Insulin-like growth factor-1, insulin-like growth factor binding protein-3 and the incidence of malignant neoplasms in a nested case-control study

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The abbreviations used are: BMI, body mass index; CI, confidence intervals; DM, diabetes mellitus; IGF, Insulin-like growth factor; GH, growth hormone; IGFBP, IGF binding protein; IGF1R, IGF-1 receptor; JACC study, Japan Collaborative Cohort Study; OR, odds ratio.

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Abstract

Insulin-like growth factor (IGF)-1 is a potent mitogen, but IGF binding protein (IGFBP)-3 inhibits IGF1. To elucidate the relationship between both (IGF)-1 and IGFBP and the risk of tumorigenesis, the association between IGF1 and IGFBP3 serum levels and of malignant tumor incidence was investigated in a prospective case-control study nested in the Japan Collaborative Cohort (JACC) Study.

A baseline survey was started in 1988-1990, 110,585 subjects were enrolled, and 35% of participants donated blood samples. Those who had been diagnosed with malignant tumors by 1997 were considered cases. The analysis involved 1,349 cases and 4,012 controls. Conditional logistic regression was used to estimate odds ratios (ORs) for cancer incidence associated with IGF-related molecules.

After controlling for alcohol intake, BMI, and smoking, participants with high total-IGFBP3 and free-IGFBP3, which is estimated by the molar difference of (IGFBP3 - IGF1), had a risk of future neoplasms (p for trend= 0.014 and 0.009, respectively), but those with IGF1 did not. People in the second to fifth quintiles had a lower risk than those in the first quintile (ORs 0.676-0.736 and 0.657-0.870, respectively). Limiting subjects to those followed for 3 years weakened the negative associations of total- and free-IGFBP3, whereas a positive relationship of free-IGF1, which was estimated by the molar ratio of IGF1/IGFBP3, was seen (p for trend= 0.0016, 0.012, and 0.013, respectively). After controlling for alcohol intake, smoking, BMI, and diabetes mellitus, the results were confirmed.

These findings suggest that serum IGF1 and IGFBP3 are related to future risk of malignant neoplasms.

(250 words)

Introduction

Malignant tumors are among the most common causes of death worldwide. Based on estimates prepared by the World Health Organization in 2015, neoplasm is the first or second leading cause of death before the age of 70 years in 91 of 172 countries(1). Therefore, we must seek new risk factors for these diseases.

In a variety of human malignancies, signals from growth factors and their receptors are required not only for tumorigenesis, but also tumor progression(2,3). The insulin-like growth factor (IGF) axis, including both ligands (IGF1 and IGF2) and the receptor (type 1 insulin-like growth factor receptor, IGF1R), is one of these systems(3-5). IGFs bind to IGF1R and then activate multiple downstream signal axes, the mechanism of which is regulated by multiple factors under normal homeostatic conditions(6,7). Growth hormone, produced in the pituitary gland, stimulates the secretion of both IGFs and IGF-binding proteins (IGFBPs) 1-6 in hepatocytes. Activation of IGF1R is tightly regulated by the amount of the free form of the ligands, which is modulated by IGFBPs and the nonstimulatory receptor of type 2 IGF receptor(6,8). IGFBPs control IGF activity by reducing its bioavailability for binding to the receptor. Most of the IGF1 in the serum is in an inactive form due to binding with IGFBPs, which form a complex with IGF in a 1:1 molar ratio. IGFBP3 is the most plentiful IGFBP and accounts for almost 80% of its binding. Proteases, including matrix metalloproteinase (MMP), control the complex of IGFs and IGFBPs(9).

Since IGFs stimulate DNA synthesis and the proliferation of cells, the IGF system plays important roles not only in homeostasis, but also in premalignancy(10). In addition to cell growth, IGF shows antiapoptotic effects and thus has survival signals in

several tumor cells(11-18). Matrilysin (MMP-7) can cleave all IGFBPs and can thus trigger IGF signal pathways(19). We have previously reported a positive feedback loop between matrilysin and IGF-IGF1R in the invasion and progression of gastrointestinal carcinoma(20,21). We also previously reported that overexpression of both IGF1R and IGFs was associated with advanced pathological parameters, higher tumor stage, recurrence, and poor prognosis(18,21).

IGF1R could be one of the next important molecular targets in cancer therapy(3,22). IGF1R blockade, such as with tyrosine kinase inhibitors, monoclonal antibodies, dominant negative for IGF1R, and IGFBP3, suppressed proliferation, stimulated apoptosis, and inhibited tumor dissemination(13,18,23,24).

IGFBP3 is a tumor suppressor molecule. Downregulation of IGFBP3 upregulated tumor growth and suppressed apoptotic activity(25). IGFBP3 has potential as a drug, and the 16-kDa $^{1\text{-}95}$IGFBP3 fragment could potentiate apoptosis(26-28). IGFBP3 overexpression enhanced chemotherapy-induced growth inhibition due to inhibiting NF-kB(29). In addition, promoter hypermethylation of IGFBP3 might be a diagnostic and predictive biomarker for colorectal cancer(30,31).

Many epidemiologic studies have reported associations between diabetes mellitus (DM) and both cancer incidences and mortalities(32,33). DM is related to increased risk for carcinomas, especially colorectal, hepatic, and pancreatic carcinomas, in Japan(34), and colorectal, hepatic, pancreatic, breast, endometrial, and bladder cancers in the USA (35). DM was correlated with an overall 20% increased risk of total tumor incidence(36). In the Japan Collaborative Cohort (JACC) study, the risk of several site-specific cancer mortalities was reported to be increased in subjects with DM(37-39).

Elevated serum IGF1 levels or free IGF1 levels, which are calculated by the molar ratio of IGF1 to IGFBP3, increase the risk of developing several cancers, including breast, colon, and prostate cancers(40-42). In addition, low serum concentrations of IGFBP3 or free IGFBP3 levels, which are estimated by the molar difference (IGFBP3 - IGF1), increase the risk of some neoplasms, such as colon and liver cancers(42,43). However, there is insufficient information about the relationship between the incidence of whole malignant neoplasms and serum levels of IGF1 or IGFBP3. In previous reports about the associations of IGF1 or IGFBP3 with mortalities of all-causes, cardiovascular diseases, and cancer, IGF1 and IGFBP3 were inversely associated with cancer death in only male subjects in one study(44), but not in either sex in another study(45). In the previous and intermediate analysis in JACC study, IGFBP3 level was inversely associated with all cancer mortality but IGF1 was not(46). Although several associations between IGFs and the risk of several site-specific malignancies from the JACC Study were published(43,47,48), the incidence of overall malignant tumors has not been reported. Thus, the aim was to investigate the relationships between these factors and malignant tumor risk in a case-control study nested in a prospective cohort study, the JACC study.

Materials and Methods

Study population and serum samples

A nested case-control study within the JACC Study, which evaluated cancer risk associated with lifestyle factors, was conducted. The details of the JACC study were previously described(49-52). In brief, a baseline survey was started in 1988 - 1990 when

110,585 apparently healthy inhabitants (40 - 79 years old) who had undergone a general health checkup were enrolled as a basic cohort population from 45 areas throughout Japan. Participants were asked to complete a questionnaire that included information about demographic characteristics, medical history, and lifestyle factors. Approximately 35% of the cohort participants (39,242 subjects) in 37 out of 45 areas voluntarily provided peripheral blood samples, which were stored at −80°C until biochemical assays were performed.

Informed consent was obtained from each participant by having the study participants sign the cover of the questionnaire in the majority of study areas. However, it was obtained at the group level in a few areas because the concept of informed consent was not popularized during the 1980s in Japan. In that case, the municipality head gave the consent to participation representing the participants living in that area. This study was conducted in accordance with International Ethical Guidelines for Biomedical Research Invoving Human Subjects. The current study was approved by the human ethics review committee at Hokkaido University and was performed after approval by the institutional review board at Sapporo Medical University.

Follow-up, identification of malignant tumors, and control selection

In 24 of 45 study areas, the incidence of tumors was followed(51). Subjects were followed from the baseline survey. Participants with any malignant tumor history at baseline were excluded. Individuals who moved away from the original study area were treated as study dropouts, since deaths after such moves could not be confirmed in this follow-up system. The occurrence of tumors was confirmed in population-based tumor

registries or by reviewing the records of local major hospitals. Malignant neoplasms were defined as C00 - C97 according to the International Statistical Classification of Diseases and Related Health Problems 10th Revision (http://www.who.int/classifications/icd/en/). Subjects diagnosed with malignant tumors by 1997 were regarded as cases in this nested case-control study. For each case, 3 control subjects who were matched for residential area, age, and sex were selected randomly; however, less than 3 controls were selected in some cases based on the selection criteria(52). This analysis involved 1,349 cases and 4,012 control subjects.

Biochemical measurement of sera

In 1999 and 2000, both serum concentrations of IGF1 and IGFBP3 were assessed at a single laboratory (SRL, Tokyo, Japan) with an immunoradiometric assay using commercially available kits (Daiichi Radioisotope Lab., Tokyo, Japan) by technicians who were blinded to case/control status. The intra-assay precision obtained using different reference sera was 2.2-3.5% of the coefficients variation value for IGF1 and 3.2-4.2% of those for IGFBP3. The inter-assay coefficient of variance was 1.1-4.2% for IGF1 and 5.3-8.8% for IGFBP3. Details of the assays for both serum levels of IGF1 and IGFBP3 were as described earlier(53).

Statistical analysis

Proportions and mean values of baseline characteristics were compared between cases and controls using the *t*-test or Fisher's exact test. Results are shown as means ± SD. *P* values of less than 0.05 were considered significant. Serum levels were divided into quintiles based on the distribution of serum levels in all control subjects, with the

first quintile used as a reference. IGF1 quintile values for quintiles 1, 2, 3, 4, and 5 were <80, 80-109, 110-130, 131-160, and >160 ng/mL, respectively. IGFBP3 quintile values for quintiles 1, 2, 3, 4, and 5 were <2.31, 2.31-2.75, 2.76-3.17, 3.18-3.66, and >3.66 μg/mL, respectively.

Because the molar ratio of IGF1/IGFBP3 is believed to correspond to the free form of IGF1, the molar ratio of IGF1/IGFBP3 was evaluated (for conversion, 1 ng/mL is 0.130 n*M* for IGFI and 0.036 n*M* for IGFBP3)(42). Because the molar difference between IGFBP3 and IGF1 is considered to reflect the free form of IGFBP3, the molar difference of (IGFBP3 – IGF1) was assessed(43).

Using conditional logistic regression, the odds ratios (ORs) for the incidences of malignant tumors associated with serum levels of IGF-related peptides were determined. ORs were controlled for body mass index (BMI, computed as weight in kilograms divided by the square of the height in meters: <18.5, 18.5-24.9, 25.0-29.9, or ≥30.0 kg/m^2, or missing), alcohol consumption (never, former, or current drinker, or missing), and cigarette smoking status (never, former, or current smoker, or missing). ORs were also adjusted for BMI, alcohol consumption, tobacco smoking status, and DM for subjects who were divided into three groups: never received treatment, previously or currently receiving treatment, or missing. The significance of trends across exposure quintiles was assessed by including ordinal terms for each serum concentration quintile and entering the variable as a continuous term in the model. The statistical interaction with gender was calculated by including interaction terms in this model. All p values and 95% confidence intervals (CIs) presented were based on two-sided tests.

Results

Baseline characteristics of both cases and controls are shown in Table 1. There were no differences in height, weight, or BMI between cases and controls. The percentage of current smokers was higher in the case group than in the control group, whereas the percentage of never drinkers was higher in the controls than in the cases. No history of DM tended to be more common in controls than in cases, but the difference was not significant. The mean serum concentration of IGF1 was not different between the two groups. The mean serum IGFBP3 level was significantly lower in cases than in controls. Table 2 shows the sites of malignant neoplasm in the present study.

Concentration of total IGF1 was not associated with the risk of malignant neoplasms in univariate or multivariate analyses (Table 3). The total IGFBP3 level was associated inversely with the risk of malignant tumors (highest compared with lowest quintile: OR = 0.693; 95% CI = 0.558-0.861; p for trend = 0.001). After adjustment for IGF1, the result was strengthened (highest compared with lowest quintile: OR = 0.667; 95% CI = 0.518-0.859; p for trend = 0.003). After full adjustment including DM, the result was similar (highest compared with lowest quintile: OR = 0.707; 95% CI = 0.546-0.916; p for trend = 0.012).

A higher molar ratio of IGF1/IGFBP3, which correspond to free IGF1, was associated with an increased risk of malignant neoplasms (highest compared with lowest quintile: OR, 1.218; 95% CI, 0.957-1.549; p for trend = 0.041, Table 4). However, the trend was not significant after adjustments for other covariates.

A higher molar difference of IGFBP3 and IGF1, which represents free IGFBP3, was associated with a decreased risk of malignant neoplasms (highest compared with lowest quintile: OR, 0.698; 95% CI, 0.563-0.866; p for trend = 0.001, Table 4). After full controlling including DM, the result was not changed (highest compared with lowest quintile: OR, 0.737; 95% CI, 0.592-0.918; p for trend = 0.007).

In order to exclude possible effects of latent malignant tumors on levels of both IGF1 and IGFBP3, the analysis was limited to subjects followed over 3 years (885 cases and 2638 controls, Table 5). Although there was no association between total IGF1 and the risk of malignancies, free IGF1 was related to the risk of malignancies (highest compared with lowest quintile: OR, 1.430; 95% CI, 1.053-1.942; p for trend = 0.013). After fully controlling for DM, free IGF1 was related to the risk of neoplasms (highest compared with lowest quintile: OR, 1.357; 95% CI, 0.994-1.853; p for trend = 0.038). This analysis strengthened the negative relationships of both total and free IGFBP3 with the risk of malignant tumors (highest compared with lowest quintile: OR, 0.674 and 0.669; 95% CI, 0.514-0.883 and 0.513-0.873; p for trend = 0.004 and 0.002, respectively). After full adjustment including DM, both total and free IGFBP3 were negatively associated with the risk of malignancies (highest compared with lowest quintile: OR, 0.680 and 0.715; 95% CI, 0.493-0.939 and 0.544-0.939; p for trend = 0.022 and 0.009, respectively).

Then, we evaluated the statistical interaction with gender. Although both total IGF1 and IGFBP3 were interacted with gender (p for interaction = 0.005 and 0.013, respectively), those interaction were not detected after adjustment each other (p for interaction = 0.109 and 0.207, respectively). Free IGFBP3 showed an interaction with gender, however free IGF1 did not (p for interaction = 0.024 and 0.645, respectively).

Then, ORs were analyzed in gender subgroups. In the male population, total IGF1 was associated inversely with the risk of tumors, but not after adjustment with IGFBP3 (highest compared with lowest quintile: OR, 0.680 and 0.979; 95% CI, 0.486-0.951 and 0.659-1.456; p for trend = 0.003 and 0.422, respectively, Table 6). Both higher total and free IGFBP3 were also inversely associated to the risk of malignancies in male subjects (highest compared with lowest quintile: OR, 0.599 and 0.609; 95% CI, 0.5440-0.815 and 0.445-0.831; respectively, p for trend < 0.001). After several adjustments, these relationships were observed again (highest compared with lowest quintile: OR, 0.678 0.705; 95% CI, 0.492-0.866 – 0.486-1.023; p for trend = 0.002 - 0.028). However, there were no relationships between both total and free IGFBP3 and the risk of malignant tumors in the female subjects. Although total IGF1 was not related to the incidence of malignancies, free IGF1 tended to be associated with the risk of neoplasms, but not significantly, even after several adjustments. These findings reinforced the association between a decrease in both total and free IGFBP3 and the risk of malignancies in males.

Discussion

IGFs play several roles in carcinogenesis and tumor dissemination, though IGFBPs can inhibit those actions(3,18,24). In the present study, although the serum level of total IGF1 was not associated with the OR for overall malignant tumors, that of IGFBP3 was associated inversely with the OR for all neoplasms. After 3 adjustments, the results were observed. Although we assessed incident of malignant tumors, the present results resemble the former studies in which IGFBP3 was associated cancer mortality, but IGF1 was not(44-46).

Although free IGF1 was associated with the risk for all cancers, the association was not significant after adjusting for BMI, alcohol intake, and smoking, with/without DM. IGFs form a complex with IGFBPs in a 1:1 molar ratio, and IGFBP3 is higher molar than IGF1. Thus, the molar difference of (IGFBP3 - IGF1) could estimate the serum level of free IGFBP3(43). Free IGFBP3 was related inversely to the risk of overall tumors, which was observed even by 2 analyses with adjustments. It might be reasonable that both total and free IGFBP3 were inversely related to the risk for overall malignancies, as IGFBP3 is a tumor suppressor molecule.

Limiting subjects to those followed over 3 years, high free IGF1 enhanced the risk of malignant neoplasms, and both high total and high free IGFBP3 reduced the risk of tumors. After adjusting for BMI, alcohol intake, and smoking, with/without DM, these results were observed again. These results suggest that there were latent patients with cancer among the subjects in the JACC study. Moreover, it might be reasonable that tumorigenic factors of IGF1 are related to the incidence of malignant tumors.

Excessive insulin action associated with insulin resistance in type 2 DM might contribute to tumorigenicity. As both insulin and IGFs have high homology, and both insulin receptor and IGF1R keep high homology, both ligands and receptors could bind to one another(54). Thus, the effects of hyperinsulinemia on carcinogenesis might be accounted for in part by IGF1R activation. After controlling for a history of DM, free IGF1 and both total and free IGFBP3 affected the future risk of tumorigenicity in the present study.

The negative relationships between total and free IGFBP3 and the incidence of malignant tumors in the total participants were only seen in the male participants in the present study. It has been reported that there were sex differences in the effects of IGFs

on the incidence of site-specific tumors(44,55,56). The current results resemble the former report in which both total IGF1 and IGFBP3 were inversely associated with cancer mortality in males but not in females(44). One of the reasons for the sex difference in the current study might depend on the sex differences in serum distributions of both IGF1 and IGFBP3. In control participants, the serum level of IGF1 was significantly lower in female than in male participants ($p < 0.001$, Table 1), and that of IGFBP3 was significantly higher in female than in male participants ($p < 0.001$). As serum IGF1 was reported to be higher in female than in male subjects in previous reports(7), it might be a feature of this cohort that the IGF1 level in female controls was low. In the present male group, levels of both IGF1 and IGFBP3 were lower in cases than in controls ($p = 0.034$ and < 0.001, respectively). However, there were no differences in the serum concentrations of IGF1 and IGFBP3 between female cases and female controls in the present study. Since levels of IGF1 and IGFBP3 levels were high in the present cases compared to cases in previous studies(7); low IGF1 levels in the male cases of this study may also be a feature of this cohort. Another reason might depend on tumor distributions. Although esophageal, gastric, liver, and lung carcinomas were more common in male than female participants, thyroid cancer was more common in female than male participants in the present cohort (Table 2).

The advantage of this study is that the samples were from a large-scale, population-based study (110,792 participants). There are, however, several limitations in the current study. The first limitation is that the serum levels of IGFBP3 and IGF1 were assayed only once, at the time of the baseline inquiry. Therefore, their chronological changes were not observed in association with the incidence of malignancies. Another limitation is that frozen serum samples were used to measure

levels of both IGFBP3 and IGF1. The IGFs have been reported to remain stable in frozen serum that was stored at −80°C for 9 years(53). The third limitation is that some data about BMI, alcohol intake, smoking, and history of DM were missing in the JACC study because of the self-administered questionnaire(49-51). The fourth limitation is that the participants were only Japanese.

In conclusion, both low serum total IGFBP3 and low free IGFBP3 (molar difference of IGFBP3 – IGF1) might be related to future risk of malignant tumors. In addition, high free-IGF1 (molar ratio of IGF1/IGFBP3) might be associated with the future risk of malignant neoplasms.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin **2018** doi 10.3322/caac.21492.
2. Baserga R. Oncogenes and the strategy of growth factors. Cell **1994**;79(6):927-30.
3. Adachi Y, Yamamoto H, Ohashi H, Endo T, Carbone DP, Imai K, *et al.* A candidate targeting molecule of insulin-like growth factor-I receptor for gastrointestinal cancers. World J Gastroenterol **2010**;16(46):5779-89.
4. Adachi Y, Yamamoto H, Imsumran A, Oka T, Oki M, Nosho K, *et al.* Insulin-like growth factor-I receptor as a candidate for a novel molecular target in the gastrointestinal cancers. Dig Endosc **2006**;18(4):245-51.
5. Yee D. A tale of two receptors: insulin and insulin-like growth factor signaling in cancer. Clin Cancer Res **2015**;21(4):667-9 doi 10.1158/1078-0432.CCR-14-2056.
6. Foulstone E, Prince S, Zaccheo O, Burns JL, Harper J, Jacobs C, *et al.* Insulin-like growth factor ligands, receptors, and binding proteins in cancer. J Pathol **2005**;205(2):145-53 doi 10.1002/path.1712.
7. Gu F, Schumacher FR, Canzian F, Allen NE, Albanes D, Berg CD, *et al.* Eighteen insulin-like growth factor pathway genes, circulating levels of IGF-I and its binding protein, and risk of prostate and breast cancer. Cancer Epidemiol Biomarkers Prev **2010**;19(11):2877-87 doi 10.1158/1055-9965.EPI-10-0507.
8. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev **2002**;23(6):824-54 doi 10.1210/er.2001-0033.
9. Miyamoto S, Nakamura M, Yano K, Ishii G, Hasebe T, Endoh Y, *et al.* Matrix metalloproteinase-7 triggers the matricrine action of insulin-like growth factor-II via proteinase activity on insulin-like growth factor binding protein 2 in the extracellular matrix. Cancer Sci **2007**;98(5):685-91 doi CAS448 [pii]10.1111/j.1349-7006.2007.00448.x.

10. Tchorzewski MT, Qureshi FG, Duncan MD, Duncan KL, Saini N, Harmon JW. Role of insulin-like growth factor-I in esophageal mucosal healing processes. J Lab Clin Med **1998**;132(2):134-41.
11. Caro JF, Poulos J, Ittoop O, Pories WJ, Flickinger EG, Sinha MK. Insulin-like growth factor I binding in hepatocytes from human liver, human hepatoma, and normal, regenerating, and fetal rat liver. J Clin Invest **1988**;81(4):976-81.
12. Thompson MA, Cox AJ, Whitehead RH, Jonas HA. Autocrine regulation of human tumor cell proliferation by insulin-like growth factor II: an in-vitro model. Endocrinology **1990**;126(6):3033-42.
13. Chen SC, Chou CK, Wong FH, Chang CM, Hu CP. Overexpression of epidermal growth factor and insulin-like growth factor-I receptors and autocrine stimulation in human esophageal carcinoma cells. Cancer Res **1991**;51(7):1898-903.
14. Remacle-Bonnet M, Garrouste F, el Atiq F, Roccabianca M, Marvaldi J, Pommier G. des-(1-3)-IGF-I, an insulin-like growth factor analog used to mimic a potential IGF-II autocrine loop, promotes the differentiation of human colon-carcinoma cells. Int J Cancer **1992**;52(6):910-7.
15. Lahm H, Suardet L, Laurent PL, Fischer JR, Ceyhan A, Givel JC, *et al.* Growth regulation and co-stimulation of human colorectal cancer cell lines by insulin-like growth factor I, II and transforming growth factor alpha. Br J Cancer **1992**;65(3):341-6.
16. Bergmann U, Funatomi H, Yokoyama M, Beger HG, Korc M. Insulin-like growth factor I overexpression in human pancreatic cancer: evidence for autocrine and paracrine roles. Cancer Res **1995**;55(10):2007-11.
17. Ajisaka H, Fushida S, Yonemura Y, Miwa K. Expression of insulin-like growth factor-2, c-MET, matrix metalloproteinase-7 and MUC-1 in primary lesions and lymph node metastatic lesions of gastric cancer. Hepatogastroenterology **2001**;48(42):1788-92.
18. Imsumran A, Adachi Y, Yamamoto H, Li R, Wang Y, Min Y, *et al.* Insulin-like growth factor-I receptor as a marker for prognosis and a therapeutic target in human esophageal squamous cell carcinoma. Carcinogenesis **2007**;28(5):947-56 doi 10.1093/carcin/bgl247.

19. Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, *et al.* Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. Biochem Biophys Res Commun **2005**;333(3):1011-6 doi S0006-291X(05)01211-8 [pii]10.1016/j.bbrc.2005.06.010.
20. Adachi Y, Li R, Yamamoto H, Min Y, Piao W, Wang Y, *et al.* Insulin-like growth factor-I receptor blockade reduces the invasiveness of gastrointestinal cancers via blocking production of matrilysin. Carcinogenesis **2009**;30(8):1305-13 doi 10.1093/carcin/bgp134.
21. Ohashi H, Adachi Y, Yamamoto H, Taniguchi H, Nosho K, Suzuki H, *et al.* Insulin-like growth factor receptor expression is associated with aggressive phenotypes and has therapeutic activity in biliary tract cancers. Cancer Sci **2012**;103(2):252-61 doi 10.1111/j.1349-7006.2011.02138.x.
22. Miller BS, Yee D. Type I insulin-like growth factor receptor as a therapeutic target in cancer. Cancer Res **2005**;65(22):10123-7.
23. Takaoka M, Harada H, Andl CD, Oyama K, Naomoto Y, Dempsey KL, *et al.* Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3. Cancer Res **2004**;64(21):7711-23.
24. Adachi Y, Ohashi H, Imsumran A, Yamamoto H, Matsunaga Y, Taniguchi H, *et al.* The effect of IGF-I receptor blockade for human esophageal squamous cell carcinoma and adenocarcinoma. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine **2014**;35(2):973-85 doi 10.1007/s13277-013-1131-2.
25. Luo LL, Zhao L, Wang YX, Tian XP, Xi M, Shen JX, *et al.* Insulin-like growth factor binding protein-3 is a new predictor of radiosensitivity on esophageal squamous cell carcinoma. Sci Rep **2015**;5:17336 doi 10.1038/srep17336.
26. Kirman I, Poltoratskaia N, Sylla P, Whelan RL. Insulin-like growth factor-binding protein 3 inhibits growth of experimental colocarcinoma. Surgery **2004**;136(2):205-9 doi 10.1016/j.surg.2004.04.020.
27. Cheung SC, Long X, Liu L, Liu Q, Lan L, Tong PC, *et al.* Inhibition of human MCF-7 breast cancer cells and HT-29 colon cancer cells by rice-produced

recombinant human insulin-like growth binding protein-3 (rhIGFBP-3). PLoS One **2013**;8(10):e77516 doi 10.1371/journal.pone.0077516.

28. H-Zadeh AM, Collard TJ, Malik K, Hicks DJ, Paraskeva C, Williams AC. Induction of apoptosis by the 16-kDa amino-terminal fragment of the insulin-like growth factor binding protein 3 in human colonic carcinoma cells. Int J Oncol **2006**;29(5):1279-86.
29. Kim MS, Lee DY. Insulin-like growth factor binding protein-3 enhances etoposide-induced cell growth inhibition by suppressing the NF-kappaB activity in gastric cancer cells. Mol Cell Biochem **2015**;403(1-2):107-13 doi 10.1007/s11010-015-2341-2.
30. Perez-Carbonell L, Balaguer F, Toiyama Y, Egoavil C, Rojas E, Guarinos C, *et al.* IGFBP3 methylation is a novel diagnostic and predictive biomarker in colorectal cancer. PLoS One **2014**;9(8):e104285 doi 10.1371/journal.pone.0104285.
31. Fu T, Pappou EP, Guzzetta AA, Calmon Mde F, Sun L, Herrera A, *et al.* IGFBP-3 Gene Methylation in Primary Tumor Predicts Recurrence of Stage II Colorectal Cancers. Ann Surg **2016**;263(2):337-44 doi 10.1097/SLA.0000000000001204.
32. Noto H, Tsujimoto T, Sasazuki T, Noda M. Significantly increased risk of cancer in patients with diabetes mellitus: a systematic review and meta-analysis. Endocr Pract **2011**;17(4):616-28 doi 10.4158/EP10357.RA.
33. Noto H, Tsujimoto T, Noda M. Significantly increased risk of cancer in diabetes mellitus patients: A meta-analysis of epidemiological evidence in Asians and non-Asians. J Diabetes Investig **2012**;3(1):24-33 doi 10.1111/j.2040-1124.2011.00183.x.
34. Kasuga M, Ueki K, Tajima N, Noda M, Ohashi K, Noto H, *et al.* Report of the Japan Diabetes Society/Japanese Cancer Association Joint Committee on Diabetes and Cancer. Cancer Sci **2013**;104(7):965-76 doi 10.1111/cas.12203.
35. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, *et al.* Diabetes and cancer: a consensus report. CA Cancer J Clin **2010**;60(4):207-21 doi 10.3322/caac.20078.

36. Sasazuki S, Charvat H, Hara A, Wakai K, Nagata C, Nakamura K, *et al.* Diabetes mellitus and cancer risk: pooled analysis of eight cohort studies in Japan. Cancer Sci **2013**;104(11):1499-507 doi 10.1111/cas.12241.
37. Khan M, Mori M, Fujino Y, Shibata A, Sakauchi F, Washio M, *et al.* Site-specific cancer risk due to diabetes mellitus history: evidence from the Japan Collaborative Cohort (JACC) Study. Asian Pac J Cancer Prev **2006**;7(2):253-9.
38. Washio M, Mori M, Khan M, Sakauchi F, Watanabe Y, Ozasa K, *et al.* Diabetes mellitus and kidney cancer risk: the results of Japan Collaborative Cohort Study for Evaluation of Cancer Risk (JACC Study). Int J Urol **2007**;14(5):393-7 doi 10.1111/j.1442-2042.2007.01744.x.
39. Tan C, Mori M, Adachi Y, Wakai K, Suzuki S, Suzuki K, *et al.* Diabetes Mellitus and Risk of Colorectal Cancer Mortality in Japan: the Japan Collaborative Cohort Study. Asian Pac J Cancer Prev **2016**;17(10):4681-8.
40. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, Wilkinson P, *et al.* Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. Science **1998**;279(5350):563-6.
41. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, *et al.* Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet **1998**;351(9113):1393-6. doi 10.1016/S0140-6736(97)10384-1.
42. Ma J, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, *et al.* 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 **1999**;91(7):620-5.
43. Adachi Y, Nojima M, Mori M, Matsunaga Y, Akutsu N, Sasaki S, *et al.* Insulin-like growth factor-related components and the risk of liver cancer in a nested case-control study. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine **2016**;37(11):15125-32 doi 10.1007/s13277-016-5360-z.
44. Friedrich N, Haring R, Nauck M, Ludemann J, Rosskopf D, Spilcke-Liss E, *et al.* Mortality and serum insulin-like growth factor (IGF)-I and IGF binding protein 3 concentrations. J Clin Endocrinol Metab **2009**;94(5):1732-9 doi 10.1210/jc.2008-2138.

45. Saydah S, Graubard B, Ballard-Barbash R, Berrigan D. Insulin-like growth factors and subsequent risk of mortality in the United States. Am J Epidemiol **2007**;166(5):518-26 doi 10.1093/aje/kwm124.
46. Pham TM, Fujino Y, Nakachi K, Suzuki K, Ito Y, Watanabe Y, *et al.* Relationship between serum levels of insulin-like growth factors and subsequent risk of cancer mortality: findings from a nested case-control study within the Japan Collaborative Cohort Study. Cancer Epidemiol **2010**;34(3):279-84 doi 10.1016/j.canep.2010.03.017.
47. Lin Y, Tamakoshi A, Kikuchi S, Yagyu K, Obata Y, Ishibashi T, *et al.* Serum insulin-like growth factor-I, insulin-like growth factor binding protein-3, and the risk of pancreatic cancer death. Int J Cancer **2004**;110(4):584-8 doi 10.1002/ijc.20147.
48. Sakauchi F, Nojima M, Mori M, Wakai K, Suzuki S, Tamakoshi A, *et al.* Serum insulin-like growth factors I and II, insulin-like growth factor binding protein-3 and risk of breast cancer in the Japan Collaborative Cohort study. Asian Pac J Cancer Prev **2009**;10 Suppl:51-5.
49. Ohno Y, Tamakoshi A, Group JS. Japan collaborative cohort study for evaluation of cancer risk sponsored by monbusho (JACC study). J Epidemiol **2001**;11(4):144-50.
50. Tamakoshi A, Yoshimura T, Inaba Y, Ito Y, Watanabe Y, Fukuda K, *et al.* Profile of the JACC study. J Epidemiol **2005**;15 Suppl 1:S4-8.
51. Tamakoshi A, Ozasa K, Fujino Y, Suzuki K, Sakata K, Mori M, *et al.* Cohort profile of the Japan Collaborative Cohort Study at final follow-up. J Epidemiol **2013**;23(3):227-32.
52. Tamakoshi A, Suzuki K, Ito Y, Watanabe Y, Inaba Y, Tajima K, *et al.* Selection of cases and controls for the nested case-control study within the Japan Collaborative Cohort Study: the First-wave. Asian Pac J Cancer Prev **2009**;10 Suppl:1-5.
53. Ito Y, Nakachi K, Imai K, Hashimoto S, Watanabe Y, Inaba Y, *et al.* Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor beta,

soluble Fas, and superoxide dismutase activity for the JACC study. J Epidemiol **2005**;15 Suppl 1:S67-73.

54. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, *et al.* Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. Embo J **1986**;5(10):2503-12.

55. Adachi Y, Nojima M, Mori M, Yamashita K, Yamano HO, Nakase H, *et al.* Insulin-like growth factor-1, IGF binding protein-3, and the risk of esophageal cancer in a nested case-control study. World J Gastroenterol **2017**;23(19):3488-95 doi 10.3748/wjg.v23.i19.3488.

56. Yamaji T, Iwasaki M, Sasazuki S, Tsugane S. Gender difference in the association of insulin and the insulin-like growth factor axis with colorectal neoplasia. Int J Obes (Lond) **2012**;36(3):440-7 doi 10.1038/ijo.2011.114.

Table 1. Selected baseline characteristics of case and control groups

		Cases	Controls	p value
Number of subjects		1349	4012	
Age (mean ± SD)		63.7 ± 8.4	63.6 ± 8.3	0.678
Male (n)		713 (52.9%)	2110 (52.6%)	0.875*
Height (cm; mean ± SD)		156.0 ± 8.3	155.4 ± 8.1	0.212
Weight (kg; mean ± SD)		55.0 ± 8.9	54.9 ± 8.7	0.571
BMI (kg/m2; mean ± SD)		22.7 ± 3.2	22.7 ± 3.0	0.922
Cigarette smoking (n)		1266	3749	< 0.001*, #
	Never	617 (48.7%)	2106 (56.2%)	
	Past	217 (17.1%)	674 (18.0%)	
	Current	432 (34.1%)	969 (25.8%)	
Alcohol intake (n)		1280	3827	0.001*, #
	Never	609 (47.6%)	1889 (49.4%)	
	Past	73 (5.7%)	129 (3.4%)	
	Current	598 (46.7%)	1809 (47.3%)	
Diabetes Mellitus (n)		1236	3679	0.057*
	Never	1151 (93.1%)	3480 (94.6%)	
	Current / Past	85 (6.9%)	199 (5.4%)	
IGF1 (ng/mL; mean ± SD)	Total	124.0 ± 56.4	125.6 ± 57.1	0.348
	Male	123.6 ± 55.1	128.9 ± 58.0	0.034 #
	Female	124.3 ± 55.9	122.0 ± 55.9	0.372
IGFBP3 (μg/mL; mean ± SD)	Total	2.93 ± 0.89	3.01 ± 0.83	0.002 #
	Male	2.73 ± 0.88	2.87 ± 0.82	< 0.001 #
	Female	3.16 ± 0.86	3.17 ± 0.81	0.801

* Chi-squared test, # $p<0.05$

Table 2. Sites of malignant neoplasm cases

Site	ICD-10 code	Total (%)	Male	Female
Esophagus	C15	26 (1.9%)	24	2
Stomach	C16	308 (22.8%)	170	138
Colorectal	C18-20	180 (13.3%)	86	94
Liver and intrahepatic bile ducts	C22	96 (7.1%)	60	36
Gallbladder and extrahepatic bile ducts	C23-24	73 (5.4%)	32	41
Pancreas	C25	72 (5.3%)	33	39
Bronchus and lung	C34	214 (15.9%)	160	54
Breast	C50	65 (4.8%)	0	65
Cervix	C53	10 (0.7%)	0	10
Uterus	C54	17 (1.3%)	0	17
Ovary	C56	13 (1.0%)	0	13
Prostate	C61	40 (3.0%)	40	0
Thyroid	C73	31 (2.3%)	3	28
Hodgkin's disease and lymphoma	C81-85	27 (2.0%)	11	16
Myeloma	C90	17 (1.3%)	5	12
Leukemia	C91-95	16 (1.2%)	7	9
Other		144 (10.7%)	82	62
Total		1349 (100%)	713	636

Table 3. Odds ratios and 95% confidence intervals for all malignant tumors with reference to serum concentrations of IGF1 and IGFBP3

		Quintile					
		1 (reference)	2	3	4	5	p for trend
IGF1							
	ng/mL (range)	< 80	80 - 109	110 - 130	131 - 160	> 160	
	No. of case / control	275 / 773	232 / 668	319 / 940	247 / 790	276 / 841	
	OR (95% CI)	1	0.942 (0.751 - 1.182)	0.909 (0.730 - 1.132)	0.829 (0.655 - 1.050)	0.869 (0.684 - 1.104)	0.156
	OR adjusted 1 (95% CI)	1	1.046 (0.829 - 1.321)	1.074 (0.849 - 1.358)	1.038 (0.798 - 1.350)	1.106 (0.836 - 1.461)	0.679
	OR adjusted 2 (95% CI)	1	1.036 (0.819 - 1.311)	1.038 (0.819 - 1.317)	1.020 (0.782 - 1.332)	1.057 (0.797 - 1.403)	0.901
	OR adjusted 3 (95% CI)	1	1.037 (0.819 - 1.312)	1.042 (0.822 - 1.321)	1.020 (0.781 - 1.331)	1.056 (0.796 - 1.401)	0.913
IGFBP3							
	μg/mL (range)	< 2.31	2.31 - 2.75	2.76 - 3.17	3.18 - 3.66	> 3.66	
	No. of case / control	345 / 808	249 / 803	261 / 810	239 / 793	255 / 798	
	OR (95% CI)	1	0.708 (0.583 - 0.859)	0.721 (0.592 - 0.878)	0.661 (0.536 - 0.815)	0.693 (0.558 - 0.861)	0.001 #
	OR adjusted 1 (95% CI)	1	0.698 (0.571 - 0.853)	0.706 (0.570 - 0.873)	0.643 (0.509 - 0.813)	0.667 (0.518 - 0.859)	0.003 #
	OR adjusted 2 (95% CI)	1	0.730 (0.595 - 0.895)	0.736 (0.593 - 0.915)	0.676 (0.532 - 0.859)	0.712 (0.550 - 0.922)	0.014 #
	OR adjusted 3 (95% CI)	1	0.729 (0.595 - 0.894)	0.733 (0.590 - 0.911)	0.673 (0.530 - 0.855)	0.707 (0.546 - 0.916)	0.012 #

adjusted 1, adjusted for IGF1 or IGFBP3; adjusted 2, adjusted for cigarette smoking, BMI, alcohol intake, and IGF1 or IGFBP3;
adjusted 3, adjusted for cigarette smoking, BMI, alcohol intake, and diabetes mellitus; # $p<0.05$

Table 4. Odds ratios and 95% confidence intervals for all malignant tumors according to molar ratio and difference of IGF1 and IGFBP3

		Quintile					
		1 (reference)	2	3	4	5	p for trend
IGF1/IGFBP3							
	molar ratio	< 0.108	0.108 - 0.138	0.139 - 0.163	0.164 - 0.193	> 0.193	
	No. of case / control	266 / 803	252 / 814	255 / 790	271 / 805	305 / 800	
	OR (95% CI)	1	0.963 (0.771 - 1.203)	1.016 (0.804 - 1.285)	1.070 (0.842 - 1.359)	1.218 (0.957 - 1.549)	0.041 #
	OR adjusted 1 (95% CI)	1	0.946 (0.755 - 1.186)	0.989 (0.780 - 1.254)	1.030 (0.808 - 1.313)	1.158 (0.907 - 1.479)	0.111
	OR adjusted 2 (95% CI)	1	0.948 (0.757 - 1.188)	0.986 (0.778 - 1.250)	1.025 (0.804 - 1.306)	1.156 (0.905 - 1.476)	0.120
IGFBP3 - IGF1							
	molar difference	< 70.14	70.14 - 83.04	83.05 - 96.84	96.85 - 112.12	> 112.12	
	No. of case / control	343 / 805	231 / 804	295 / 800	225 / 803	225 / 800	
	OR (95% CI)	1	0.663 (0.546 - 0.807)	0.834 (0.690 - 1.009)	0.625 (0.507 - 0.771)	0.698 (0.563 - 0.866)	0.001 #
	OR adjusted 1 (95% CI)	1	0.687 (0.564 - 0.838)	0.870 (0.717 - 1.056)	0.657 (0.531 - 0.813)	0.742 (0.596 - 0.924)	0.009 #
	OR adjusted 2 (95% CI)	1	0.689 (0.565 - 0.839)	0.867 (0.714 - 1.053)	0.656 (0.530 - 0.812)	0.737 (0.592 - 0.918)	0.007 #

adjusted 1, adjusted for cigarette smoking, BMI, and alcohol intake

adjusted 2, adjusted for cigarette smoking, BMI, alcohol intake, and diabetes mellitus ; # $p<0.05$

Table 5. Odds ratios and 95% confidence intervals for all malignant tumors followed over 3 years.

		Quintile					
		1 (reference)	2	3	4	5	p for trend
IGF1							
	ng/mL (range)	< 80	80 - 109	110 - 130	131 - 160	> 160	
	No. of case / control	161 / 492	158 / 427	219 / 601	162 / 539	185 / 579	
	OR (95% CI)	1	1.145 (0.858 - 1.529)	1.114 (0.843 - 1.471)	0.913 (0.677 - 1.230)	0.963 (0.712 - 1.302)	0.274
	OR adjusted 1 (95% CI)	1	1.281 (0.952 - 1.725)	1.317 (0.978 - 1.775)	1.157 (0.828 - 1.617)	1.258 (0.886 - 1.787)	0.573
	OR adjusted 2 (95% CI)	1	1.266 (0.937 - 1.710)	1.281 (0.946 - 1.734)	1.126 (0.802 - 1.582)	1.211 (0.848 - 1.732)	0.733
	OR adjusted 3 (95% CI)	1	1.267 (0.938 - 1.712)	1.290 (0.952 - 1.746)	1.127 (0.803 - 1.583)	1.212 (0.848 - 1.733)	0.733
IGFBP3							
	μg/mL (range)	< 2.31	2.31 - 2.75	2.76 - 3.17	3.18 - 3.66	> 3.66	
	No. of case / control	218 / 526	167 / 516	175 / 533	162 / 529	163 / 534	
	OR (95% CI)	1	0.761 (0.600 - 0.966)	0.755 (0.591 - 0.963)	0.688 (0.531 - 0.891)	0.674 (0.514 - 0.883)	0.004 #
	OR adjusted 1 (95% CI)	1	0.726 (0.567 - 0.928)	0.715 (0.548 - 0.933)	0.655 (0.490 - 0.875)	0.633 (0.462 - 0.868)	0.007 #
	OR adjusted 2 (95% CI)	1	0.766 (0.596 - 0.984)	0.752 (0.574 - 0.986)	0.697 (0.518 - 0.937)	0.688 (0.499 - 0.950)	0.028 #
	OR adjusted 3 (95% CI)	1	0.766 (0.596 - 0.984)	0.746 (0.569 - 0.979)	0.691 (0.513 - 0.929)	0.680 (0.493 - 0.939)	0.022 #
IGF1/IGFBP3							
	molar ratio	< 0.108	0.108 - 0.138	0.139 - 0.163	0.164 - 0.193	> 0.193	
	No. of case / control	152 / 504	172 / 538	167 / 524	190 / 528	204 / 544	
	OR (95% CI)	1	1.156 (0.872 - 1.532)	1.184 (0.878 - 1.597)	1.358 (1.004 - 1.837)	1.430 (1.053 - 1.942)	0.013 #
	OR adjusted 4 (95% CI)	1	1.131 (0.850 - 1.505)	1.157 (0.854 - 1.566)	1.305 (0.960 - 1.774)	1.358 (0.995 - 1.855)	0.036 #
	OR adjusted 5 (95% CI)	1	1.133 (0.852 - 1.507)	1.151 (0.850 - 1.559)	1.296 (0.954 - 1.762)	1.357 (0.994 - 1.853)	0.038 #
IGFBP3 - IGF1							
	molar difference	< 70.14	70.14 - 83.04	83.05 - 96.84	96.85 - 112.12	> 112.12	
	No. of case / control	221 / 529	157 / 508	194 / 530	149 / 534	164 / 537	
	OR (95% CI)	1	0.724 (0.569 - 0.922)	0.836 (0.661 - 1.058)	0.624 (0.481 - 0.808)	0.669 (0.513 - 0.873)	0.002 #
	OR adjusted 4 (95% CI)	1	0.753 (0.590 - 0.961)	0.881 (0.693 - 1.120)	0.659 (0.506 - 0.857)	0.723 (0.550 - 0.948)	0.012 #
	OR adjusted 5 (95% CI)	1	0.755 (0.591 - 0.965)	0.875 (0.688 - 1.113)	0.656 (0.504 - 0.854)	0.715 (0.544 - 0.939)	0.009 #

adjusted 1, adjusted for IGF1 or IGFBP3; adjusted 2, adjusted for cigarette smoking, BMI, alcohol intake, and IGF1 or IGFBP3;
adjusted 3, adjusted for cigarette smoking, BMI, alcohol intake, diabetes mellitus, and IGF1 or IGFBP3;
adjusted 4, adjusted for cigarette smoking, BMI, and alcohol intake; adjusted 5, adjusted for cigarette smoking, BMI, alcohol intake, and diabetes mellitus ; # $p<0.05$

Table 6. Odds ratios and 95% confidence intervals for all malignant tumors among subgroup

		Quintile					
		1 (reference)	2	3	4	5	p for trend
IGF1							
(Male)	ng/mL (range)	< 80	80 - 109	110 - 130	131 - 160	> 160	
	No. of case / control	139 / 371	129 / 311	168 / 506	136 / 447	141 / 475	
	OR (95% CI)	1	1.017 (0.737 - 1.405)	0.789 (0.576 - 1.082)	0.708 (0.508 - 0.988)	0.680 (0.486 - 0.951)	0.003 #
	OR adjusted 1 (95% CI)	1	1.143 (0.822 - 1.589)	0.990 (0.705 - 1.389)	0.988 (0.678 - 1.438)	0.979 (0.659 - 1.456)	0.422
	OR adjusted 2 (95% CI)	1	1.596 (0.815 - 1.596)	0.939 (0.663 - 1.331)	0.959 (0.652 - 1.411)	0.917 (0.611 - 1.376)	0.279
	OR adjusted 3 (95% CI)	1	1.138 (0.813 - 1.594)	0.941 (0.663 - 1.334)	0.955 (0.649 - 1.405)	0.913 (0.608 - 1.372)	0.269
IGFBP3							
(Male)	μg/mL (range)	< 2.31	2.31 - 2.75	2.76 - 3.17	3.18 - 3.66	> 3.66	
	No. of case / control	249 / 548	143 / 478	132 / 401	87 / 354	102 / 329	
	OR (95% CI)	1	0.636 (0.498 - 0.811)	0.678 (0.524 - 0.878)	0.483 (0.358 - 0.653)	0.599 (0.440 - 0.815)	<0.001 #
	OR adjusted 1 (95% CI)	1	0.645 (0.498 - 0.836)	0.700 (0.526 - 0.931)	0.504 (0.358 - 0.709)	0.625 (0.435 - 0.899)	0.004 #
	OR adjusted 2 (95% CI)	1	0.704 (0.540 - 0.918)	0.764 (0.570 - 1.024)	0.557 (0.391 - 0.789)	0.705 (0.486 - 1.023)	0.028 #
	OR adjusted 3 (95% CI)	1	0.707 (0.542 - 0.921)	0.764 (0.570 - 1.024)	0.555 (0.391 - 0.789)	0.701 (0.483 - 1.017)	0.025 #
IGF1/IGFBP3							
(Male)	molar ratio	< 0.108	0.108 - 0.138	0.139 - 0.163	0.164 - 0.193	> 0.193	
	No. of case / control	101 / 320	104 / 307	135 / 425	170 / 495	203 / 563	
	OR (95% CI)	1	1.151 (0.801 - 1.654)	1.087 (0.759 - 1.557)	1.181 (0.822 - 1.697)	1.232 (0.863 - 1.759)	0.265
	OR adjusted 4 (95% CI)	1	1.125 (0.776 - 1.632)	1.027 (0.710 - 1.484)	1.099 (0.758 - 1.593)	1.134 (0.787 - 1.634)	0.568
	OR adjusted 5 (95% CI)	1	1.115 (0.768 - 1.617)	1.013 (0.700 - 1.465)	1.080 (0.744 - 1.567)	1.120 (0.777 - 1.614)	0.607
IGFBP3- IGF1							
(Male)	molar difference	< 70.14	70.14 - 83.04	83.05 - 96.84	96.85 - 112.12	> 112.12	
	No. of case / control	252 / 569	141 / 489	146 / 404	82 / 338	92 / 310	
	OR (95% CI)	1	0.644 (0.507 - 0.820)	0.778 (0.607 - 0.996)	0.518 (0.385 - 0.698)	0.607 (0.445 - 0.831)	<0.001 #

	OR adjusted 4 (95% CI)	1	0.688 (0.538 - 0.878)	0.851 (0.660 - 1.098)	0.560 (0.413 - 0.759)	0.683 (0.496 - 0.942)	0.003 #
	OR adjusted 5 (95% CI)	1	0.689 (0.539 - 0.880)	0.849 (0.658 - 1.095)	0.559 (0.413 - 0.759)	0.678 (0.492 - 0.935)	0.002 #
IGF1							
(Female)	ng/mL (range)	< 80	80 - 109	110 - 130	131 - 160	> 160	
	No. of case / control	136 / 402	103 / 357	151 / 434	111 / 343	135 / 366	
	OR (95% CI)	1	0.863 (0.625 - 1.192)	1.041 (0.766 - 1.414)	0.981 (0.701 - 1.371)	1.142 (0.809 - 1.614)	0.294
	OR adjusted 1 (95% CI)	1	0.908 (0.650 - 1.267)	1.120 (0.806 - 1.555)	1.073 (0.741 - 1.554)	1.276 (0.860 - 1.894)	0.140
	OR adjusted 2 (95% CI)	1	0.907 (0.649 - 1.269)	1.119 (0.804 - 1.557)	1.075 (0.741 - 1.561)	1.276 (0.857 - 1.901)	0.144
	OR adjusted 3 (95% CI)	1	0.915 (0.654 - 1.281)	1.138 (0.817 - 1.585)	1.085 (0.748 - 1.575)	1.291 (0.867 - 1.923)	0.133
IGFBP3							
(Female)	μg/mL (range)	< 2.31	2.31 - 2.75	2.76 - 3.17	3.18 - 3.66	> 3.66	
	No. of case / control	96 / 260	106 / 325	129 / 409	152 / 439	153 / 469	
	OR (95% CI)	1	0.876 (0.634 - 1.212)	0.849 (0.619 - 1.164)	0935 (0.681 - 1.238)	0.875 (0.633 - 1.210)	0.651
	OR adjusted 1 (95% CI)	1	0.882 (0.633 - 1.229)	0.813 (0.582 - 1.137)	0.876 (0.620 - 1.235)	0.784 (0.543 - 1.134)	0.258
	OR adjusted 2 (95% CI)	1	0.874 (0.626 - 1.222)	0.798 (0.569 - 1.119)	0.861 (0.607 - 1.220)	0.776 (0.534 - 1.127)	0.240
	OR adjusted 3 (95% CI)	1	0.869 (0.622 - 1.215)	0.785 (0.559 - 1.102)	0.850 (0.600 - 1.205)	0.764 (0.526 - 1.111)	0.215
IGF1/IGFBP3							
(Female)	molar ratio	< 0.108	0.108 - 0.138	0.139 - 0.163	0.164 - 0.193	> 0.193	
	No. of case / control	165 / 483	148 / 507	120 / 365	101 / 310	102 / 237	
	OR (95% CI)	1	0.855 (0.644 - 1.136)	0.971 (0.709 - 1.331)	0.971 (0.698 - 1.353)	1.318 (0.929 - 1.872)	0.088
	OR adjusted 4 (95% CI)	1	0.848 (0.637 - 1.128)	0.959 (0.699 - 1.316)	0.979 (0.701 - 1.365)	1.304 (0.916 - 1.858)	0.092
	OR adjusted 5 (95% CI)	1	0.856 (0.643 - 1.139)	0.967 (0.704 - 1.371)	0.983 (0.704 - 1.371)	1.319 (0.926 - 1.879)	0.086
IGFBP3- IGF1							
(Female)	molar difference	< 70.14	70.14 - 83.04	83.05 - 96.84	96.85 - 112.12	> 112.12	
	No. of case / control	91 / 236	90 / 315	149 / 396	143 / 465	163 / 490	
	OR (95% CI)	1	0.735 (0.523 - 1.033)	0.965 (0.708 - 1.315)	0.789 (0.572 - 1.088)	0.847 (0.614 - 1.170)	0.559
	OR adjusted 4 (95% CI)	1	0.727 (0.515 - 1.026)	0.951 (0.695 - 1.300)	0.787 (0.569 - 1.088)	0.842 (0.607 - 1.169)	0.565
	OR adjusted 5 (95% CI)	1	0.731 (0.518 - 1.031)	0.945 (0.691 - 1.293)	0.786 (0.568 - 1.087)	0.837 (0.603 - 1.162)	0.531

adjusted 1, adjusted for IGF1 or IGFBP3; adjusted 2, adjusted for smoking, BMI, alcohol intake, and IGF1 or IGFBP3;
adjusted 3, adjusted for smoking, BMI, alcohol intake, diabetes mellitus, and IGF1 or IGFBP3;
adjusted 4, adjusted for smoking, BMI, and alcohol intake; adjusted 5, adjusted for smoking, BMI, alcohol intake, and diabetes mellitus; # $p<0.05$