) where the cohort of men were heavy smokers and asbestos workers.

46. Chen *et al*., (2009) conducted a meta- analysis of six nested case-control studies (Lukanova *et al*., 2001; London *et al*., 2002; Spitz *et al*., 2002; Waikai *et al*., 2002; Ahn *et al*., 2006; Morris *et al*., 2006) giving a total number of 1,043 cases and 11, 472 controls. Heterogeneity was assessed by the chi-square based Q test and publication bias was assessed by Eggers test and Begg's funnel plot. After performing the tests for heterogeneity, a fixed effects model was used to obtain summary statistics. It was concluded that IGF-I levels were not associated with lung cancer risk (OR; 95%CI 0.87 (95%CI 0.60-1.13) and WMD (weighted mean difference); 95%CI-3.05; 17.10—1.02, p= 0.14), but that IGFBP-3 acted as a tumour suppressor and had an inverse correlation with the risk of lung cancer 0.68; 0.48-0.88 and -112.28; 165.88- - 58.68), p= 0.0001).

47. Cao *et al*., 2012 conducted a meta-analysis of six nested case-control studies (1,043 cases and 11, 472 controls) and eight case-control studies (401 cases and 343 controls) were included. The nested case-control studies were Lukanova *et al*., 2001; London *et al*., 2002; Spitz *et al*., 2002; Wakai *et al*., 2002; Ahn *et al*., 2006; Morris *et al*., 2001 and the case-control studies were Bhatavdekar *et al*., 1994; Lee *et al*., 1999; Wu *et al*., 2000; Wang *et al*., 2004; Unsal *et al*., 2005 and Izycki *et al*., 2006. Heterogeneity between trials was assessed by the I^2 statistic. Depending on this statistic, a fixed or random effect model was used. Publication bias was assessed by Egger's test. IGFBP-3 was tested to have publication bias in the case control studies. Of the nested case control studies, the multivariate adjusted OR; (95%CIs for the highest vs lowest levels of IGF-I and IGFBP-3 were 1.047; 0.802-1.367, *p*=0736 and 0.960; 0.591-1.559, *p*=0.868 respectively. Standard mean differences (SMDs) were calculated to indicate the difference of the circulating IGF-I and IGFBP-3 concentrations between the lung cancer case group and the control group. For the nested case-control studies these were -0.079; -0.169-0.011, *p*= 0.086 and -0.097; -0.264- 0.071, *p*= 0.258 for IGF-I and IGFBP-3 respectively. For the case control studies the SMDs were 0.568; -0.035-1.171, p= 0.065 and -0.780; -1.358-0.201, *p*= 0.008 for IGF-I and IGFBP-3 respectively. It was concluded that there was an inverse association between IGFBP-3 and lung cancer risk in the case control studies but there was no association between IGF-I and lung cancer risk (consistent with the findings of the individual studies. The circulating level of IGFBP-3 underwent a decline during tumorigenesis and development of lung cancer which suggested IGFBP-3 was a promising candidate as a biomarker of lung cancer.

Summary and discussion: IGF-I and risk of lung cancer

48. Lung cancer is the third most common cancer in the UK with very low survival rates. Lung cancer can be divided into two types – Non Small Cell Lung Cancer and Small Cell Lung Cancer. Lung cancer is considered to be 89% avoidable with risk factors including smoking, occupational exposure and exposure to ionising radiation being associated with an increased risk of the condition.

49. It has been suggested that IGF-I may act with tobacco carcinogens to promote lung cancer and that it could also be involved in tumour de-differentiation.

50. Studies comparing circulating serum or plasma IGF-I levels in patients with lung cancer and controls have reported increased, decreased and no difference in the levels of IGF-I in the cancer patients. Since cancers may produce their own growth factors, the results are difficult to interpret.

51. The results of the prospective studies investigating levels of IGF-I and lung cancer risk are also inconsistent. Some studies report an association between IGF-I but the majority report no association.

52. One explanation for this variability may be the range of different study designs and the range of potentially confounding factors that may influence the results. These might include the classification of smoking or other relevant exposures and time between sample and diagnosis. Data on ethnicity are often absent which may be important if particular polymorphisms are relevant to IGF-I levels. The choice of assay may also be important since it is unclear to what extent active IGF-I is measured by the different procedures. IGF-I concentrations may be measured and reported as total or free IGF-I or this may not be specified. Some studies adjust the IGF-I results for IGFBP-3 and vice versa, and others present information on the IGF-I/IGFBP-3 molar ratio.

53. Several meta-analyses have also been performed. These produced results which generally did not show any association.

54. Results for an association with IGFBP-3 are more consistent. It has been suggested that high IGFBP-3 is protective by taking free IGF-I out of circulation, and this seems to be consistent with the available data.

Secretariat
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REFERENCES

Ahn, J., Weinstein, S., Snyder, K., Pollak, M., Virtamo, J., Albanes. (2006). No association between serum insulin-like growth factor (IGF)-I, IGF-Binding Protein-3 and lung cancer risk. Cancer Epidemiol. Biomarkers Prev. 15, 2010-2012.

Bhatavdekar, J.M., Patel, D.D., Chikhlikar, P.R., Mehta, R.H., Vora, H.H., Karelia, N.H., Hosh, >, Shah, N.G., Suthar, T.P., Neema, J.P. et *al*., 1994. Levels of circulating peptide and steroid hormones in men with lung cancer. Neoplasma, 41, 101-3.

Cancer Research UK (2016) http://www.cancerresearchuk.org/health-professional/lung-cancer-statistics accessed February 2016.

Cao, H., Wang, G., Meng, L., Shen, H., Feng, Z., Liu, G., Du J. (2012). Association between circulating levels of IGF-I and IGFBP-3 and lung cancer risk; A meta-analysis. PLOS one, 7, 1-8.

Chang, Y.S., Wang, L., Liu, D., Mao, L., Hong, W.K., Khuri, F.R., Lee, H-Y. (2002a). Correlation between Insulin-like Growth Factor-binding Protein 3 Promoter Methylation and Prognosis of Patients with Stage 1 Non-Small Cell Lung Cancer. Clinical Cancer Research, 8, 3669-3675.

Dilli, U.D., Yildirim, M., Suren, D., Alikanoglu, A., Kaya, V., Goktas, S., Yildiz, M., Cezer, C., Gunduz, S. (2014). Lack of any Prognostic role of Insulin-like growth factor-I receptor in Non-small cell lung cancer. Asian Pacific Journal of Cancer prevention, 15, 5753-5757.

Dziadziuszko R., Camidge, D.R., Hirsch, F.R. (2008). The insulin-like growth factor pathway in lung cancer. J. Thorac. Oncol, 3, 815-818.

El Aziz, S., Chadli, A., El Ghomari, H., Farouqi, A. (2012)/ Pulmonary epidermoid carcinoma in a patient with acromegaly: a rare entity. Pan African Medical Journal, 2012; 12: 27. Published online 2012 June 10.

Favoni, R.E., de Cupis, A., Ravera, F., Cantoni, C., Pirani, P., Ardizzoni, A., Noonan, D., Biassoni, R. (1994). Expression and function of the insulin-like growth factor I system in human non-small cell lung cancer and normal lung cell lines. Int. J. Cancer, 15, 858-866.

Fu, S.L., Tang, H.X., Liao, Y.D., Xu, Q.Z., Deng, Y., Fu, X.N. (2013) Association of pre-operative serum IGF-I concentration with clinicopathological parameters in patients with non-small cell lung cancer. J Huazhong Univ. Sci. Technolog. Med Sci, 33, 224-7.

Gualberto, A., Hixon, M.L., Karp, D.D., Green, S., Dolled-Filhart, M., Paz-Ares, L.G., Novello, S., Blakely, J., Langer, C.J., Pollak, M.N. (2011). Pre-treatment level of circulating free IGF-I identify NSCLC patients who derive clinical benefit from figitumumab. Br. J. Cancer, 131, E105-E112.

Han, J-Y., Choi, B.G., Choi, B.G., Lee, S.Y., Ju, S.Y. 2006. The prognostic significance of pre-treatment plasma levels of insulin-like growth factor (IGF)-I, IGF-“ and IGF binding protein-3 in patients with advanced non-small cell lung cancer. Lung Cancer, 54, 227-234.

Han SG, Park KH, Sung JS, Whang YM, Kim YJ, Shin ES, Lee JW, Lee HJ, Ryu JS, Choi IK, Seo JH, Kim YH. Single nucleotide polymorphisms of IGFBP-3 gene and lung cancer risk in a Korean population. Lung Cancer. 2008 Nov;62(2):152-61

Izycki, T., Chyczewska, E., Naumnik, W., Ossolinska, M. (2006) Serum levels of IGF-I and IGFBP-3 in patients with lung cancer during chemotherapy. Oncol. Res. 16, 49-54.
Kaiser, U., Schardt, C., Brandscheidt, D., Wollmer, E., Havemann K. (1993). Expression of insulin-like growth factor receptors I and II in normal human lung and in lung cancer. J Cancer Res. Clin. Oncol., 119, 665-8.

Kim, J-S., Kim, E.S., Liu, D., Lee, J.J., Solis, L., Behrens, C., Lippman, S.M., Hong, W.K., Wistuba, I.I., Lee, H-Y. (2014). Prognostic implicayions of Tumoral Expression of insulin-like growth factor (IGF)-I and 2 in patients with Non-small cell lung cancer. Clin. Lung Cancer, 15, 213-221.

Kim, W-Y., Jin, Q., Oh, S-H., Kim, E.S., Yang, Y.J., Lee, D.H., Feng, L., Behrens, C., Prudkin, L., Miller, Y.E., Lee, J.J., Lippman, S.M., Hong, W.K., Wistuba, I.I., Lee, H-Y. (2009). Elevated epithelial Insulin-like Growth Factor Expression is a risk for lung cancer development. Cancer Res., 69, 7439-7448.

Kim, W-Y., Kim, M.J., Moon, H., Yuan, P., Kim, J.S., Woo, J.K., Zhang, G., Suh, Y.A., Feng, L., Behrens, C., Van Pelt, C.S., Kang, H., Lee, J.J., Hong, W.K., Wistuba, I.I., Lee, H-Y. (2011). Differential impacts of Insulin-like Growth Factor-binding protein-3 (IGFBP-3) in epithelial IGF-induced lung cancer development. Endocrinology, 152, 2164-2173.

Lee, D-Y., Kim, S-J., Lee, Y-C. (1999). Serum Insulin-like Growth factor (IGF)-I and IGF-Binding Proteins in Lung Cancer Patients. J. Korean Med. Sci., 14, 401-404.

London SJ, Yuan JM, Travlos P, Akhmedkhanov A, *et al*., 2002, “Insulin-like growth factor-I, IGF-binding protein 3 and lung cancer risk in a prospective study of men in China”, J. Natl. Cancer Inst., 94: 749-754. .

Lukanova A, Toniolo P, Akhmeedkhanov A, *et al*., 2001, “A prospective study insulin-like growth factor-I, IGF-binding proteins-1, -2 and -3 and lung cancer risk in women”, Int. J. Cancer, 92: 888-892.

Matuschek, C., Rudoy, M., Peiper, M., Gerber, P.A., Hoff, N.P., Buhren, B.A., Flehmig, B., Budach, W., Knoefel, W.T., Bojar, H., Prisack, H.B., Steinbach, G., Shukla, V., Schwarz, A., Kammers, K., Erhardt, A., Scherer, A., Bölke, E., Schauer, M. (2011). Do Insulin-like growth factor associated proteins qualify as a Tumour marker? Results of a prospective study in 163 cancer patients. European Journal of Medical Research,. 16, 451-456.

Mazzoccoli G, Sothern RB, Pazienza V, Piepoli A, Muscarella LA, Giuliani F, Tarquini R. Circadian aspects of growth hormone-insulin-like growth factor axis function in patients with lung cancer. Clin Lung Cancer. 2012 Jan;13(1):68-74.

Morris JK, George LM, Wu T and Wald NJ, 2006, “Insulin-like growth factors and cancer: no role in screening. Evidence from the BUPA study and meta-analysis of prospective epidemiology studies”, Br. J. Cancer, 95: 112–117.

Pavelić, J., Križanac, Š., Kapitanović, S., Pavelić, L., Samaržija, M., Pavičić, F., Spaventi, Š., Jakopović, M., Herceg-Ivanovii, Z., Pavelić, K. (2005). The Consequences of insulin-like growth factors/receptors dysfunction in lung cancer. Am. J. Respir. Cell Mol. Biol. 32, 65-71.

Peng, Y., Dai, Y., Hitchcock, C., Yang, X., Kassis, E.S., Liu, L., Luo, Z., Sun, H-L., Cui, R., Wei, H., Kim, T., Lee, T.J., Jeon, Y-J., Nuovo, G.J., Volinia, S., He, Q., Yu, J., Nana-Sinkam, P., Croce, C.M. (2013). Insulin growth factor signalling is regulated by microRNA-486, an underexpressed microRNA in lung cancer. PNAS, 110, 15043-15048.

Rajski, M., Zanetti-Dällenbach, R., Vogel, B., Hermann, R., Rochlitz, C., Buess, M. (201). IGF-I induced genes in stromal fibroblasts predict the clinical outcome of breast and lung cancer. BMC Medicine, 8:1.

Renehan AG, Zwahlen M, Minder C, O’Dwyer ST, Shalet SM and Egger M, 2004, “Insulin-like growth factor (IGF)-I, IGF binding protein-3 and cancer risk: systematic review and meta-regression analysis”, Lancet, 363: 1346-1353.

Scagliotti, G.V., Novello, S. (2012), The role of the insulin-like growth factor signalling pathway in non-small cell lung cancer and other solid tumours. Cancer Treatment Reviews, 38, 292-302.

Shersher DD, Vercillo MS, Fhied C, Basu S, Rouhi O, Mahon B, Coon JS, Warren WH, Faber LP, Hong E, Bonomi P, Liptay MJ, Borgia JA. Biomarkers of the insulin-like growth factor pathway predict progression and outcome in lung cancer. Ann Thorac Surg. 2011 Nov;92(5):1805-11;

Spitz MR, Barnett MJ, Goodman GE, Thornquist MD, Wu, X, and Pollak M, 2002, “Serum insulin-like growth factor (IGF) and IGF-binding protein levels and risk of lung cancer: a case-control study nested in the beta-Carotene and Retinol Efficacy Trial Cohort”, Cancer Epidemiol. Biomarkers Prev., 11: 1413-1418.

Tang, H., Liao, Y., Chen, Z. (2012). Estrogen upregulates the IGF-I signalling pathway in lung cancer through estrogen receptor β. Med. Oncol, 29, 2640-2648.

Tang, H., Liao, Y., Xu, L., Zhang, C., Liu, Z., Deng, Y., Jiang, Z., Fu, S., Chen, Z., Zhou, S. (2013). Estrogen and insulin-like growth factor 1 synergistically promote the development of lung adenocarcinoma in mice. Int. J Cancer, 133, 2473-2482.

Ünsal, E., Köksal, D., Yurdakul, A.S., Atikan, Ş., Ciaz, P. (2005) Analysis og insulin like growth factor 1 and insulin like growth factor binding protein 3 levels in

bronchiolar lavage fluid and serum of patients with lung cancer. Respiratory Medicine, 99, 559-565.

Velchetti, V., Govindan, R. (2006). Insulin- like Growth Factor and Lung Cancer. Journal of Thoracic Oncology, 1, 607-610.

Vlachostergios PJ, Gioulbasanis I, Kamposioras K, Georgoulias P, Baracos VE, Ghosh S, Maragouli E, Georgoulias V, Papandreou CN. Baseline insulin-like growth factor-I plasma levels, systemic inflammation, weight loss and clinical outcome in metastatic non-small cell lung cancer patients. Oncology. 2011;81(2):113-8.

Wakai K, Ito Y, Suzuki K, Tamakoshi A, Seki N, Ando M, Ozasa K, Watanabe Y, Kondo T, Nishino Y, Ohno Y; JACC Study Group. Jpn J Cancer Res. 2002 Dec;93(12):1279-86. Serum insulin-like growth factors, insulin-like growth factor-binding protein-3, and risk of lung cancer death: a case-control study nested in the Japan Collaborative Cohort (JACC) Study.

Wang, H., Wan, Y.X., Zhang, QX. (2004). Significance and expression of insulin-like growth factor 1 and IGF binding protein 3 in serum of patients with lung cancer. Ai Zheng, 23, 710-714.

Wu X, Yu H, Amos CI, Hong Wk and Spitz MR, 2000, “Joint effect of insulin-like growth factors and mutagen sensitivity in lung cancer risk”, J. Natl. Cancer Inst., 92: 737-743.

Yu H, Spitz MR, Mistry J, Gu J, Hong WK and Wu X, 1999, “Plasma levels of IGF-1 and lung cancer risk”, J. Natl. Cancer Inst., 91(2): 151-156.

Zhang, M., Hu, Z., Huang, J., Shu, Y., Dai, J., Jin, G., Tang, R., Dong, J., Chen, Y., Xu, L., Huang, X,, Shen H. (2010) 3’ untranslated region polymorphism in IGF-I predicts survival of non-small cell lung cancer in a Chinese population. Clin. Cancer Res. 16, 1236-1244.

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET – Part 3

IGF-I and colorectal cancer and lung cancer.

DETAILS OF LITERATURE SEARCH

1. Question to be addressed: "Does the ingestion of IGF-I in the diet cause an increased risk of cancer in consumers?"

2. The starting point for obtaining documents on the dietary effects of IGF-I was the book "Your Life In Your Hands" by Jane Plant. All cited articles that referred to IGF-I were obtained. These articles were often not primary references to original research, so the original reports that were cited in the articles were obtained also.

3. Several searches of the literature were performed on computer by the FSA's Information Unit. The databases searched included PubMed and the British Library ETOC. Several combinations of keywords were used, including:

- IGF-I (title) AND food,
- IGF-I (title) AND cancer (all fields) filtered by Cancer Cells,
- IGF-I (title) factor AND digestion OR breakdown,
- IGF-I (title) AND absorption, IGF-1 (title) OR insulin-like growth factor AND gut AND lining OR lumen.

4. Less formal searches were also performed by the Secretariat using Google.

5. Articles were chosen from the results of the literature search according to the relevance of their titles and/or abstracts to:

- concentrations of IGF-I in foodstuffs,
- endogenous levels of IGF-I
- association of endogenous IGF-I levels with cancers,
- association of eating particular foods with cancer risks,
- toxicological or pharmacokinetic studies of IGF-I,
- possible mechanisms of action.

6. The selected articles were obtained. Further relevant articles were cited in the articles that had been obtained and copies of these too were ordered.

7. The Secretariat was unsuccessful in its attempt to obtain original copies of the full reports of toxicological studies submitted in support of the authorisation of use of IGF-I as a medicine for human patients. However, published summaries of the studies were available.

8. Not all of the obtained articles were cited in the review. Some did not meet the selection criteria (the same as the above list of relevances), despite their titles. Some repeated information given elsewhere. Wherever possible, the primary source of information was used.

9. In updating the search, a more ad hoc approach was taken working topic by topic. When the review of IGF-I is completed, the entire search will be updated to ensure that the available information is complete.